Science of the Total Environment 640–641 (2018) 429–441

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Methane and nitrous oxide cycling microbial communities in soils above septic leach fields: Abundances with depth and correlations with net surface emissions

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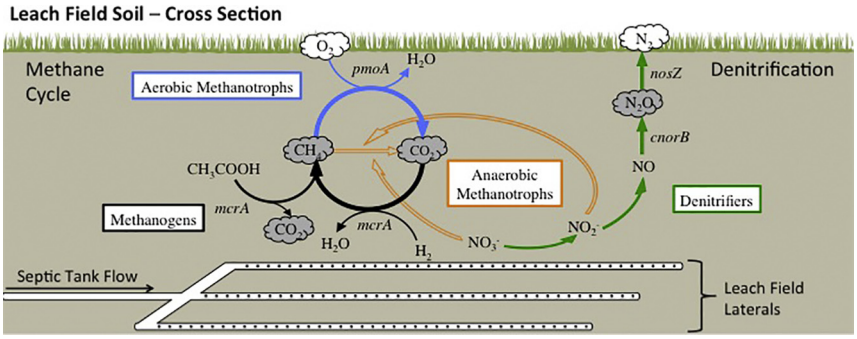
HIGHLIGHTS

- GHG emissions and microbial communities were studied in 9 leach and control soils.
- CH_4 and N_2O cycling biomarker genes were present in all soil sites and depths.
- Microbial community composition was driven by soil VWC, CH_4 and N_2O fluxes.
- Leach field presence did not affect community structure or GHG fluxes.
- Ratio of methanogen to methanotroph abundance correlated with CH_4 fluxes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 April 2018
Received in revised form 23 May 2018
Accepted 24 May 2018
Available online xxxx

Editor: Jay Gan

Keywords:
Methane
Nitrous oxide
Septic leach field
Methanogen
Methanotroph
Denitrifier

ABSTRACT

Onsite septic systems use soil microbial communities to treat wastewater, in the process creating potent greenhouse gases (GHGs): methane (CH_4) and nitrous oxide (N_2O). Subsurface soil dispersal systems of septic tank overflow, known as leach fields, are an important part of wastewater treatment and have the potential to contribute significantly to GHG cycling. This study aimed to characterize soil microbial communities associated with leach field systems and quantify the abundance and distribution of microbial populations involved in CH_4 and N_2O cycling. Functional genes were used to target populations producing and consuming GHGs, specifically methyl coenzyme M reductase (*mcrA*) and particulate methane monooxygenase (*pmoA*) for CH_4 and nitric oxide reductase (*cnorB*) and nitrous oxide reductase (*nosZ*) for N_2O. All biomarker genes were found in all soil samples regardless of treatment (leach field, sand filter, or control) or depth (surface or subsurface). In general, biomarker genes were more abundant in surface soils than subsurface soils suggesting the majority of GHG cycling is occurring in near-surface soils. Ratios of production to consumption gene abundances showed a positive relationship with CH_4 emissions (*mcrA*:*pmoA*, $p < 0.001$) but not with N_2O emission (*cnorB*:*nosZ*, $p > 0.05$). Of the three measured soil parameters (volumetric water content (VWC), temperature, and conductivity), only VWC was significantly correlated to a biomarker gene, *mcrA* ($p = 0.0398$) but not *pmoA* or either of the N_2O cycling genes ($p > 0.05$ for *cnorB* and *nosZ*). 16S rRNA amplicon library sequencing results revealed soil VWC, CH_4 flux

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https://doi.org/10.1016/j.scitotenv.2018.05.303

and N_2O flux together explained 64% of the microbial community diversity between samples. Sequencing of *mcrA* and *pmoA* amplicon libraries revealed treatment had little effect on diversity of CH_4 cycling organisms. Overall, these results suggest GHG cycling occurs in all soils regardless of whether or not they are associated with a leach field system.

1. Introduction

Septic systems account for approximately 25% of U.S. wastewater treatment however they are estimated to be responsible for almost 65% of domestic wastewater greenhouse gas (GHG) emissions (US EPA, 2012). Septic systems typically consist of two parts: the septic tank and the subsurface soil dispersal system hereafter referred to as the leach field. Some newer systems have an additional sand filter between the tank and the leach field to improve discharged water quality (USEPA, 2002). Both the septic tank and leach field portions of the system use microbial communities to degrade complex organics in wastewater and mineralize nutrients. Together they provide both anaerobic and aerobic treatment to effectively reduce organic carbon (C), nitrogen (N) and phosphorus (P) loads.

As a consequence of transforming C and N, microorganisms can produce the potent greenhouse gases methane (CH_4) and nitrous oxide (N_2O) with global warming potentials (GWP) of 20 and 200 times that of CO_2 over a 25-year time span, respectively. Still, only a handful of studies have quantified GHG emissions from septic systems (Diaz-Valbuena et al., 2011; IPCC, 2006; Kinnicutt et al., 1919; Leverenz et al., 2010; Winneberger, 1983). Greenhouse gases from these systems escape primarily through the roof vent, however a portion of these gases can be released through supersaturated septic tank effluent or be produced subsurface in soils surrounding leach field laterals. Truhlar et al. (2016) provided the first measurements of GHG emissions from two key septic system outlets: the roof vent and leach field soils. They found that CO_2 emissions from the roof vent and soils above leach fields were comparable. In contrast, CH_4 and N_2O emissions were significantly greater from the roof vent as compared to the leach field. The discrepancy between roof vent and leach field emissions suggests that microbial GHG cycling in soils above leach fields is, in part, responsible for mitigating CH_4 and N_2O emissions from leach field systems. This study aims to examine whether the abundance of key GHG cycling microbial populations in leach field soils correlates to measured CH_4 and N_2O emissions from these systems.

For microbial CH_4 production and aerobic consumption, biomarker selection is relatively straightforward. In methanogens, the α-subunit of the methyl-coenzyme M reductase (*mcrA*) enzyme, involved in the final step of methanogenesis, is well conserved across all known methanogens with the exception of anaerobic methane oxidizing (ANME) archaea (Friedrich, 2005; Luton et al., 2002; Steinberg and Regan, 2009). Similarly, all aerobic methane-oxidizing bacteria (MOB) contain the enzyme methane monooxygenase (MMO). MMO catalyzes the first step in CH_4 oxidation and the particulate form (pMMO) of the enzyme is found in the majority of cultivated methanotrophs (Dedysh et al., 2003; Semrau et al., 1995, 2010). The *pmoA* gene, encoding the α-subunit of pMMO, has near universal presence in both aerobic and nitrite-reducing bacterial methanotrophs and has been used as a biomarker for their presence and activity (Freitag and Prosser, 2009; Lee et al., 2014; Seo et al., 2013; Tate, 2015).

Recent discoveries of anaerobic methanotrophs make the CH_4 cycle more complex than previously thought. Nitrite-dependent anaerobic methane oxidizing (n-damo) bacteria of the NC10 phylum and anaerobic methane oxidizing (ANME) archaea - are phylogenetically diverse groups that have the potential to contribute significantly to CH_4 mitigation globally. N-damo processes are carried out by *Candidatus Methylomirabilis oxyfera*–like bacteria, which couple methane oxidation to nitrite reduction. These bacteria have previously been identified and quantified by targeting their *pmoA* or 16S rRNA gene (Ettwig et al., 2009; Luesken et al., 2011). ANME-2D archaea are thought to couple methane oxidation to nitrate reduction in a reverse methanogenesis pathway and can be studied using the same *mcrA* gene used to for methanogens (Ettwig et al., 2010; Hallam et al., 2004; Haroon et al., 2013; Wu et al., 2011). Both types of anaerobic methanotrophs have been found across a wide variety of soil systems and may be well suited for the leach field soil environment (Beal et al., 2009; Hui et al., 2017; Meng et al., 2016; Orphan et al., 2001; Shen et al., 2015, 2016; Vaksmaa et al., 2016; Wang et al., 2012; Weber et al., 2017).

The N_2O cycle is more complex than the CH_4 cycle and represents only a portion of the full denitrification pathway. Denitrification is the sequential reduction of nitrate to dinitrogen (N_2) gas. Each reduction step is catalyzed by one of four enzymes: nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos). Many denitrifying bacteria lack the genes encoding for Nos making partial or incomplete biological denitrification a significant source of N_2O (Henry et al., 2006; Sanford et al., 2012). Quantifying the microbial populations directly involved in production (Nor) and consumption (Nos) of N_2O is therefore key to understanding N_2O cycling in soils (Levy-Booth et al., 2014). Two types of bacterial nitric oxide reductases exist: cNor (cytochrome *c* electron donor) and qNor (quinol electron donor) (Braker and Tiedje, 2003; Dandie et al., 2007). cNor is specific to denitrifier populations and the *cnorB* gene has previously been used as a biomarker for N_2O production (Braker and Tiedje, 2003; Hendriks et al., 2000). For N_2O consumption, the *nosZ* gene has proven to be a suitable biomarker (Henry et al., 2006; Levy-Booth et al., 2014).

Studying the microbial populations involved in GHG cycling is essential to gaining greater insight into the factors controlling GHG emissions from soil systems. This study provides the first examination of presence, abundance, distribution, and characterization of GHG cycling microbial populations associated with septic system leach field soils. We quantified four functional gene biomarkers involved in GHG production and consumption in leach field systems: *mcrA* and *pmoA* for CH_4 and *cnorB* and *nosZ* for N_2O. Statistical models were used to investigate whether treatment type or measured soil environmental parameters control the abundance of these GHG cycling microbes. Additional statistical models were created to examine the relationship between functional gene abundances and net GHG fluxes from leach field soils. Furthermore, we examined microbial community composition in these soils by sequencing and analyzing 16S rRNA, *mcrA*, and *pmoA* amplicon libraries.

2. Materials and methods

2.1. Site descriptions

Nine homes in central New York using septic systems for onsite wastewater treatment volunteered to participate in this study. The characteristics for 8 of the 9 sites are summarized in Truhlar et al. (2016). Site 9, which was previously omitted due to a saturated leach field system, is included in this study and had a leach field area of 168 ft^2. Gas flux measurements, soil samples, and other relevant environmental parameters were taken between June and August of 2014. Three soil treatments were examined: leach field, sand filter and control