Metal Ions in Life Sciences

Ecological Determinants of Methane Oxidation and Denitrification Processes Across Global Drylands

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Abstract

Aim: Microorganisms carrying *pmoA* and *nosZ* genes are major drivers of methane and nitrous oxide fluxes from soils. However, most studies on these organisms have been conducted in mesic ecosystems so little is known about the factors driving their distribution in drylands, the largest biome on Earth. We conducted a global survey to evaluate the role of climate- and soil-related variables as predictors of the richness, abundance and community structure of bacteria carrying *pmoA* and *nosZ* genes.

Location: Eighty dryland ecosystems distributed worldwide.

Time period: From February 2006 to December 2011

Major taxa studied: Methanotrophic (carrying the *pmoA* gene) and denitrifiying (carrying the *nosZ* gene) bacteria.

Methods: We used data from a field survey and structural equation modelling to evaluate the direct and indirect effects of climatic (aridity, rainfall seasonality, mean annual temperature) and soil (organic carbon, pH and texture) variables on the total abundance, richness and community structure of microorganisms carrying *pmoA* and *nosZ* genes.

Results: Taxa related to *Methylococcus capsulatus* or *Methylocapsa* sp., often associated with mesic environments, were common in global drylands. The abundance and richness of methanotrophs were not associated with climate or soil properties. However, mean annual temperature, rainfall seasonality, organic C, pH and sand content were highly correlated with their community structure. Aridity and soil variables such as sand content and pH were correlated with the abundance, community structure and richness of the *nosZ* bacterial community.

Main conclusions: Our study provides novel insights on drivers of the abundance, richness and community structure of soil microorganisms carrying *pmoA* and *nosZ* genes in drylands worldwide. Highlighting how ongoing climate change will alter the structure of soil

microorganisms, which might affect the net CH_4 exchange, and will likely reduce the capacity of dryland soils to carry out the final step of the denitrification, favouring net N_2O emissions.

Key words: abundance, richness, community structure, drylands, *methanotrophs, denitrifiers*.

Introduction

Microbial communities are the main biotic drivers of methane (CH_4) and nitrous oxide (N_2O) emissions from soils (Dalal & Allen, 2008). For instance, aerobic soils are the only biological sink for atmospheric CH_4, and about two thirds of the CH_4 found in the atmosphere derives from microbial metabolism (Dalal & Allen, 2008; Conrad, 2009). Methanogens produce CH_4 in anaerobic conditions (e.g. rice fields, lake sediments or wetlands) through a process called methanogenesis (Le Mer & Roger, 2001). As such, CH_4 production in drylands (arid, semi-arid and dry-subhumid ecosystems), environments characterized by having low and infrequent rainfall regimes, has been traditionally considered to be low (Castaldi, Ermice, & Strumia, 2006). Conversely, methanotrophs possess the methane monooxygenase encoded by the *pmoA* gene, which oxidises CH_4 in aerobic conditions, therefore potentially reducing atmospheric CH_4 concentrations. As a result of their aerobic requirements, we expect methanotrophs to be important members of microbial communities in these ecosystems (Safriel & Adeel, 2005). Alternatively, NH_4^+, competes with CH_4 for the methane monooxigenase producing hydroxylamine, which is metabolised further to nitrite (King & Schnell, 1994). However, this alternative in natural systems appear to be very minor (Stein, Roy, & Dunfield, 2012; Nazaries, Murrel, Millard, Baggs, & Singh, 2013). In contrast, microbial processes such as nitrification and denitrification are responsible for over two-thirds of the soil N_2O emissions to the atmosphere (Dalal & Allen, 2008; Butterbach-Bahl, Baggs, Dannenmann, Kiese, & Zechmeister-Boltenstern, 2013). Nitrifying microorganisms transform NH_4^+ to NO_3^-, releasing N_2O to the atmosphere as a by-product (Bremner, 1997). Denitrification (i.e., the reduction of NO_3^- to NO, N_2O and finally to N_2) is a multi-step process carried out by different microorganisms and their enzymes, including the nitrous oxide reductase (*nosZ*) that catalyses the last step of this process.

Many studies have explored what are the main abiotic and biotic drivers of carbon dioxide [CO_2; the major anthropogenic greenhouse gas (GHG)] fluxes, but much less is known about the factors controlling fluxes of CH_4 and N_2O, which have global warming potentials 28 and 265 times greater than that of CO_2, respectively (Nakicenovic & Swart, 2000). The traditional consensus that GHGs fluxes are likely to be small in drylands has resulted in a bias in which the vast majority of knowledge about the exchange of CH_4 and N_2O between the soil and the atmosphere, and about its associated microbial ecology, comes from studies carried out in mesic

ecosystems (Oertel, Matschullat, Zurba, Zimmermann, & Erasmi, 2016). However, drylands cover 45% of the land surface (Prăvălie, 2016) and sustain > 40% of human population (Reynolds et al., 2007). Furthermore, these numbers are likely to increase substantially in coming decades as a result of climate change-driven increases in aridity (Huang, Yu, Guan, Wang, & Guo, 2015) and projected human population growth rates (United Nations, 2017). In addition, recent evidence suggests that CH_4 and N_2O fluxes in drylands might be relevant at the global scale, given both the reported process rates and their extent worldwide (e.g. Martins, Nazaries, Macdonald, Anderson, & Singh, 2015; Weber, et al., 2015; Hu Trivedi, He, & Singh, 2017).

The abundance of *pmoA* and *nosZ* genes is strongly related to GHG fluxes, and have been used to predict them (Nazaries, Pan, et al., 2013; Powell, Welsh, & Hallin, 2015; Martins, et al., 2017). Although the major ecological drivers of CH_4 oxidation and denitrification rates (e.g., soil moisture and texture) are starting to be assessed (Butterbach-Bahl et al., 2013; Nazaries, Murrell, et al., 2013), much less is known about the distribution and drivers of the richness, abundance and community structure of *pmoA*- and *nosZ*-carrying microorganisms (i.e. methanotrophs and denitrifiers, respectively) across the globe.

Global-scale studies conducted in recent years have emphasized the role of climatic factors (e.g. aridity) and of soil properties (e.g., pH) as main drivers of soil microbial communities, both in drylands (Maestre et al., 2015) and elsewhere (Delgado-Baquerizo et al., 2018). However, to the best of our knowledge, no similar studies have been conducted on *pmoA*- and *nosZ*-carrying microorganisms to date. To fill this knowledge gap, the aim of this study was to identify major biotic and abiotic factors that affect the abundance, richness and/or community structure of functional genes involved in CH_4 (*pmoA*) and N_2O (*nosZ*) fluxes in dryland soils globally. We did so using a dataset including 80 dryland sites from six continents. Given the potential importance of functional genes as modulators of CH_4 and N_2O fluxes (Martins, Macdonald, Anderson, & Singh, 2016), a better understanding of what drives the abundance, richness and community structure of the soil microorganisms containing these genes across large environmental gradients is of paramount importance to predict GHG emissions in global drylands under climate change.

Material and Methods

Study sites

This study was carried in 80 dryland ecosystems of 12 countries (Argentina, Australia, Chile, China, Iran, Israel, Mexico, Morocco, Spain, Tunisia, USA and Venezuela: see Supporting Information Map S1). The locations surveyed encompass a wide variety of the biotic and abiotic conditions that can be found in drylands worldwide. Study sites had an aridity index (AI = mean

precipitation/potential evapotranspiration) ranging from 0.06 to 0.50, mean annual temperature ranging from -1.8 °C to 24.2 °C, and precipitation values ranging from 67 mm to 766 mm, and included major vegetation types (grasslands, shrublands, savannahs and dry forests). Field sampling took place between February 2006 and December 2011 in 30 m x 30 m plots representative of the vegetation found at each site, following a standardized protocol (for more details, see Maestre et al, 2012). In short, to ensure that we captured the spatial heterogeneity and to avoid bias in the sampling, five 50 cm x 50 cm quadrats were randomly placed under the canopy of the dominant perennial species and in open areas devoid of perennial vascular vegetation at each plot; when more than one dominant plant species was present, five additional quadrats were established under the canopy of the co-dominant perennial species. At each sampling quadrat, a composite topsoil sample (five 145 cm^3 soil cores 0-7.5 cm depth) was collected, bulked and homogenised in the field and taken to the laboratories of Rey Juan Carlos University (Spain). These samples were used to obtain a composite sample per microsite (vegetated and open areas) and site. Field samples were sieved (2 mm mesh) and split into two. One portion was air dried and used to analyse soil physicochemical properties, and the other was frozen and stored at -20°C for molecular analyses.

Environmental and soil properties

Standardized climate data (mean annual temperature and rainfall seasonality) for all sites were obtained from Worldclim (www.worldclim.org), a high resolution (30 arc seconds or ~ 1km at equator) database generated from a high number of climate observations and topographical data (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). We estimated the degree of aridity of each site by obtaining its aridity index (precipitation/potential evapotranspiration) from Trabucco & Zomer (2009). For clarity, we used aridity [1- AI], meaning that higher values indicate increasing aridity (Delgado-Baquerizo et al, 2013). Soil organic C was determined by colorimetry after oxidation with a mixture of potassium dichromate and sulphuric acid (Anderson & Ingram, 1993). Soil pH was measured with a pH meter, in a 1:2.5 (mass:volume, soil:water) suspension. Soil sand content was estimated according to Kettler, Doran, & Gilbert (2001).

Functional microbial communities

We extracted soil total genomic DNA from 0.5 g of frozen soil using the PowerSoil DNA Isolation kit (MOBIO Laboratories, Inc. Carlsbad, CA, USA) following the manufacturer's protocol, except for modifications in the cell lysis step [we used a tissue homogenizer (Precellys 24- dual. Bertin technologies, Montigny-le-Bretonneux, France) at 4500 rpm for 45 s, twice]. The abundance of

functional genes related to methanotrophs (*pmoA*) and N_2O reducing bacteria (*nosZ*) was determined with real-time quantitative PCR (q-PCR) using 96-well plates on a BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA). These genes were quantified in duplicate (and then pooled, standard approach; Lammel, Feigl, Cerri, & Nüsslein, 2015; Martins et al., 2015; Wen et al., 2018) using the pmo189f/pmo650r (Bourne, McDonald, & Murrel, 2001) and nosZ2f/nosZ2r (Henry, Bru, Stres, Hallet, & Philippot, 2006) primers, respectively (Table S1).

We used a well-established approach to study methanotrophs (Nazaries et al., 2011, Nazaries, Karunaratne, Delgado-Baquerizo, Campbell, & Singh, 2018) and denitrifiers (Singh, Tate, Thomas, Ross, & Singh, 2011) using terminal restriction fragment length polymorphism (T-RFLP). Soil DNA samples were amplified with PCR using specific primers for methanotrophs and N_2O reducing bacteria (Supporting Information Table S2). The PCR products were purified using a FavoPrep GEL/PCR purification kit (FAVORGEN Biotech Corporation, Taiwan) following the manufacturer's instructions. The PCR-purified products were digested with HhaI and MspI restriction enzymes (for *pmoA* and *nosZ*, respectively) in an 11 µl reaction mixture containing 9.4 ng μl^{-1} of PCR product, bovine serum albumin, 10x NH_4 buffer and 0.5 ng μl^{-1} restriction enzyme (New England Biolabs, UK). Samples were incubated at 37ºC for 3h, which was followed by a deactivation at 95ºC for 10 min. After digestion, 2 µl of each sample was mixed with 0.3 µl of Genescan-600 LIZ size marker and 10 µl of Hi-Di formamide (Applied Biosystems, Warrington, United Kingdom). Before fragment analysis, samples were denatured at 95°C for 5 min and then chilled on ice for 5 min. A fragment size analysis was carried out with an Applied Biosystems 3500 Genetic Analyser. Terminal restriction fragments (T-RFs) generated by the sequencer were analysed using GeneMapper v.4.0 (Applied Biosystems, Warrington, United Kingdom) and raw data originated from GeneMapper were processed with the T-REX online software (Culman, Bukowski, Gauch, Cadillo-Quiroz, & Buckley, 2009). To control data quality, noise was filtered (peak area above the fluorescence noise), T-RFs were aligned (2 bp as clustering threshold) and T-RFs present in fewer than three sites were discarded. We estimated the community structure (size clades within a community, i.e., number of base pairs within a T-RF) and richness (number of clades) according to the number of unique T-RFs and their abundance based on the q-PCR analyses. A total of 116 (*pmoA*) and 118 (*nosZ*) operational taxonomic units (OTUs) were found in the 160 soil samples (80 sites x 2 microsites) analysed.

Statistical analyses

We used structural equation modelling (SEM; Grace, 2006) to evaluate the direct and indirect effects of geographical location (latitude and longitude), aridity, mean annual temperature,

rainfall seasonality, microsite (open/vegetated areas) and soil properties (pH, sand content and organic C) as predictors of the abundance, richness and the community structure of *pmoA* and *nosZ* genes (for our *a priori* model, see Supporting information Figure S1). As a preliminary step, to create an expression of community structure compatible with SEM, we used a three dimensional non-metric multidimensional scaling (NMDS) ordination, based on Bray-Curtis distance, to summarize the structure of the community of methanotrophs (Supporting Information Figure S2a) and *nosZ* carrying denitrifiers (related to N_2O reducing bacteria; Supporting Information Figure S2b; Paliy & Shankar, 2016). The NMDS was performed in R with the vegan package (Oksanen et al., 2013). We included the three axes obtained from NMDS analyses in the SEM. We expressed the geographical latitude as decimal degrees and decomposed longitude in its sine and cosine to account for the spatial autocorrelation of our data. We included microsite as a binary variable (one and zero denote samples coming from under the canopy of the dominant vegetation and plant interspaces, respectively) to capture the variability between vegetated microsites and plant interspaces microsites (Ochoa-Hueso et al., 2017). The remaining variables were transformed using natural logarithms (i.e., rainfall seasonality, *pmoA* and *nosZ* gene abundances), the square root (i.e., soil organic C and *pmoA* gene richness) and exponential (pH) transformations to improve normality. All variables (except microsite) were centred prior to SEM analysis to facilitate the interpretation of parameter estimates. By default, SEM is based on an underlying linear model, an assumption that must be checked. Overall, the relationships expressed in our model were well-approximated linearly, except that we observed a quadratic relation between aridity and the abundance of *nosZ* carrying denitrifiers (Supporting Information Figure S3a). To include this curvilinear relationship in our otherwise linear model, we used the method suggested by Grace (2006). Briefly, aridity and its square (having first been centred to zero) were entered into the model as predictors, and their effects on the response of interest were pooled using a composite variable.

To test the goodness of fit of our SEMs, we used the χ^2 test (the model has a good fit when χ^2/df is low, i.e., *c*. ≤ 2, and p is high, traditionally > 0.05), the root-mean-square error of approximation (RMSEA; the model has a good fit when RMSEA is indistinguishable from zero, and p is high, traditionally > 0.05), and the Bollen-Stine bootstrap tests (Schermelleh-Engel, Moosbrugger, & Müller, 2003). All indices suggested adequate model fit (Figures 1 and 2); therefore, we were free to interpret the path coefficients of the model and their associated *p*-values. A path coefficient is analogous to the partial correlation coefficient or regression weight and describes the strength and sign of the relationships between two variables (Grace, 2006). The probability that a path coefficient differs from zero was tested using bootstrap tests, because our data were not always normally distributed (Schermelleh-Engel et al., 2003; Kline,

2011). We calculated the standardized total effects of all drivers on the selected functional gene attributes (Grace, 2006). The net influence that one variable had upon another was calculated by summing all direct and indirect pathways (effects) between two variables. All SEM analyses were conducted using AMOS 24.0 (IBM SPSS, Chicago, IL, USA).

Differences across vegetation types (grasslands, shrublands and open forests) and aridity classes (arid and semi-arid), regardless of the microsite considered, were compared with one-way ANOVA; the Tukey's post-hoc HSD test was used to assess significant differences among them. These analyses were performed in R (R Core Team, 2017). Data associated with this study are available from figshare (Lafuente et al., 2019).

Results

Dominant taxa of pmoA- and nosZ- carrying bacteria in drylands

Seven (79, 86, 35, 245, 27, 83 and 31) and six (32, 73, 107, 35, 47 and 39) dominant T-RFs accounted for >75% relative abundance of *pmoA* and *nosZ* genes, respectively, across global drylands (Figure 3).

The first and second axes of the NMDS for methanotrophs (MCS_1) were positively (Spearman's $\rho = 0.34$) and negatively ($\rho = -0.68$) correlated with T-RF_31, respectively, which is related to USCα, a distant relative of *Methylocapsa* sp (a type-II methanotroph). The second methanotroph NMDS axis (MCS_2) was also positively correlated with T-RF_79 (Spearman's $\rho = 0.41$), which is related to type-II methanotrophs associated with the Methylocystaceae family (Nazaries, Pan, et al., 2013). The third methanotroph NMDS axis (MCS_3) was positively correlated with T-RF_79 and T-RF_ 245 ($\rho = 0.46$ and $\rho = 0.49$, respectively); the later belongs to type-I methanotrophs and is closely related to *Methylococcus capsulatus* (Nazaries et al., 2011).

Likewise, the first axis of the NMDS for *nosZ* carrying denitrifiers (DCS_1) was positively correlated to T-RF_88 and T-RF_132 ($\rho = 0.66$ and $\rho = 0.54$, respectively); these fragments were present in more than 70% of the samples analysed. The second denitrifier NMDS axis (DCS_2) was positively correlated with T-RF_471, T-RF_73, T-RF_107 and T-RF_331 ($\rho = 0.66$, $\rho = 0.54$, $\rho = 0.53$, and $\rho = 0.51$, respectively), fragments present in ≥ 86% of the samples analysed, and negatively correlated with T-RF_35 ($\rho = -0.57$), which was found in all the soil samples evaluated. The third denitrifier NMDS axis (DCS_3) was negatively correlated with T-RF_104 ($\rho = -0.45$). These affiliations are based on previous studies using the exact same protocol that we used (Nazaries et al., 2011, Nazaries, Pan, et al., 2013). Unfortunately, to our knowledge, there is no published work that provides information to relate these fragments to specific taxonomical groups for *nosZ* genes.

Effects of vegetation type and climate on dominant taxa on pmoA- and nosZ- carrying bacteria

On average, we found $5.1x10^7$ and $5.8x10^8$ copies of *pmoA* and *nosZ*, respectively. Changes in total abundance and richness of *pmoA*, and in richness of *nosZ*, genes were not detectable across either vegetation types or aridity classes (Supporting Information Figure S4). However, we detected an increased *nosZ* gene abundance in semiarid grasslands and shrublands (Supporting Information Figure S4). The dominant *pmoA* gene T-RFs were highly variable across vegetation types and aridity classes considered (Figure 3a), in contrast with the *nosZ* gene, where the dominant T-RFs were T-RF_32 and T-RF_73, regardless of the vegetation types and aridity classes considered. The abundance of the dominant T-RFs across vegetation types and aridity classes was very consistent, and we detected changes in the abundance of only T-RF_32 (Figure 3b). In semiarid open forests, T-RF_32 was less abundant than in arid grasslands and semiarid shrublands (Figure 3b).

Direct and indirect effects of climate and soil properties on pmoA- and nosZ- carrying bacteria

Increases in aridity showed a quadratic relation with *nosZ* abundance (Supporting Information Figure S3a). Increases in soil organic C and pH were linearly associated with increases in *nosZ* abundance (Supporting Information Figure S3b). Increases in sand content were linearly associated with reductions in *nosZ* abundance (Supporting Information Figure S3c).

Our SEM provided a comprehensive view of the major ecological predictors of the richness, abundance and community structure of *pmoA*- and *nosZ*- carrying bacteria. It was able to explain only 6% of the variance of the abundance of methanotrophic bacteria, and 10% of their richness, but it was more successful explaining their community structure (18- 37% of NMDS axes; Figure 1). Neither the abundance nor the richness of the *pmoA* gene was directly explained by any of the soil or climate variables evaluated, nor by the microsite (vegetation vs. bare soil) considered. The only detectable, albeit weak, effect was a positive effect of latitude on *pmoA* gene richness, indicating a higher richness for methanotrophs in the Northern Hemisphere. Climate and soil were roughly equal predictors of the community structure of methanotrophs. Specifically, we found that the MCS_1 axis (potentially associated with genera *Methylocapsa*) was directly and positively associated with mean annual temperature, and directly and negatively related to the amount of soil organic C. In other words, taxa related to this axis (see Table S3) might prefer locations with higher temperature and low soil C. Aridity had a direct negative effect on the MCS_1 axis, and an indirect positive effect on this axis by reducing the content of soil organic C. The MCS_2 (potentially associated with taxa *Methylocystaceae*) axis was directly and positively associated with soil pH, and directly and

negatively related to mean annual temperature and soil texture. Thus, taxa related to this axis (see Supporting Information Table S3) might prefer locations with lower temperature and high pH. Mean annual temperature also indirectly and positively influenced the MCS_2 axis via increases in soil pH. The MCS_3 axis (potentially associated with taxa *Methylocystaceae* and *Methyloccoccus capsulatus*) was directly and positively associated with aridity, mean annual temperature, rainfall seasonality and soil organic C, but was negatively associated with the proximity of vegetation. This means that taxa related to this axis might prefer both unvegetated surfaces with higher temperature and rainfall seasonality and soils with higher amounts of organic C. Aridity also indirectly and negatively influenced the MCS_3 axis via reductions in the amount of soil organic C. The standardised total effects highlighted soil variables as major drivers of MCS_1 (organic C and sand content, Figure 4) and MCS_2 (soil pH and texture, Figure 4); mean annual temperature, rainfall seasonality and soil organic C were major predictors of MCS_3 (Figure 4).

Our SEM explained 34% of the variance of the abundance of *nosZ* carrying denitrifiers, 24% of their richness and 22-52% of their structure (NMDS axes, Figure 2). Aridity and soil variables were equally informative determinants of the abundance and richness of the *nosZ* gene. The abundance of this gene was positively associated with soil organic C, indicating that *nosZ* carrying denitrifiers might prefer soils with higher organic C content. Aridity had a direct and curvilinear influence on the total abundance of *nosZ* (i.e., intermediate aridity values were associated with the greatest *nosZ* abundance). Furthermore, aridity had an indirect negative effect associated with this variable via its negative effect on soil organic C (Figure 5). The richness of *nosZ* was directly and positively influenced by soil properties (pH and soil texture) and directly and negatively associated with aridity. Put simply, the richness of *nosZ* was higher in coarser soils with higher pH levels. Aridity also had an indirect effect on the richness of *nosZ* gene through its direct positive effects on soil pH and texture and direct negative effect on organic C (Figure 5).

The structure of *nosZ*-carrying bacterial communities was influenced by both climatic and soil variables, with aridity, soil pH and sand content being the most influential drivers of the NMDS axes. The DCS_1 axis was directly and negatively associated with aridity, and positively associated with soil pH and texture. In other words, taxa related with this axis might prefer coarser soils with higher pH levels. Aridity also had an indirect effect on DCS_1 through its positive effect on soil pH and texture (Figure 5). The DCS_2 axis was directly and negatively associated with rainfall seasonality, and positively associated with vegetation, suggesting that taxa related to this axis might prefer vegetated sites with lower rainfall seasonality. Rainfall seasonality also had a positive indirect effect on DCS_2 through its direct negative effect on soil

texture (Figure 5). The DCS_3 axis was directly and positively associated with aridity, mean annual temperature, soil pH and organic C. In simple terms, taxa related to this axis might prefer warmer and more arid locations, in addition to basic soils with higher organic C content. Also, mean annual temperature was indirectly and negatively associated with DCS_3 through its positive effect on soil pH (Figure 5). The standardised total effects showed aridity and soil organic C as the major drivers of *nosZ* abundance (Figure 4). Soil variables (soil texture and pH) were the major drivers of *nosZ* richness (Figure 5) and of DCS_1 (Figure 4). Vegetation and rainfall seasonality were the major drivers of DCS_2 (Figure 4), and mean annual temperature was the major driver of DCS_3, followed by soil pH, aridity and soil organic C (Figure 5).

Discussion

Understanding the main ecological drivers of the abundance, richness, and community structure of soil methanotrophs and *nosZ*-carrying denitrifiers is crucial to improve our understanding of how global change will affect the distribution of these functionally important microbial communities in drylands worldwide. Our study provides the first global assessment of bacterial taxa carrying *pmoA* and *nosZ* genes in drylands. Moreover, our findings provide new insights into the ecological predictors of these organisms at a global scale, which can be used to model the abundance and richness of methanotrophs and *nosZ*-carrying denitrifiers under global change scenarios. We observed that taxa often associated with more mesic environments [e.g., T-RFs 31 (*Methylocapsa* sp.) and 245 (*Methylococcus capsulatus*); Nazaries et al, 2011, 2018], are also common in drylands across the globe. We also found that climate (i.e., mean annual temperature, rainfall seasonality or aridity) and soil properties (i.e., organic C, pH or sand content) were important predictors of the community structure of both *pmoA* and *nosZ* genes. Our results suggest that many of the effects of climate on the community structure of methanotrophs and *nosZ* carrying denitrifiers are driven indirectly by changes in soil variables such as organic C, pH and texture. Furthermore, our modelling approach was more effective in explaining the richness, abundance and community structure of the *nosZ*- than the *pmoA*-carrying bacteria. Despite the known effects of climate on soil microorganisms, we could not detect any significant direct effect of climate on methanotrophic bacteria comparable to the strong effects observed for *nosZ*-carrying denitrifiers, suggesting that methanotrophs have lower sensitivity to key climatic drivers than *nosZ*-carrying denitrifiers (Martins et al., 2017).

Direct and indirect effects on methanotrophic bacteria

Despite the commonly held assumption that climate is a crucial determinant of the abundance of methanotrophs (Mohanty, Bodelier, & Conrad, 2007), we did not find strong direct effects of either temperature or aridity on the abundance and richness of the *pmoA* gene (Figure 1). These results suggest that, unlike the situation in more mesic ecosystems (Le Mer & Roger, 2001), methanotrophic bacterial abundance and richness are not well predicted by averaged annual climatic parameters (aridity index and temperature) and that, in drylands, these organisms could be largely driven by more stochastic events (e.g., water pulses; Martin*s* et al., 2015) not properly captured by climatic interpolations. With regard to community structure, some of the most dominant *pmoA* T-RFs observed in our study (i.e., T-RFs 31 and 245) have also been described as dominant T-RFs in more mesic and humid systems (Nazaries et al., 2011, 2018; Nazaries, Pan, et al., 2013). These results indicate that methanothrophs can succeed in a wide range of environmental conditions. In particular, T-RF_245, potentially associated with *Methylococcus capsulatus*, which is widely described in mesic ecosystems (Bourne et al., 2001; Nazaries et al., 2011), was also one of the most abundant T-RFs observed in our study (Figure 3). This finding highlights the global distribution of this methanotroph taxon and suggests that methanotrophic communities might consistently be abundant in drylands, confirming that the low and infrequent rainfall regimes characterizing these ecosystems support the maintenance of methanotrophic communities (Striegl, MCSonnaughey, Thorstenson, Weeks, & Woodward, 1992). Despite the consistent high abundance of T-RF_245 in drylands, this taxon was associated with more arid sites with higher temperatures (Figure 1), which matches our current knowledge on T-RF_245, which is associated with *Methylococcus capsulatus*, a thermophile (growth > 45ºC) methanotroph from the Methylococcaceae family (Nazaries, Murrell et al., 2013).

In addition to some direct effects of climate on the methanotrophic community, we found that some effects of climate were indirect and driven by changes in soil properties. Different levels of soil organic C, pH and texture associated with changes in aridity and mean annual temperature were major environmental predictors of the structure of methanotrophic communities. Soil properties and climate are expected to be among the major predictors of methanotrophs (Le Mer & Roger, 2001; Sullivan, Selmants, & Hart, 2013). The negative effect of soil organic C on the MCS_1 axis could be related to the existence of high affinity methanotrophs, which are capable of using atmospheric CH_4 as a carbon source (Bender & Conrad, 1992). We expect these taxa to be major constituents of the methanotrophic community in dryland soils, although further work is needed to support this assumption. However, in addition to CH_4, methane-oxidising bacteria are able to use organic C sources facultatively (Sullivan, Selmants, & Hart, 2013). Supporting previous research suggesting that soil pH is a strong driver of bacterial abundance and diversity (Fierer & Jackson, 2006; Maestre

et al., 2015), we observed a significant association of pH with the MCS_2 axis describing the methanotrophic community structure, which was correlated to T-RF_31 and T-RF_79, potentially related to the family Methylocystaceae. This family encompasses several acidophilic species (growth at a pH of 3.8-5.5), which can explain our results, at least in part. Finally, atmospheric CH_4 consumption occurs in the first centimetres of the soil profile, which makes it highly dependent on physical factors controlling gas diffusion (Koschorreck & Conrad, 1993). Indeed, our SEM pointed towards soil texture and climate as major drivers of the methanotrophic community structure, highlighting the importance of soil aeration for these microorganisms.

Direct and indirect effects on nosZ carrying bacteria

Unlike the situation for methanotrophs, our SEM provided detailed information on the major drivers of the abundance and richness of *nosZ*-carrying denitrifiers. For instance, we found a strong effect of aridity on the abundance of the *nosZ* gene (owing to the second-order polynomial relation between aridity and *nosZ* gene abundance, this path has an interpretable sign), and a negative effect of aridity on its richness. Aridity also showed a strong influence on the community structure of *nosZ*-carrying denitrifiers. In drylands, aridity is a major driver of nitrogen availability, as increasing aridity reduces organic matter, nitrogen availability and soil microbial activity associated with N cycling (Delgado-Baquerizo et al., 2013, 2016). Our study shows an interesting relationship between aridity and *nosZ*-carrying denitrifiers at the global scale, which might result in potential implications for the capacity of drylands to exchange N_2O with the atmosphere in a changing world. As aridity continues to increase worldwide owing to climate change (Hu et al., 2017), the changes in the abundance and decrease in the richness of the *nosZ* gene with aridity may reduce the potential of drylands to support complete denitrification of N_2O to N_2. Interestingly, unlike a study that has observed a negative impact of increasing soil temperature on the abundance of *nosZ* genes in a boreal–temperate ecotone (Martins et al., 2017), we could not detect such an effect in global drylands. We hypothesize that restrictions in water availability imposed in drylands overcome the known effects of temperature on the abundance of *nosZ* gene found in more mesic areas.

Soil properties were also important drivers of the abundance, richness and community structure of *nosZ*-carrying denitrifiers and, indirectly, expressed the effects of climate on *nosZ* genes. Denitrification processes, which produce N_2O or N_2 as by-product, have been shown to be highly dependent on soil properties (Andersen & Petersen, 2009). Accordingly, in our global study, the abundance of the *nosZ* gene was strongly driven by soil organic C, a common surrogate of soil fertility (Delgado-Baquerizo et al., 2017; Figure 5), suggesting that this microbial

functional group is nutrient limited in global drylands and respond positively to increases in organic C contents. Soil pH and climate-related variables (i.e., aridity), which are also known to alter denitrifier activity and determine the capacity of N_2O reductase to assemble (Liu, Mørkved, Frostegård, & Bakken, 2010), were also positively and negatively, respectively, related to the richness (but not to the abundance) of the *nosZ* gene. Below optimal pH (6.0), the assemblage of the *nosZ* protein is impaired (Liu et al., 2010); consequently, higher pH might enhance the richness of *nosZ* gene as shown in our SEM. Sand content and pH were also major drivers of the structure of the *nosZ*-carrying denitrifying community (Figure 2). The denitrification rate is reduced with decreasing pH (Burford & Bremner, 1975). However, not all denitrification steps are equally affected by soil pH, and thus, N_2O production could increase under lower pH values (Burford & Bremner, 1975). On the contrary, denitrification products shift towards N_2 production as pH increases (Dalal & Allen, 2008). Our results are in agreement with Zeng et al., (2017), who also observed a strong positive effect of soil pH on the N_2O reducing bacterial community.

Concluding remarks

Here we report, for the first time, the direct and indirect effects of climate- and soil-related variables on the abundance, richness and community structure of two functional genes related to methanotrophy and denitrification in global drylands. First, we demonstrate that *pmoA* and *nosZ* associated microbial communities are widely distributed in global drylands, highlighting the importance of including methanotrophs and *nosZ*-carrying denitrifiers in dryland biogeochemical models. Second, we provide strong observational evidence that both climate- (i.e., aridity, mean annual temperature and rainfall seasonality), and soil-related (i.e., soil organic C, pH and soil texture) variables are important predictors of the abundance and richness of *nosZ* gene and of the community structure of methanotrophs and *nosZ*-carrying denitrifiers. Finally, we show that *nosZ*-carrying denitrifiers might be more sensitive than *pmoA*-carrying bacteria to the known drivers of microbial abundance and richness.

Climatic models forecast widespread increases in aridity by the end of the 21st century, which will increase the extension of drylands worldwide (Huang et al, 2016). Here, we demonstrate that these changes could alter methanotrophic and *nosZ*-carrying denitrifying communities. Increases in aridity associated with climate change are expected to reduce vegetation cover (Delgado-Baquerizo et al., 2013) (Ulrich et al., 2014), reducing organic C inputs into the soil and thus soil organic C contents (Delgado-Baquerizo et al., 2013). We found a negative effect of microsite on the methanotrophic bacterial community structure, suggesting that the expected reduction in vegetation cover owing to climate change will directly and indirectly alter the structure of methane-oxidising bacterial communities, which might affect the

net CH_4 exchange to the atmosphere. Our results also point towards changes in the *nosZ*-carrying denitrifying community owing to forecasted increases in aridity and reductions in soil organic C with climate change. These changes are likely to result in a reduced capacity of dryland soils to carry out the final step of the denitrification (reduction of N_2O to N_2), favouring net N_2O emissions to the atmosphere. Together, our results provide a better understanding of the environmental factors driving variation in the abundance, richness and structure of *nosZ* and *pmoA* genes in dryland soils, which is of paramount importance to forecast changes in the emission of greenhouse gases in the future.

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Data Accessibility Statement

Data associated with this manuscript are available from figshare:
https://figshare.com/s/e58abe2e99ea44eb221e

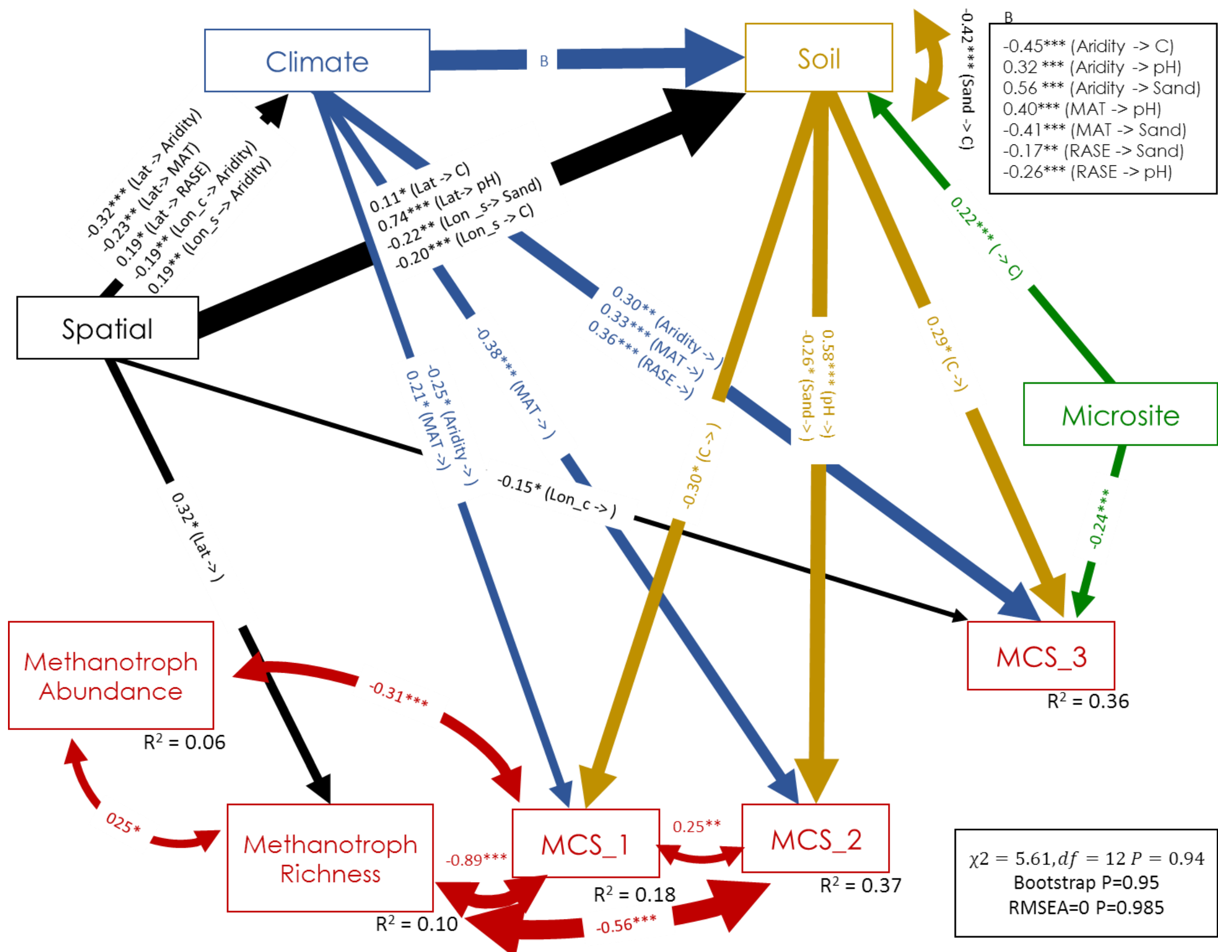


Figure 1. Structural equation model, describing the effects of spatial location (latitude [Lat], longitude sine [Lon_s] and longitude cosine [Lon_c]), climate (aridity, mean annual temperature [MAT] and rainfall seasonality [RASE]), soil properties (organic carbon [C], pH and sand content [Sand]) and microsite (open/vegetated areas) on methanotrophic bacteria abundance, richness and community structure. MCS = NMDS ordination axis for Methanotrophic bacteria community structure. The components within spatial location, climate and soil properties are included in the model as independent observed variables, but in this figure are grouped for simplicity. Numbers within the arrows show standardised path coefficients and indicate the effect size of the relationship among variables. Arrow widths are proportional to the strength of the relationship. Only significant relationships are shown ($P < 0.05$) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. The goodness of fit of the model is shown in the bottom right hand corner of the figure (df, degrees of freedom; RMSEA, root mean square error of approximation).

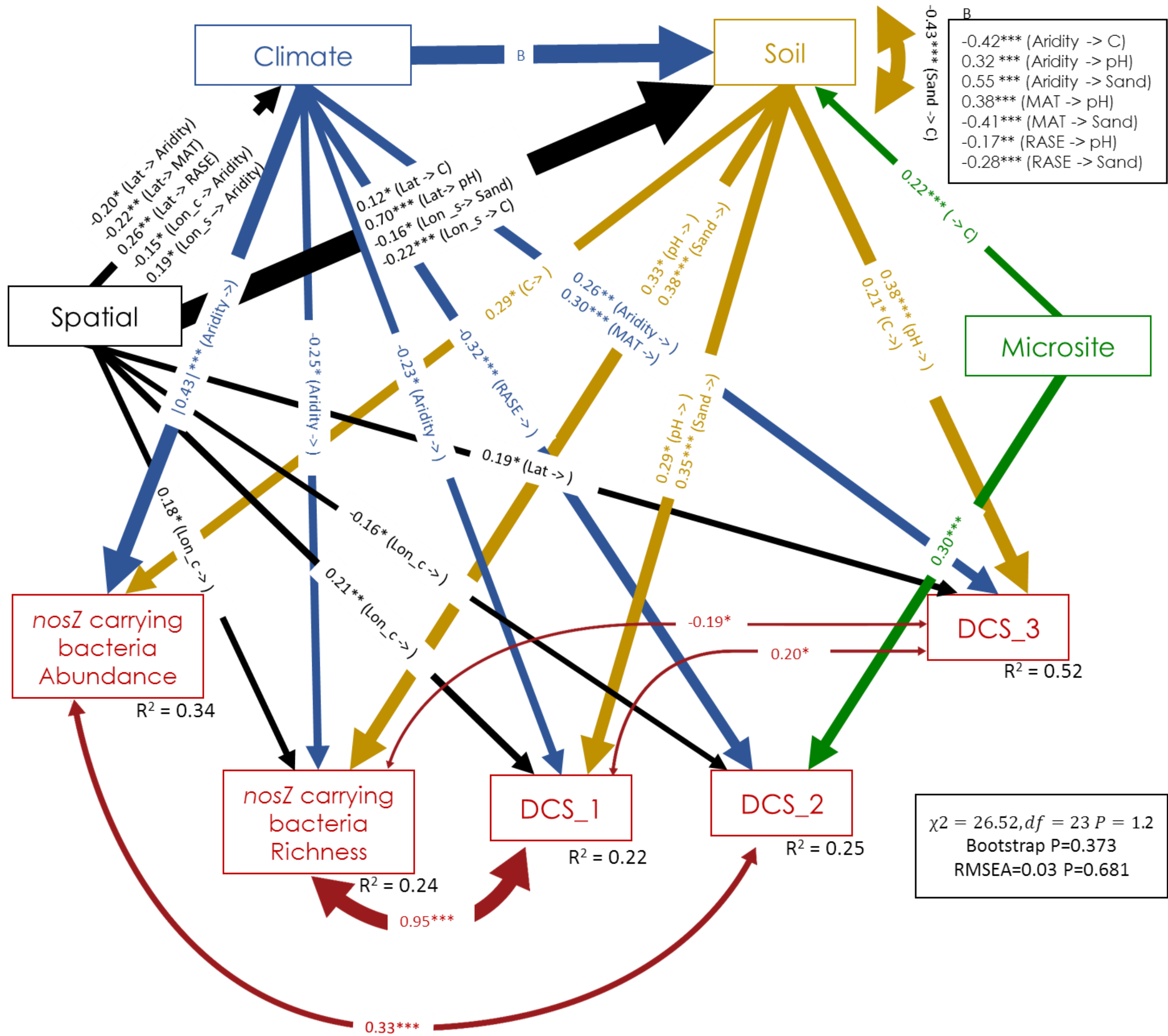


Figure 2. Structural equation model, describing the effects of spatial location, climate, soil properties and microsite on *nosZ* carrying bacteria abundance, richness and community structure. DCS = NMDS ordination axis for *nosZ* carrying bacteria community structure. The path coefficient for the direct effect of climate on *nosZ* carrying bacteria is expressed as an absolute value because the relationship is curvilinear, rendering the sign uninterpretable. Rest of caption as in Figure 1.

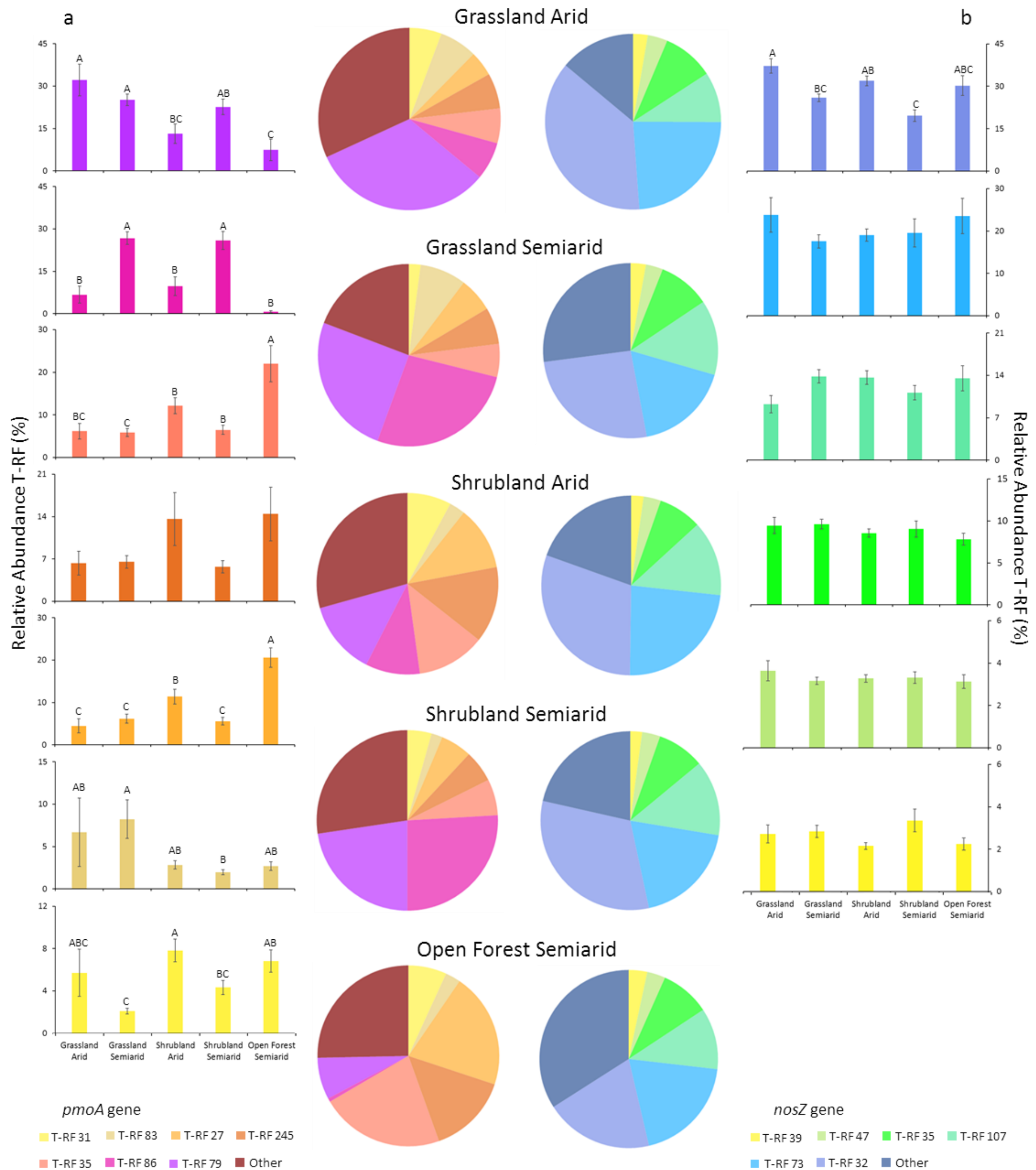


Figure 3. Relative abundance of major T-RFs of the *pmoA* (a) and *nosZ* (b) genes across major vegetation and aridity categories [n =12 (11 for *nosZ*), 56 (53 for *nosZ*), 26 (22 for *nosZ*), 52 and 14 for arid grasslands, semiarid grasslands, arid shrublands, semiarid shrublands and semiarid open forest sites, respectively]. Letters over the bars indicate significant differences across vegetation biomes and aridity classes (p-value <0.05, post-hoc test after ANOVA).

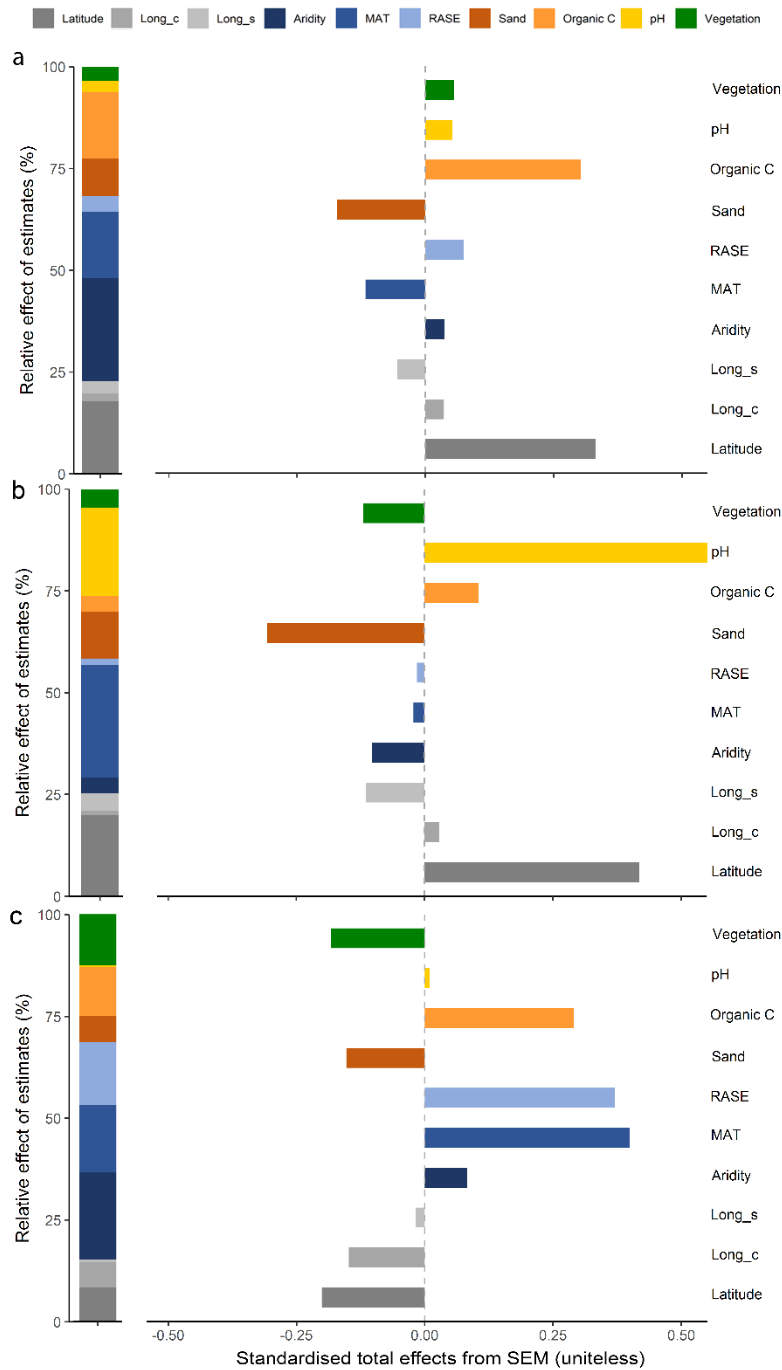
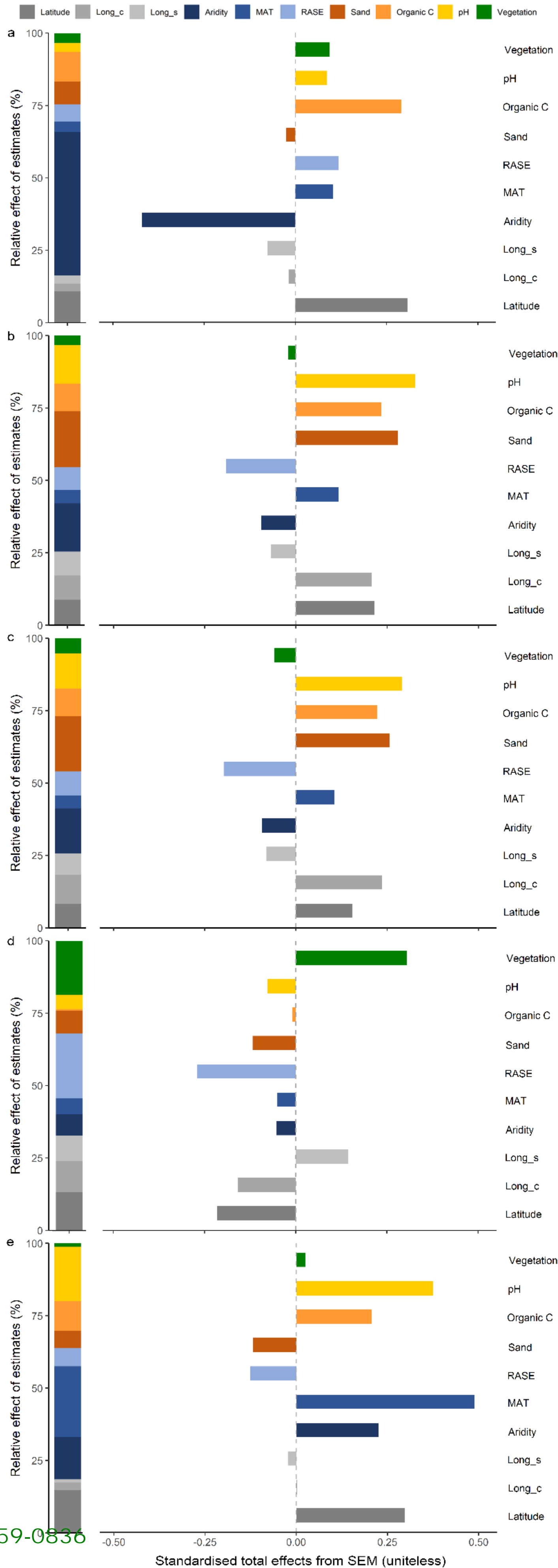


Figure 4. Absolute effects (sum in absolute value; left panels) and standardised total (direct plus indirect effects; right panels) of the estimates derived from the structural equation modelling, including latitude [Lat], longitude cosine [Lon_c]), longitude sine [Lon_s], aridity, mean annual temperature [MAT], rainfall seasonality [RASE], sand content, organic carbon, pH and microsite (open/vegetated areas) on MCS_1 (a), MCS_2 (b) and MCS_3 (c) of methane-oxidising bacteria.


Latitude
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Sand
Organic C
pH
Vegetation
a
b
c
d
e
Relative effect of estimates (%)
100
75
50
25
0
Vegetation
pH
Organic C
Sand
RASE
MAT
Aridity
Long_s
Long_c
Latitude
-0.50
-0.25
0.00
0.25
0.50
Standardised total effects from SEM (uniteless)

Figure 5. Absolute effects (sum in absolute value; left panels) and standardised total (direct plus indirect effects; right panels) of the estimates derived from the structural equation modelling, including latitude [Lat], longitude cosine [Lon_c]), longitude sine [Lon_s], aridity, mean annual temperature [MAT], rainfall seasonality [RASE], sand content, organic carbon, pH and microsite (open/vegetated areas) on the abundance (a), richness (b), DCS_1 (c), DCS_2 (d) and DCS_3 (e) of *nosZ* carrying bacteria.