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Impact of preferential methane flow through soil on microbial community composition

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ABSTRACT

The anaerobic microbial degradation of waste organic fractions in landfills constitutes one of the principal anthropogenic methane sources. Microbial oxidation of methane in optimized landfill covers or biofilters has been listed as key mitigation technology for the reduction of methane fluxes from landfills that are no longer suitable for energy recovery or flaring. Therefore, it is vital to understand what influences distribution of methane oxidizers and their activity in landfill soils. Here we describe the impact of gas fluxes through preferential pathways (hotspots) in the cover soil of a municipal solid waste landfill in north-western Germany on the soil properties and the microbial communities that colonize the upper soil crust in these environments. Two sites with high surface methane concentrations (>14,000 ppm), two sites with moderate surface methane concentrations (~400 ppm) and two sites without measurable methane emissions at the surface were investigated. It was found that elevated average soil methane concentrations coincided with increased levels of TOC and TN and the TOC/TN ratio in the topsoil. The increase of the latter posits a change in the composition of the organic matter towards increasing levels of nitrogen-poor components as for example EPS, which were observed in the samples with higher TOC/TN ratios. Elevated average soil methane concentrations were also accompanied by a decrease in the overall bacterial diversity. The community at these sites were dominated by a few lineages such as methanotrophs, particularly of type II, *Burkholderiales*, *Rhodospirillales* and *Bradyrhizobiaceae*. This dominance may have contributed to the purple discoloration at the soil surface at the sites with the highest surface methane concentrations.

1. Introduction

Under the anaerobic conditions prevailing in landfills, the microbial degradation of the waste organic fraction yields a gas mixture typically composed of 55–60% v/v CH_4 and 40–45% v/v CO_2, accompanied by various trace gases. Along with agriculture, fossil fuel extraction and biomass burning, waste management and disposal, i.e. landfills, constitute one of the principal anthropogenic methane sources. They are estimated to be responsible for emissions as high as 35–69 Tg CH_4/a and are ranked as second largest anthropogenic methane emission source in Europe, making up 22% of the total anthropogenic methane emissions at approximately 3.6 Tg/a (~90 Tg CO_2 equivalents/a; [1]). The microbial oxidation of methane in optimized landfill covers or biofilters has been listed as key mitigation technology for the reduction of methane fluxes from landfills that are no longer suitable for energy recovery or flaring [2]. Methane oxidation thus presents an “end-of-pipe” option both for older, non-sanitary sites but also for sanitary landfills after active gas extraction has ended but gas production continues for decades or even centuries on a lower level.

As a result of the high source strength of methane, landfill soil covers harbor an abundant methanotrophic community, usually dominated by *Methylosinus* and *Methylocystis* species [3–6], classified as type II methanotrophs. Landfill cover soils show potential methane oxidation rates in the order of several hundred g CH_4 m^{-2} d^{-1} [7,8] which, however, are subject to large spatial variability [9], resulting from the spatial variability of gas fluxes through the cover soil, caused by preferential flow paths in the soil. These are formed by differences in soil texture and compaction, through soil aggregation, rootage [10] or animal burrows, providing pathways of enhanced gas permeability and hence for preferential gas [11,12]

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and water [13] flow. Thus, methane emissions are not spatially homogeneous but occur through small-scale areas, often referred to as emission hotspots [14], creating small-scale patterns of habitats with varying environmental conditions.

Hotspots of gas emission can often be identified visually, for example by a change, damage or lack of vegetation, likely due to the shortage of oxygen in the root zone as a result of strong advective flow of gas from the underlying waste body [15]. Moreover, a distinct discoloration of the topsoil towards purple colors is frequently encountered at locations of increased gas emission (author's personal observation). In contrast, such discoloration was never observed at methanotrophically very active sites, harboring an abundant methanotrophic population but not showing high emissions [16]. It is hypothesized that the discoloration stems from the particular microbial community established as a result of the specific habitat posed by the enhanced gas fluxes. Characteristics of these habitats comprise, for example, excess availability of methane-derived carbon and of hydrogen sulfide (both components of landfill gas) and possible changes between anaerobic and aerobic conditions over very short distances on the micrometer to centimeter scale (soil pore system to secondary macropores such as cracks). However, to date, the microbial community of such discolored patches has never been investigated. The aim of this study was to elucidate possible differences between microbial community compositions from methane emitting hotspot and neighboring non-methane-emissive locations, consisting of the same original type of soil. The focus lay particularly on those parts of the community that were affected directly or indirectly by methane concentrations.

2. Materials and methods

2.1. Study site

The investigated site is a 1.5 ha old municipal solid waste landfill located approximately 70 km southwest of the city of Hamburg in northwestern Germany that was operated between 1970 and 1982. The waste, a mixture of household (37 mass-%), construction and demolition (27 mass-%), industrial (10 mass-%), other inert waste (11 mass-%) and sludges (15 mass-%) [17], was disposed of in a former sand pit on top of which waste was further piled up to form a mound.

Base or surface liners are missing so that landfill gas can migrate freely from the landfill body through the cover. The gas extraction system was operated only in the first few years after closure. Cover soil properties and cover thickness vary strongly and basically reflect whatever material was available to the landfill operator at the time of cover construction. Soil texture is mostly sandy, however, bulk density, pores size distribution, as well as soil chemical properties such as carbon and nitrogen content vary strongly (data given in Ref. [9]). The site is vegetated, mostly with grassland interspersed with shrubs, bushes and small trees. Previous analyses of the methanotrophic community using diagnostic microarray revealed a preponderance of type II methanotrophs [6] and a methane oxidation rate of 0.3–19.5 μg CH_4 g_{dw}^{-1} h^{-1} [9]. Assuming a thickness of the methane oxidation horizon of 40 cm and a dry bulk density of the soil of * g cm^{-3}, these oxidation rates translate to 0.17 and 9.80 g CH_4 m^{-2} d^{-1}. Actual gas production was estimated at 5700 m^3 CH_4 per year, using the IPCC (Intergovernmental Panel on Climate Change) gas production model [17,18], yielding a hypothetical spatial load to the cover soil of 0.72 g CH_4 m^{-2} d^{-1}, assuming an even spatial distribution of this load to the landfill cover. However, due to the above mentioned preferential nature of gas flow, emissions escaped through hotspots with fluxes of up to 155 g CH_4 d^{-1}, emanating from areas of a few square centimeters in size [14].

2.2. Sampling locations and sampling strategy

Gas and soil samples were collected on the 26th of April, 2012 from two areas (1 and 2) at the south eastern slope of the landfill. On this slope, several hotspots of gas emission had been detected during a previous monitoring campaign [14]. The two areas were 3 m apart on the same height of the slope. Emissions in this area, monitored by means of static chambers, ranged between 2.7 and 62.5 g CH_4 d^{-1} with an average of 32.1 g CH_4 d^{-1} ($n = 31$, measured biweekly to monthly from 2008 to 2010). The methodology of the chamber measurement is described in Ref. [14].

Within these two areas six sampling locations were chosen: two were hallmarked by distinct purple discoloration of the soil (1A and 2A), a lack of vegetation and very high surface methane concentrations. Within a maximum distance of 80 cm from 1A to 2A, two further sampling sites, namely 1B and 1C (near 1A) and 2B and 2C (near 2A), were selected, which did not exhibit this typical purple discoloration, bore fresh grass vegetation and showed no detectable surface methane concentration. Sites 1C and 2B exhibited intermediary features, showing lower levels of surface methane concentrations. For details of sampled soils and depths see Table 1.

2.3. Soil properties and gas composition

Soils were analyzed for texture according to DIN ISO 11277 [19], organic carbon and total nitrogen according to DIN ISO 10694 [20] and DIN ISO 13878 [21] using a CHN analyser vario Max (Elementar Analysensysteme GmbH). Measurement error was 0.08% TOC and 0.01% TN. Moisture was determined by gravimetric analysis following oven-drying at 105 °C.

Composition of the soil gas phase was sampled using a gas probe that was inserted into 4, 10, 20, 30 and 40 cm below soil surface. Gas was collected by probing through the probe septum with a needle connected to a 60 ml syringe and analyzed on-site using a biogas analyzer (BM 2000, Geotechnical Instruments (UK) Ltd.; detection limit = 0.1%). The concentration of N_2 was calculated by subtracting the sum of the measured concentrations of CH_4, CO_2 and O_2 from 100.

Methane concentrations at the soil surface were analyzed using a portable flame ionization detector (FID; Sewerin GmbH, Gütersloh). The reading was recorded when the FID gave a stable value. The lower detection limit was 1 ppm, the upper detection limit 14,000 ppm of volatile organic compounds.

2.4. Microbial community analyses

Following collection, the soil samples were frozen at −20 °C immediately until microbial community analyses were performed.

2.4.1. DNA extraction, PCR, cloning, and sequencing

Around 0.5 g soil from eight samples, i.e. 1A, 1B (consisting of parallels 1B1 and 1B2), 1C1, 1C2, 2A, 2B and 2C (for soil depths see Table 1) was used to extract DNA with the UltraClean Soil DNA Isolation Kit (MoBio, Solana, CA, USA) according to the manufacturer's instructions. Bacterial 16S rRNA genes were PCR-amplified in three parallels using the oligonucleotide primer sets consisting of 27F and 1492R [22]. All amplifications were performed in triplicate, products pooled and separated by gel electrophoresis. Purified PCR products were ligated into the pGEM®-T vector system (Promega, Madison, WI, USA) and transformed into competent *Escherichia coli* DH5α. PCR products of the correct size were partially sequenced using the primer 27F [[22], sequence length = 331 to 856 base pairs]. Sequencing was performed with