Soil Biology & Biochemistry 105 (2017) 92–95

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Short Communication

Indirect microbial effects on methane flux are stronger when the environmental influence is weaker in a temperate forest ecosystem

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ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form
8 November 2016
Accepted 14 November 2016
Available online 23 November 2016

Keywords:
Structural equation modelling
Mediation
Methane
mcrA

ABSTRACT

Microbial gene markers are hypothesized to be mediating factors between environment factors and methane flux, but mediation is not typically modeled directly in ecosystem studies using graphic models. Structural equation modelling (SEM) was used to test if *mcrA* and *16S* markers were mediating the effects of soil moisture on methane flux in two ecosystem types, Upland and Wetland Forest. SEM results indicated that *mcrA* functional marker was a mediator in the Upland Forest but not in the Wetland Forest. In the Upland Forest, the 16S marker indirectly effected methane flux though its effect on the mcrA marker. The results suggest that functional genes are mediating drivers in ecosystems where environmental factors are weak drivers of methane fluxes. Our results highlight the importance of testing for microbial indirect pathways in assessing drivers of methane cycling and provide a basis for more complex modelling of mediated pathways in analysis of ecosystem processes.

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1. Introduction

Methane flux from soils are biologically mediated processes, with net emissions resulting from complex interactions between microbes and the environment (Le Mer and Roger, 2001). In principle, assessment of functional gene abundances and activity allows for characterization of microbial populations that drive biogeochemical cycling (Levy-Booth et al., 2014). However, Rocca et al. (2015) found an overall weak relationship between functional genes and ecosystem processes. Where researchers have found strong correlations between functional genes implicated in methane cycling and methane fluxes, these effects have been highly context dependent (for example, Freitag and Prosser, 2009). Studies linking functional genes with methane flux typically only assess direct relationships between functional genes and methane flux, but indirect effects can also drive ecosystem processes (Classen et al., 2015; Clough, 2012), including carbon cycling (Trivedi et al., 2016). Using a Structural equation modelling (SEM) framework provides a basis for testing for indirect linkages between microbes and ecosystem processes. Several recent papers use SEM to model complex interactions between environment, functional genes, and methane (Lammel et al., 2015; Niklaus et al., 2016). However, none of these papers explicitly report results for indirect pathways for methane specific pathways.

SEM allows for testing for direct (X → Y) and indirect relationship pathways (X → Z → Y). An important feature of SEM is the specification of *a priori* hypothesized causal pathways, which are tested against observed data (Grace et al., 2012; Sutton-Grier et al., 2010). Here we test if microbial gene markers are indirect drivers of methane fluxes using SEM (Fig. 1). Christiansen et al. (2016) found that qPCR-based abundances of mcrA (methanogenisis) and 16S (bacteria) markers were strongly associated with methane fluxes and soil moisture, but the direct and indirect interactions between the factors were not clear. We modeled soil environmental factors as primary drivers of methane flux, with mcrA and 16S markers as mediating factors (Fig. 2). Using the gene abundance data from Christiansen et al. (2016), we then tested the SEM model on two forest types: Upland and Wetland Forest, respectively. We predicted that gene markers would influence methane fluxes through indirect pathways as shown in Fig. 2.

2. Methods

We used the R (ver. 3.2) lavaan package (Rosseel, 2012) to test our conceptual model against observed data. Lavaan uses a maximum likelihood estimation method, which compares an

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http://dx.doi.org/10.1016/j.soilbio.2016.11.013
0038-0717/Published by Elsevier Ltd.

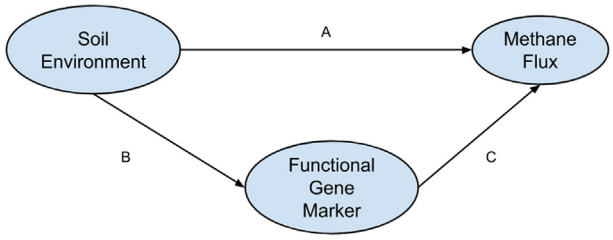

Fig. 1. Conceptual model with soil environmental driver directly affecting methane flux (A) and functional gene markers (B). The functional gene marker is a direct pathway (C) and a mediated pathway (B * C).

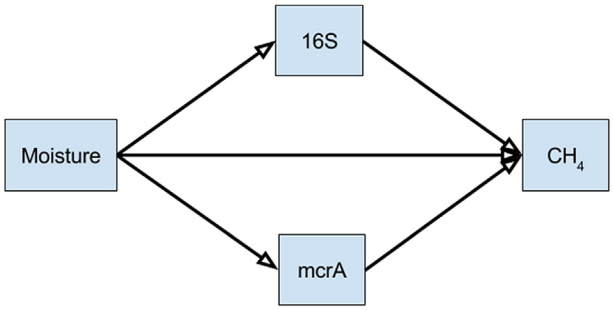

Fig. 2. Modified conceptual model testing if *mcrA* and *16S* marker abundances are mediating the influence of moisture on methane fluxes at the Beaver Lake site.

expected covariance matrix derived from a hypothesized *a priori* model to the observed covariance matrix of the data (Grace, 2006). For testing specific pathways, we built upon the results of Christiansen et al. (2016). Moisture was the major environmental driver of surface methane flux, while *mcrA* and *16S* bacteria gene abundances were also strongly associated with methane flux. The mcrA functional marker serves as a proxy for methanogenic activity (Nazaries et al., 2013) while the 16S marker is a coarser proxy for bacterial activity. Our conceptual model (Fig. 1) has direct pathways connecting soil environmental variables (A) and functional markers (C) to methane fluxes. Soil moisture directly affects microbial groups (B), methane fluxes and indirectly through microbes (B * C) (Fig. 1). Total effects are calculated by adding direct effects and indirect effects.

Data for observed variables was obtained from Christiansen et al. (2016). The Beaver Lake site (50°35′51.69″N, 127°18′19.06″W) was located near Port Hardy, Vancouver Island, Canada. We used moisture for the soil environmental variable and DNA abundances of *mcrA* and *16S* for functional markers. Fig. 2 shows in the initial conceptual model as modified to test for mediation effects in the Beaver Lake dataset. Moisture was logged transformed to meet assumptions of linearity. The final models for the Wetland and Upland Forest types had adequate fit when tested against the Beaver Lake data (Table 1). Further details of model development are found in Supplement 1.

Table 1
Model fit indices for the hypothesized relationship between moisture, functional gene markers, and methane flux for the Upland Forest and Wetland Forest type. CFI = Comparative Fit Index. SRMR = Standardized Root Mean Square Residual.

Model			
	P-value (Chi-square)	CFI	SRMR
Upland Forest	0.53	1.0	0.023
Wetland Forest	0.10	0.87	0.09

3. Results

Moisture in the Wetland Forest directly affected *mcrA* marker and methane (Fig. 3a). The indirect pathway effect (moisture * *mcrA*) was large but not significant (Fig. 3b). The 16S marker had a positive effect on methane and was not directly driven by moisture. The mcrA marker had a negative effect on methane, which was driven by moisture. The total pathway (moisture + moisture * 16S) significantly affected methane fluxes, but the effect was driven by moisture (Fig. 3b).

A major difference between the two forest types was that the initial model (Fig. 2) did not support a direct connection between 16S and methane flux (Supplement 1). Instead, in the Upland Forest 16s marker directly affected the mcrA marker, having an equivalent effect to moisture. The *mcrA* marker was a negative direct driver of methane fluxes (Fig. 4a). Both indirect pathways (moisture * *mcrA*, 16S * *mcrA*) affected methane fluxes in the Upland Forest (Fig. 4b).

4. Discussion

The Upland Forest model results partially confirmed that moisture effects on methane flux were mediated by methanogenic potential, assessed through the mcrA marker. In the Upland Forest type, moisture's effect was only weakly mediated by methanogenic potential. The much larger moisture effect on both methane flux and the mcrA functional gene marker in the Wetland Forest Type overwhelmed any mediating role of the marker. The larger effect of environmental factors over functional genes in the Wetland Forest is consistent with the Lammel et al. (2015) SEM analysis of methane flux. The direct effects of moisture on methane flux could have resulted from physical effects, such as methane diffusion through the soil. However, as we did not test all possible mediation pathways, we cannot rule out a stronger role for microbial mediation in the Wetland Forest.

McrA functional marker had a negative relationship on methane. At first glance, this negative relationship might seem counterintuitive given the positive association between mcrA abundance/transcription and methane emissions found in other studies (Lammel et al., 2015; Freitag and Prosser, 2009, Ma et al., 2012). One possibility is that increasing methanogenic activity was countered-balanced by methanotrophic activity. In both ecosystem types, methanotrophs were present, indicated pmoA marker, which was relatively constant compared to mcrA (Christiansen et al., 2016). Further, Christiansen et al. (2016) found highest methanotrophic abundances in the Wetland Forest, which would contribute to the negative relationship as methanotrophs would be able to respond to methanogenic activity. A key consideration in evaluating the model results is that functional gene abundances do not always reflect activity (Ma et al., 2012; Rocca et al., 2015). Differences in enzymatic efficiencies may help to explain why assessments of genetic potential do not always perfectly correlate with activity. These differences could have contributed to the observed weak relationship between pmoA abundances and methane fluxes.

Increased abundance of mcrA was associated with increasing variability of methane flux in the Wetland Forest type (Christiansen et al., 2016). This suggests that methanogensis was limited by factors, such as microsite variation in soil aeration (Galand et al., 2003) or nutrients, such as Fe (Ma et al., 2012) and nitrogen (Andert et al., 2012). In contrast to *mcrA*, the *16S* marker directly affected Wetland Forest emissions but was unaffected by moisture. The decoupling of moisture and methane flux in the *16S* pathway further suggests that other factors, such as nitrogen, drove the association between the bacterial community and methane flux. Christiansen et al. (2016) found that total nitrogen had a positive association with