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Spatial and phyloecological analyses of *nosZ* genes underscore niche differentiation amongst terrestrial N_2O reducing communities

Jaanis Juhanson [a, c], Sara Hallin [a], Mats Söderström [b], Maria Stenberg [b], Christopher M. Jones [a, *]

[a] Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Box 7026, 750-07 Uppsala, Sweden
[b] Swedish University of Agricultural Sciences, Department of Soil and Environment, Box 234, 53223 Skara, Sweden
[c] Institute of Molecular and Cell Biology, University of Tartu, Riia 23, 51010 Tartu, Estonia

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ABSTRACT

The only known biological sink for the greenhouse gas nitrous oxide (N_2O) is its reduction to nitrogen gas (N_2) by bacteria and archaea that possess the *nosZ* gene conferring this trait. Phylogenetic and genomic evidence indicates that N_2O reducing communities can be divided into two genetically distinct groups, termed clade I and II. Differing abundance or diversity of each clade may have significant consequences for N_2O emissions from soil. However, whether the two groups respond similarly to gradients of environmental or biotic factors in soils remains unclear. Here, we explore spatial patterns of *nosZ* clade I and II abundance, phylogenetic diversity and community structure across a 44-ha farm, and compare with edaphic factors and abundances of ammonia oxidizing and denitrifying communities, which are the main N_2O producers in soil. Contrasting spatial distributions of the total abundance and phylogenetic diversity of each clade, as well as disparate associations with various edaphic and biotic factors indicated potential niche differentiation between the two clades. This is supported by the greater occurrence of significant phylogenetic clustering or overdispersion in clade I communities compared to clade II, indicating differences in the underlying mechanisms of community assembly for each group. Variance partitioning of phylogenetic community structure further showed that biotic factors, particularly the abundance of denitrifiers, played a more substantial role in explaining clade II community structure compared to that of clade I. Finally, identification of *nosZ* lineages driving differences in community structure and spatial distribution patterns of gene abundances suggests a shift in the genetic potential for N_2O production and consumption dynamics across the farm.

1. Introduction

Agricultural soils are a significant source of nitrous oxide (N_2O) (Thomson et al., 2012), a potent greenhouse gas and the leading cause of stratospheric ozone depletion (Ravishankara et al., 2009). Emissions from agriculture are expected to increase nearly 83% by 2050, largely due to increased use of nitrogen (N) fertilizers (Bouwman et al., 2013). Thus, there is substantial interest in the development of soil management practices that aim to reduce N_2O emissions while still maintaining high crop yields (Snyder et al., 2014). Since transformation of N compounds by microbial consortia is the primary driver of N_2O emissions from soils, a better understanding of the ecology of these communities could aid in the development of effective N_2O mitigation strategies. While several N-cycle pathways are known to contribute to the production of N_2O in soil (Butterbach-Bahl et al., 2013), the only known sink for N_2O in soils is its reduction to N_2 by microorganisms that possess the *nosZ* gene encoding the nitrous oxide reductase. This step has classically been associated with organisms capable of complete denitrification, in which nitrate or nitrite are reduced to N_2, with N_2O as an intermediate (Zumft, 1997). However, recent studies have shown that the diversity of microbial communities that carry *nosZ* is greater than previously expected (Jones et al., 2013) and that the capacity to reduce N_2O is not solely associated with denitrifying organisms (Sanford et al., 2012; Mania et al., 2014; Domeignoz-Horta et al., 2016).

* Corresponding author. Swedish University of Agricultural Sciences, Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Box 7026, 750 07 Uppsala, Sweden.
E-mail address: Chris.Jones@slu.se (C.M. Jones).

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Nitrous oxide reducers can be divided into two distinct groups, designated clades I and II based on the phylogeny of the *nosZ* gene, with clade I consisting of *nosZ* from proteobacteria and halophilic archaea whereas clade II includes a more diverse range of bacterial and archaeal taxa (Sanford et al., 2012; Jones et al., 2013). In an analysis of 652 microbial genomes including one or more of the enzymatic steps in the denitrification pathway, Graf et al. (2014) demonstrated that patterns of co-occurrence between *nosZ* and genes involved in denitrification differed substantially between the two *nosZ* clades. Organisms with clade I were more likely to be complete denitrifiers, while a large fraction of organisms with clade II *nosZ* lacked the genetic capacity to produce N_2O. This suggests that the relative proportion of N_2O reducing organisms with either clade I or clade II *nosZ* may have consequence for potential N_2O emissions from arable soils. In agreement, several studies have shown that increased diversity or abundance of clade II *nosZ* is associated with increased potential N_2O consumption or lower net N_2O production (Jones et al., 2014; Domeignoz-Horta et al., 2015; Samad et al., 2016). We also recently confirmed experimentally that the N_2O:N_2 ratio from soil is decreased when the proportion of bacteria with clade II increases, showing a direct causal link between clade II *nosZ* and lowered potential N_2O emissions (Domeignoz-Horta et al., 2016). A recent review summarizing studies examining N_2O reducing communities in a range of soils and other environments indicates that organisms with either *nosZ* variant occupy different niches (Hallin et al., 2017 in press). However, there are no studies that have examined the potential for niche differentiation at spatial scales relevant to agricultural management practices. Moreover, little is known about the relative importance of biotic factors, in particular the abundance of different N_2O producing communities in soil, compared to edaphic factors in determining the structure, abundance and diversity of each N_2O reducing community.

To address current knowledge gaps and further our understanding of the ecology of N_2O reducers in agricultural soils, we revisit the Logården experimental site in western Sweden, a 44-ha farm. From previous studies examining spatial patterns at this site, we have soil chemical and physical data as well as abundances of genes associated with the two main guilds involved in N_2O production, namely the denitrifier and ammonia oxidizer communities (Enwall et al., 2010; Wessén et al., 2011). In the present study, we sequenced and quantified clade I and clade II *nosZ* to obtain estimates of the community structure, diversity and abundance of the two clades of N_2O reducers. We hypothesized that i) clade I and clade II communities exhibit contrasting spatial structures of diversity and abundance in relation to edaphic factors due to niche differention and ii) the structure, abundance, and diversity of each clade are differentially associated with shifts in biotic factors, in this case the abundance of *nirS*, *nirK*, and *amoA* genes associated with different N_2O producing communities. To test this, we used phyloecological and variance partitioning methods to identify key biotic and abiotic factors that drive niche differentiation between and within N_2O reducers in clade I and II. A spatially explicit framework of analysis was used, in which spatial structure is treated as a covariate or predictor of ecological variation in addition to environmental or biotic factors (Dray et al., 2006). This has been shown to be highly useful for detecting differences in resource use or habitat preference amongst taxonomically or functionally defined microbial groups (Philippot et al., 2009; Bru et al., 2010; Griffiths et al., 2011).

2. Material and methods

2.1. Field description and soil sampling

The Logården research farm site is located in western Sweden (58°20′N, 12°38′E) and consists of a southern 26-ha area with seven fields under conventional farm management, and an adjoining northern 18-ha area with seven fields managed according to the Swedish criteria for organic farming (http://www.krav.se) since 1991. Each farming system uses a seven-year crop rotation that includes green manure leys and pulses. Thus, each field represents different years in the rotation in each system. The soil type varies from silty clay loam to silty clay with an average clay content of approximately 40%, and an organic matter content of 2–3%. Throughout the site, a total of 51 fixed points (Fig. 1) were selected in 2003 for soil sampling based on fuzzy k-means clustering of soil electrical conductivity, field topography and phosphorous (P) content measurements, which allowed for identification of potential environmental gradients within the field (Söderström and Lindén, 2004). Composite samples of twelve soil cores (20 mm) were taken to 10 cm depth at each point in April 2007, and an extensive survey of physical and chemical soil properties for each site was performed. Full descriptions of the experimental site, analyses of physical and chemical soil parameters, and soil sampling are provided in Enwall et al. (2010).

2.2. Quantification of 16S rRNA genes, amoA, nirS, nirK, and nosZ clade I and II

Soil DNA extraction was performed in duplicate from a total of 1 g of soil for each sample (Enwall et al., 2010) using the FastDNA SPIN for Soil kit (MP Biomedicals, Santa Ana, CA, USA) according to manufacturer instructions. The two DNA extractions were then pooled and quantified by spectrophotometry using a BioPhotometer (Eppendorf, Hamburg, Germany).

Quantification of 16S rRNA and *nosZ* genes was based on SYBR green assays using 1X DyNAmo Flash SYBR Green qPCR master mix (Finnzymes OY, Espoo, Finland), 1 μg μL^{-1} bovine serum albumin (New England Biolabs, Ipswich, MA, USA), 20 ng template DNA and either 0.4 μM of 341f and 534r primers for 16S rRNA gene (Muyzer et al., 1993), or 0.8 μM of nosZ2F and nosZ2R (Henry et al., 2006) or nosZ-II-F and nosZ-II-R (Jones et al., 2013) for *nosZ* clades I and II, respectively. Information on primer sequences and complete thermal cycling conditions are listed in Table S1 (Supporting information). Standard curves for each assay were generated by preparing 10-fold serial dilutions of linearized plasmids containing either cloned 16S rRNA genes from *Pseudomonas aeruginosa* PAO1, or cloned *nosZ* genes from either *Pseudomonas fluorescens* AF197468 (clade I) or *Dydadobacter fermentas* DSM 18053 (clade II). Two independent qPCR assays were performed for each gene and three no-template controls were run within each assay, which gave null or negligible values. The presence of PCR inhibitors in DNA extracted from soil was tested for by mixing a known amount of plasmid DNA with environmental DNA extracts, followed by amplification using plasmid-specific primers (Wessén et al., 2011). No inhibition was detected, and PCR efficiencies for 16S rRNA, *nosZ* clade I and clade II were 95%, 88% and 86% respectively, with an r^2 of >0.99 for each gene. The abundances of *nirS* and *nirK* genes was determined previously in (Enwall et al., 2010), while that of ammonia oxidizing bacteria (AOB) and archaea (AOA) was determined by quantification of *amoA* genes in (Wessén et al., 2011).