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Stimulation of methane consumption by endogenous CH_4 production in aerobic grassland soil

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ABSTRACT

The net CH_4 flux in terrestrial ecosystems is mostly the result of simultaneous gross CH_4 production (P_{CH_4}) and gross CH_4 consumption (C_{CH_4}) rates. However, it is not well-understood how endogenous P_{CH_4} may influence C_{CH_4} and hence the net CH_4 flux outcome in upland soils. Gross CH_4 consumption may be either stimulated or outweighed by P_{CH_4}, resulting in higher or lower net CH_4 uptake rates, respectively. To investigate gross CH_4 fluxes, we incubated intact soil cores taken from a permanent grassland with and without difluoromethane (DFM; blocks CH_4 oxidation). The gross CH_4 consumption was calculated by taking the DFM-measured rate of P_{CH_4} into account. Most of the oxic cores exhibited a more than 30 times higher C_{CH_4} than P_{CH_4} rate. However, four cores showed a considerably higher gross CH_4 production, accompanied by a stimulated gross CH_4 uptake which was entirely masked in the net CH_4 flux (C_{CH_4}/P_{CH_4} ratios of 0.9–2.3). In three of the four cores macro-fauna (earthworms and Scarab beetle larvae) were present. Removing the animals and homogenizing the intact core structure lowered P_{CH_4} by 27–100%. Subsequent incubation of Scarabidae larvae such as *Pachnoda* sp., *Cetonia* sp. and others revealed animal CH_4 and N_2O production rates within the range of those observed in the inhabited cores. Moreover, in-situ soil air CH_4 concentrations obtained from four depth profiles (0–50 cm depth) at the grassland site also indicated that temporarily, small-scale local hot spots of CH_4 and N_2O production existed within otherwise aerobic soil profiles. These spots indicated a stimulated CH_4 uptake after the CH_4 source (of whatever nature) had ceased. The results suggest that soil macro-fauna, anaerobic soil microsites or both combined may provide suitable conditions for CH_4 production in otherwise oxic soil environments. Hence, endogenous CH_4 production in upland soil may partially be related to the density and species composition of soil invertebrates, with a potential impact on the CH_4 sink capacity of these soils.

1. Introduction

Oxidation of the greenhouse gas methane (CH_4) in soils by methanotrophic bacteria currently removes 30 Tg annually from the atmosphere which equals 5.4% of the global CH_4 sink (Denman et al., 2007). Methanotrophs are active or at last present in almost all (aerobic) soils from desert to arctic ecosystems (e.g. Striegl et al., 1992; Whalen, 2005). Net CH_4 consumption rates are known to be related to ecosystem type, soil moisture, water table depth, soil texture, and agricultural management practices (Czepiel et al., 1995; Boeckx et al., 1997; Kammann et al., 2001a; Powlson et al., 1997). A major factor governing the actual CH_4 uptake is the diffusion characteristics of O_2 and CH_4 to the site of bacterial consumption. Hence the water content or water table depth often is a good predictor of net CH_4 uptake rates (Steudler et al., 1989; Castro et al., 1994; Kammann et al., 2001a,b; Conrad, 2007).

Another major factor is the composition of the methanotrophic community itself. In addition to morphological, physiological and phylogenetic differences of types I and II Methanotrophs (belonging to the γ- and α-subdivisions of the Proteobacteria, respectively) there are two methanotrophic activity patterns related to the ecosystems' dominant oxic conditions (Bender and Conrad, 1992, 1995). Methanotrophs with a comparably high affinity of the methane monooxygenase (MMO) for the substrate CH_4 are able to oxidise atmospheric CH_4 concentrations. They are common in upland soils, have not been cultured so far, and probably belong to the α-Proteobacteria (Dunfield et al., 1999; Ricke et al., 2005; Knief et al., 2006). In contrast, low-affinity

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Methanotrophs with a much larger apparent half-saturation constant (k_m) of the MMO require much higher CH_4 concentrations to thrive; these can be found in habitats with CH_4 production – oxic boundaries (e.g. rice paddies, swamps, landfill cover soils) and have been cultured and characterized (Conrad, 2007).

Methane production by methanogenic Archaea can occur in all soils (Peters and Conrad, 1995), provided that soils contain degradable carbon substrate and are exposed to anoxic conditions over a sufficiently long time period for the onset of methanogenesis (Conrad, 2007). However, small endogenous rates of CH_4 production in oxic soil environments (dominantly consuming methane) have been reported which may be related to anaerobic microsites (Andersen et al., 1998; Wagner et al., 1999; von Fischer and Hedin, 2002; Megonigal and Guenther, 2008). Therefore, net CH_4 fluxes between soil surfaces and the atmosphere are regarded as the outcome of gross CH_4 production (P_{CH_4}) and gross CH_4 consumption (C_{CH_4}), i.e. the net CH_4 flux is zero when both gross rates balance each other. However, C_{CH_4} may increase locally in response to P_{CH_4} in anaerobic microsites in the soil, either due to growth stimulation of low-affinity Methanotrophs (Bender and Conrad, 1995; Dunfield et al., 1999) or due to an increase in the activity of high-affinity Methanotrophs (Bender and Conrad, 1995), or both.

Earthworms have not been closely linked to *in-situ* CH_4 production so far (Karsten and Drake, 1997; Borken et al., 2000; Drake et al., 2006) but they significantly increased denitrification and the emissions of another potent greenhouse gas, N_2O, indicating low-oxygen conditions associated with earthworm activity (Elliott et al., 1990; Karsten and Drake, 1997; Borken et al., 2000; Speratti et al., 2007). In contrast, soil-dwelling (mostly tropic) invertebrates such as termites or humus-feeding Scarabidae larvae (*Pachnoda* sp.) have been described to produce and emit methane via methanogenic Archaea in their gut systems (Hackstein and Stumm, 1994; Egert et al., 2003; laboratory incubations). However, we are not aware of reports of endogenous *in-situ* CH_4 production (apart from termites) in temperate oxic soils due to soil macro-fauna.

During a long-term experiment at the grassland site under investigation (Kammann et al., 2008), closed-chamber measurements of net CH_4 fluxes as well as soil air CH_4 concentration measurements repeatedly suggested that small-scale hot spots of CH_4 production may temporarily exist in well-aerated soil (unpublished results). Therefore, the aim of this study was to investigate the autumnal gross CH_4 production and consumption rates of intact grassland topsoil cores in the laboratory where any influence of the water table (e.g. vertical diffusion of CH_4) can be excluded.

2. Materials and methods

2.1. Site description and core incubation

The extensively managed grassland site (*Arrhenatheretum elatioris* Br.-Bl.) near Gießen, Germany, has been fertilized with 40 kg Calcium-Ammoniumnitrate in mid-April since 1996 and is mown twice a year at the beginning of June and September. The N-limited grassland is intermediate species rich and has not been ploughed for at least 100 years. The soil is a stagno-fluvic gleysol over a clay layer with about 10% sand, 30% clay and 60% silt and mean C and N contents of 4.5% and 0.45%, respectively (Jäger et al., 2003; Kammann et al., 2008). Net CH_4 consumption at the site is in the upper range of values reported for grasslands in the literature (Kammann et al., 2001a) with a diverse methanotrophic community (types I and II genera: *Methylocystis*, *Methylomicrobium* and *Methylocapsa*) with CH_4 consumption characteristics that range between communities of wetland and upland soils (Horz et al., 2002).

Eight soil cores were sampled from 0 to 7.5 cm depth (8 cm diameter; Eijkelkamp agrisearch equipment, The Netherlands) on 27 October 2006 from each of three locations (L1–L3) which cover a slight soil moisture gradient at the site. Cores from L1 and L2 plots had encountered the lowest and highest mean soil moisture and water table depth throughout annual courses, respectively, while the L3 plot experienced intermediate conditions. Immediately after sampling, aboveground biomass was clipped. The intact cores, still in their steel sampling rings and capped at the bottom with a plastic cap, were placed in air-tight 2.6 L jars (Weck®, Germany) with a septum in the glass lid for sampling, and repeatedly incubated for 60–70 h in the laboratory at 22 °C. Three control jars were included that did not contain any soil. Air samples were taken with 60-ml polyethylene syringes (55 ml) roughly every 12 h for 3 days, and the sampled gas amount was replaced by synthetic air (without CH_4). For this, a half-filled, diffusion-tight FlexFoil grab bag (3 L; SKC Inc., USA) was connected via needle/septum and closed stopcock of the bag to the incubation jar. Directly after sampling, the stopcock was opened to allow the influx of synthetic air according to the actual atmospheric pressure (which was recorded and used in the calculations). Jars were incubated without and with the application of 0.8 ml DFM (difluoromethane, CH_2F_2, i.e. an effective concentration of 0.03–0.05 kPa) to inhibit CH_4 oxidation (Miller et al., 1998). Finally, soil cores were hand-homogenized, soil animals were removed if present, and the homogenized soil was incubated again in the presence of 0.8 ml DFM per jar. All CH_4 concentration results were corrected for the dilution (replacement with CH_4-free air) and/or CH_4 concentrations in the control jars (Fig. 1). The application of DFM indeed completely blocked the CH_4 uptake in the intact cores, and the inhibition developed quickly enough to prevent the decline of CH_4 concentrations in the incubation jars to lower-than-control concentrations (Fig. 1b and c).

2.2. In-situ soil air CH_4 concentrations and gas analysis

Soil air was sampled during July–November 2006 at a fourth location, L4 (in soil moisture between L1 and L3) in the field every 1–3 weeks with soil air samplers (Kammann et al., 2001b) installed in September 2001 in four soil profiles with samplers at 5, 10, 20, 30, 40 and 50 cm depth, respectively (5 and 50 cm: $n = 7$; all other depths: $n = 4$).

The samplers consist of closed silicone tubes that allow the diffusion of the greenhouse gases CO_2, N_2O and CH_4 from the soil atmosphere of the respective depth into the samplers, but no mass flow, or diffusion of liquid or vapour H_2O (method: Kammann et al., 2001b). The horizontally installed, flat silicone coils had a diameter and height of about 20 and 2 cm, respectively, integrating across an area of at least 635 cm^2 (sampler–soil matrix interface).

Gas samples were analyzed within 12 h in the lab on a GC (Shimadzu GC-14B) fitted with an FID and an ECD (carrier gas N_2 at 30 ml min^{-1}) and an automated sampling unit (Loftfield et al., 1997).

2.3. Calculations

The calculation of gross CH_4 flux rates followed the approach of von Fischer and Hedin (2002) with the difference that we used DFM blocking instead of $^{13}CH_4$ pool dilution to determine gross CH_4 production (P_{CH_4}). Results are based on the assumptions that (i) DFM does completely block CH_4 oxidation, (ii) DFM does not impact CH_4 production (Miller et al., 1998), and that (iii) P_{CH_4} is constant over time.

First, P_{CH_4} was calculated from a linear increase in CH_4 concentrations in the jars after DFM application, i.e. inhibition of C_{CH_4} (see Fig. 1b). For P_{CH_4} of core L2_8, only the first five samplings were taken (Fig. 1b) for the linear regression, because we consider it likely in retrospect that the soil animals most likely responsible for