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Soil methanotroph abundance and community composition are not influenced by substrate availability in laboratory incubations

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ABSTRACT

Variations in the rates of atmospheric CH_4 uptake in upland soils can arise from both abiotic and biotic factors. Among the less-studied biotic factors is the degree to which methanotroph activity and community composition interact with supply of CH_4 to the soil. Here, we investigated whether the abundance of high affinity methanotrophs in a range of soils representing different land use types is substrate (CH_4) dependent. Field replicates of three soils sampled from deciduous forest, spruce forest and agricultural sites were incubated in columns flushed continuously for 24 days with air at one of four CH_4 concentrations: <1 ppm (starvation), 1.8 (ambient), 30 (low elevated) and 60 (high elevated) ppm. In all soils, CH_4 oxidation rates increased linearly with CH_4 supply. For all levels of CH_4 supply, CH_4 oxidation rates were the highest in deciduous forest soil followed by spruce forest and agricultural soils. Terminal restriction fragment length polymorphism (T-RFLP) analysis indicated that the agricultural soil had a distinct methanotrophic community compared to the two forest soils. In particular, the T-RFs (Terminal restriction fragments) associated with *USCα* and *Type II* methanotrophs (*Methylocystis* sp, *Methylosinus* sp.) were the most abundant in forest soils while *Type 1a* associated T-RFs dominated in agricultural soil. The agricultural and forest soils also differed in their fractionation of stable isotopes, ^{13}C and ^{2}H, during CH_4 oxidation. Altering CH_4 concentration in the inlet air did not change methanotroph abundance, as evidenced by three different assays, two qPCR and T-RFLP, that recorded no changes in the number of *pmoA* genes and/or the relative abundance of T-RFs. Altogether, it is proposed that intrinsic differences in CH_4 oxidation rates between soils, particularly between temperate agricultural and forest soils, are driven by methanotroph community structure. The population size of methanotrophs in upland soils did not respond to CH_4 availability and is most probably regulated by other factors, such as the availability of nitrogen, cross-feeding or other carbon sources.

1. Introduction

Methane (CH_4) is a potent greenhouse gas with a global warming potential ~ 23 times greater than CO_2. Methane concentrations in the atmosphere have more than doubled since 1800 AD, and contribute about 15% to the current enhanced greenhouse effect (IPCC, 2013). Currently CH_4 concentrations in the atmosphere are increasing at 0.4% per year (Kirschke et al., 2013), after nearly a decade where they stayed constant. Biological oxidation of methane in upland soils by high affinity methanotrophs, specialized microorganisms that are capable of consuming low concentrations of CH_4, represents the second largest sink for atmospheric CH_4 after tropospheric chemical oxidation (Brevik, 2012). Global estimates of the soil sink vary more than five-fold, between 9 and 47 Tg yr^{-1} (Kirschke et al., 2013), and the large uncertainties are mainly due to poor understanding of the factors that regulate CH_4 uptake rates.

Upland soils vary in their rates of atmospheric CH_4 oxidation, and the mechanisms underlying these differences can be broken down into factors that affect soil diffusivity and factors that affect microbial (methanotrophic) activity (Smith et al., 2003; Curry,

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2007). Soil diffusivity controls the rate at which CH_4 and O_2 are supplied from the atmosphere to the methanotroph community. The rate of gas diffusion through soil is mainly controlled by soil moisture, although the relationship between diffusivity and soil moisture differs among soil types (Hiltbrunner et al., 2012; Luo et al., 2013). The role of methanotrophic activity is rather complex and reflects a suite of underlying features of the biological community. Methanotroph activity can be broken down into the per-capita rate of activity, which may be thought of as physiological state (e.g., in terms of response to temperature) or it may be a function of enzyme kinetics and therefore respond to substrate supply and/or inhibitor concentration (e.g., ammonium) (King and Schnell, 1994; Bodelier and Laanbroek, 2004; Menyailo et al., 2012). Variation in the per-capita activity may further be influenced by which individual strains comprise the methanotroph community because enzyme kinetics and physiological responses appear to vary among taxa or even genera (Knief and Dunfield, 2005; Mohanty et al., 2006; Tate, 2015).

In recent studies, two main explanatory factors have been proposed to regulate methanotroph activity in soils under contrasting land use: methanotrophic community structure and methanotroph abundance. It is experimentally evident that particulate methane monooxygenase (*pMMO*) enzyme facilitates the conversion of CH_4 to methanol, which is the first step of CH_4 oxidation (Hanson and Hanson, 1996). The ability of the enzyme to perform at different substrate concentrations is indicated by its Michaelis-Menten constant (Km), with lower Km values indicating higher affinity. A range of Km values have been measured in different soils (Knief and Dunfield, 2005; Knief et al., 2006; Tate et al., 2012), indicating that *pMMO* associated with different methanotrophs may vary in its affinity for CH_4. In this regard, identified groups of methanotrophs such as the upland soil cluster (*USCα, USCγ*), *Methylocystis* spp have particularly gained attention as their ability to oxidize atmospheric concentrations of CH_4 has been confirmed by stable isotope probing (Kolb, 2009; Nazaries et al., 2013). In addition, the inconsistent response of different soils to NH_4^+ amendment has also been related to the dominant methanotroph community, with some taxa having higher tolerance to NH_4^+ compared to others (Jang et al., 2011).

Overall methanotroph abundance can be positively correlated with CH_4 uptake rates (Knief and Dunfield, 2005; Degelmann et al., 2009; Ho et al., 2013; Bárcena et al., 2014). For example, the effects of land management practices, such as fertilizer use in cultivated lands, afforestation or the type of vegetation, on the intrinsic CH_4 oxidation capacity of soil have been linked to altered biomass of the methanotrophic community (Singh et al., 2007; Maxfield et al., 2008b; Menyailo et al., 2010). However, the mechanisms underlying these relationships remain unknown. Given the very low concentrations of methane in the atmosphere and soil air, we hypothesize that methane supply could limit methanotroph community size and activity. Previously, a few attempts have been made to link CH_4 availability with biomass or abundance of high affinity methanotrophs (Roslev et al., 1997; Bull et al., 2000). But the major drawbacks of such studies are their methodological limitations in determining methanotrophic community size (based on PLFA) and experimental design (exposure to high CH_4 concentrations without phylogenetic analysis to test for shifts in methanotrophs community structure).

Atmospheric CH_4 oxidation results in isotopic enrichment of residual CH_4 due to discrimination against heavy isotopes (^{13}C & ^{2}H) by methane oxidizing enzyme (pMMO) and molecular diffusion (Feisthauer et al., 2011). Thus enrichment of residual CH_4 is a quantitative indicator for presence of microbial sink in soils. However, its quantitative use to estimate rates of microbial CH_4 oxidation requires associated parameters known as fractionation factors or kinetic isotopic effects (Snover and Quay, 2000; Templeton et al., 2006; Feisthauer et al., 2011; Kato et al., 2013). Under field conditions, the magnitude of fractionation factors (microbial and diffusion) could be affected by a number of biological and physical drivers (i.e. temperature, soil type, methanotroph community composition or abundance). However, the degree to which such parameters can combine to influence the observed kinetic isotopic factor (α) is also not well known.

To test the methane limitation hypothesis, i.e. that the abundance of methanotrophs depends on CH_4 availability, and to link overall microbial isotopic effects to methanotroph community, we conducted an incubation study using three upland soils that differed in a range of soil properties, including land use. After prolonged exposure to high and/or low CH_4 levels (24 days), all samples were tested for shifts in their capacity to consume methane at the initial, atmospheric, level for three days. We used DNA based phylogenetic tests to observe whether shifts in methanotrophic community size and composition occurred due to altered rates of CH_4 supply.

2. Material and methods

2.1. Site description and soil sampling

For this study we chose soils from three land uses common in central Europe, including deciduous and spruce forests and an agricultural field sampled in the state of Thüringen, Germany. Preliminary experiments with soils from these sites indicated that they had different intrinsic CH_4 oxidation rates, and we have considerable information on their chemical and physical properties. The agricultural soil was collected from the site of the Jena biodiversity experiment, and includes a C3–C4 vegetation shift experiment established in 2003 (Steinbeiss et al., 2009). At the time of sampling, the plot was sown with maize (*Zea mays* L.), which was at the seedling stage. The two forest sampling sites are part of the Biodiversity Exploratories (Fischer et al., 2010) and the vegetation at the soil sampling sites were monocultures of deciduous beech (*Fagus Sylvatica* L.) and spruce (*Picea abies* (*L.*) Karst), respectively, located near the Hainich National Park.

At each site, 16 soil samples (0–10 cm) were acquired from an area of 20 × 20 m using a spade. Separate samples were taken to determine field soil moisture and bulk density. Soil sampling was done 2–5 days before starting incubations and all samples were kept at room temperature. Each sample was separately passed through 4 mm sieve and large plant particles were removed by hand. The characteristics of the three soils are presented in Table 1.

2.2. Soil incubation

Approximately 1 kg of field moist, sieved and thoroughly mixed soil was placed in custom made PVC columns (10 cm diameter and 20 cm height) and soil moisture was increased to 50% WFPS by adding distilled water using a sprinkler for even distribution. All soil columns were placed in a climate chamber at constant temperature (25 °C). The bottom of each column was connected to an air inlet system that continuously flushed the soil columns (30 ± 3 mL min^{-1}) with air of known composition (see below). The headspace (top) of each column was connected to a gas analysis system via an automated multiport stream selection valve (Valco). The concentration of CH_4 in the airstream leaving the headspace of each column was analyzed in a cavity ring down spectrometer (PICARO 2301). For stable isotopic analysis of CH_4 ($\delta^{13}CH_4$, $CH_3\delta D$), headspace air was sampled with 1 L glass flasks (see below). Moisture loss (~1 g H_2O per day) due to continuous flushing with dry air was compensated by adding water on every 4th day using a water spray bottle.