Annals of Microbiology (2019) 69:329–339
https://doi.org/10.1007/s13213-018-1421-4

ORIGINAL ARTICLE

Increased methane concentration alters soil prokaryotic community structure along an artificial pH gradient

Petr Heděnec[1,2,3] · Roey Angel[4] · Qiang Lin[4] · Junpeng Rui[1] · Xiangzhen Li[1]

Received: 6 July 2018 / Accepted: 6 December 2018 / Published online: 2 January 2019

Abstract

Global climate change may have a large impact on increased emission rates of carbon dioxide and methane to total greenhouse gas emissions from terrestrial wetlands. Methane consumption by soil microbiota in alpine wet meadows serves as a biofilter for the methane produced in the waterlogged soil below. Altered pH regimes change microbial community composition and structure by exerting selection pressure on soil microorganisms with different ecological strategies and thus affect greenhouse gas emissions resulting from the metabolic activity of soil microorganisms. However, responses of prokaryotic communities to artificial pH shift under elevated methane concentration remain unclear. In this study, we assessed diversity and relative abundance of soil prokaryotes in an alpine meadow under elevated methane concentration along an artificial pH gradient using laboratory incubation experiments. We established an incubation experiment treated with artificial pH gradient (pH 4.5–8.5). After 3 months of incubation, 300 ml of methane at a concentration of 20,000 ppm was added to stimulate potential methanothrophs in topsoil. Sequencing of 16S rRNA gene indicated increasing of relative abundances of Crenarchaeota, Chloroflexi, Bacteroidetes, and Planctomycetes in soil after addition of methane, while the relative abundances of Actinobacteria and Gemmatimonadetes did not significant change before and after methane treatment. Results of phylogenetic relatedness of soil prokaryotes showed that microbial community is mostly shaped by deterministic factors. Species indicator analysis revealed distinct OTUs among various pH and methane treatments. Network analysis revealed distinct co-occurrence patterns of soil prokaryotic community before and after methane addition, and different correlation patterns among various prokaryotic taxa. Linear regression model revealed significant decrease of methane oxidation along elevated pH gradient. Soil pH constituted a strong environmental filter in species assembly of soil prokaryotic community. Methane oxidation rates decreased significantly with elevated pH. The interactive effects of elevated methane concentration and pH are therefore promising topic for future research.

Keywords Elevated methane oxidation · Soil prokaryotes · pH gradient · Methanotrophs

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-018-1421-4) contains supplementary material, which is available to authorized users.

✉ Petr Heděnec
petr.hedenec@centrum.cz

[1] Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China

[2] Institute for Environmental Studies and SoWa Research Infrastructure, Faculty of Science, Charles University in Prague, Benátská 2, 128 44 Prague 2, Czech Republic

[3] Laboratory of Soil Biodiversity, University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, Switzerland

[4] SoWa Research Infrastructure and Institute of Soil Biology, Biology Centre of the Czech Academy of Sciences, Na Sádkách 702/7, 370 05 České Budějovice, Czech Republic

Introduction

Soil microbiome and its associated functions, e.g., increased methane emission or in contrast methane oxidation, are sensitive to various aspects of environmental changes such as water regime (Evans et al. 2014), temperature (Zhou et al. 2016), and soil acidification or alkalization (Lauber et al. 2009; Rousk et al. 2010; Heděnec et al. 2018). Both alkalization and acidification may change diversity and abundance of soil microbes by exerting selection pressure on soil microbiota with different ecological strategies and thus affect greenhouse gas emissions resulting from its metabolic activity (Heděnec et al. 2018). However, little effort has been paid to investigate effect of increased methane concentration on composition and structure of soil prokaryotic communities in soils treated by various pH.

Global climate change may have a large impact on increased emission rates of methane to total greenhouse gas emissions from terrestrial wetlands (Yvon-Durocher et al. 2014). Alkalization or acidification changes prokaryotic community composition by selecting species or groups with different metabolic and ecological strategies to optimize their fitness. Study of Heděnec et al. (2018) showed altered methane emission along artificial pH gradient (4.5–8.5). The highest methane emissions were detected at pH 8.5 (2.2 nmol g^{-1} h^{-1}) and the lowest methane emissions were detected at pH 7.5 (1.98 nmol g^{-1} h^{-1}) and 6.8 (1.87 nmol g^{-1} h^{-1}) respectively (Heděnec et al. 2018). However, little is known about the effect of pH and increased methane concentration on change of methane oxidation rates under laboratory conditions.

Methane is a critical greenhouse gas contributing to global climate change (Yvon-Durocher et al. 2014). It absorbs infrared radiation nearly 25 times more efficiently than carbon dioxide (Ward et al. 2013). Alpine wetlands are a major source of methane emissions globally (Yun et al. 2012; Gao et al. 2013). For example, wetlands represent one of the biggest methane emission centers in China (Yun et al. 2012). The annual methane emissions from this area have been estimated to be between 0.56 and 1 Tg (Fang et al. 2011; Gao et al. 2013). In addition, these wetlands become extremely vulnerable to acidification resulted from increased fertilizing of agricultural landscape (Kemmitt et al. 2006; Lue and Tian 2007; Gao et al. 2013).

Methanotrophs are ubiquitous in soils and utilize methane as carbon and energy source (Liu et al. 2014). Methanotrophs mainly include type I (Gammaproteobacteria), type II (Alphaproteobacteria), and uncultured microorganisms associated with pxmA gene expression (Pandey et al. 2014; Kou et al. 2017). Consumption of methane by soil microbes in alpine wet meadows serves as a biofilter for the mitigation of methane emissions into atmosphere (Conrad 2007; Kögel-Knabner et al. 2010). Soil pH is a major factor affecting the diversity and activity of soil microbiota at local (Heděnec et al. 2018) and/or regional scale (Rousk et al. 2010). Soil pH directly affects microbial physiological functions and the accessibility of nutrients and growth factors of soil microbiota (Angel et al. 2010; Rousk et al. 2010).

Type I methanotrophs are more active under changing environmental conditions (Henckel et al. 2000), while type II methanotrophs favor low pH, low O_2 habitats, and high methane concentrations (Henckel et al. 2000; Putkinen et al. 2014; Singh et al. 2007). Thus, the responses of prokaryotic community structure and activity (including methanotrophs) to increased methane concentration may vary with soil pH. However, it is still unclear how increased methane concentration impact on the diversity and structure of soil prokaryotes along a pH gradient.

Microbial communities are simultaneously influenced by deterministic (moisture, pH, and nutrient flow) and stochastic factors (speciation, extinction, and ecological drift) (Tilman 2004; Vellend 2010; Stegen et al. 2012; Nemergut et al. 2013; Zhang et al. 2016). Deterministic theory proposes that the abundance and distribution of a species is mainly driven by a set of niche conditions that the species adapts, and resources that the species utilize (Tilman 2004; Gilbert et al. 2012).

Stochastic models originally based on Hubbell's neutral theory (Hubbell et al. 2001) assume that community dynamics are the consequences of individual stochastic events at spatial-temporal scales, e.g., natality, mortality, and migration of individuals (Vellend 2010; Stegen et al. 2012; Nemergut et al. 2013). Usually, microbial communities shaped by deterministic factors show stronger phylogenetic relatedness than those dominantly shaped by stochastic factors (Kembel et al. 2011; Stegen et al. 2012).

In this study, we assessed the diversity and relative abundance soil of prokaryotes in an alpine meadow soil to elevated methane concentration along an artificial pH gradient using laboratory incubation experiments. We aimed to answer the following questions. (i) How elevated methane concentration changes community structure and diversity of soil prokaryotic community along a pH gradient? (ii) Which factors affecting soil microbial communities under increased methane concentration and altered pH regime? (iii) How the changes of soil pH affect methane oxidation rates?

Material and methods

Study site and description

The sampling site is a natural alpine meadow (not permanently waterlogged) in Hongyuan County, Sichuan Province, China, which locates at the eastern edge of Qinghai-Tibetan Plateau (33° 05′ N, 102° 35′ E). The average altitude of the sampling site is 3462 m above sea level. The average annual temperature is 1.4 °C, and annual rainfall is approximately 752 mm (Gao et al. 2013). The dominant plant species in this region are perennial grasses *Clinelymus nutans* and *Roegneria nutans*, accompanied by *Koeleria litwinowii*, *Agrostis schneideri*, *Kobresia setchwanensis*, and perennial herb *Anemone rivularis* with average vegetation coverage over 90% (Gao et al. 2013). Soil is rich in organic matter and classified as Mat-cry-gelic-cambisols based on Chinese soil classification system (Gao et al. 2013). Soil moisture (measured gravimetrically) was 40%, pH 6.8 (measured using pH meter at soil:water ratio of 1:5), conductivity 35 cm s^{-1} (measured simultaneously as pH at soil:water ratio of 1:5), and soil organic matter (SOM) 12.2% (measured by dichromate digestion method (Jenkinson and Powlson 1976)). Soil properties were measured once again by the end of experiments. We did not find any significant differences in SOM and conductivity

among treatments at the start and the end of the experiment (data not shown).

Experimental design

Soil samples were taken during vegetation season in May 2014 (Heděnec et al. 2018). In total, five random soil samples (1 kg) to a depth of 15 cm from the soil surface were collected with iron soil corer (10 cm in diameter) and pooled to one composite sample (5 kg) (Heděnec et al. 2018). This was done to avoid having spatial variation within the site as a factor in our subsequent statistical analyses. Soil samples were sieved with 2-mm mesh to remove visible stones and plant residuals. Fifty grams of fresh, sieved soil was weighted into a glass bottle (310 ml) and sealed with a rubber stopper. Soil pH was adjusted to final pH levels of 4.5, 5.5, 6.8, 7.5, and 8.5, whereas soil at pH 6.8 represented the control treatment (Heděnec et al. 2018). Each pH treatment consisted of four independent replicates (bottles). The pH was further adjusted each month during 3 months of pre-incubation to maintain constant pH (Heděnec et al. 2018).

To manipulate soil pH, we first tested how much HCl (0.1 M) or NaOH (0.5 M) solution was needed (Nelson and Su 2010). To achieve pH 4.5 and 5.5, the 220 and 181 ml of 0.1 M HCl solution respectively were added to 780 and 819 ml of distilled water (Nelson and Su 2010; Heděnec et al. 2018). To achieve pH 7.5 and 8.5, the 465 and 393 ml of 0.5 M NaOH solution respectively were added to 535 and 607 ml of distilled water (Nelson and Su 2010; Heděnec et al. 2018). Then, we added 15 ml of diluted solution to the soil to achieve a final 40% of water holding capacity (Heděnec et al. 2018). Bottles were incubated at 25 °C for 3 months in the dark to promote long-term effect of various pH treatments. To allow gas exchange, bottles were opened for 10 min and slowly shaken every second day of pre-incubation. Methane emission rates under laboratory conditions ranged from 2 to 2.2 nmol g^{-1} h^{-1} (Heděnec et al. 2018).

Measurement of methane oxidation rates

After 3 months of incubation, 310 ml of air from headspace was evacuated and immediately replaced by 300 ml lab air containing 20,000 ppm of CH_4. We incubated soils in glass bottles at room temperature (25 °C) and extracted a 1-mL gas sample from the headspace at 0, 2, 6, 24, 30, and 48 h after bottle closure. Gas samples were manually injected into the gas chromatograph (Shimadzu GC-2014 gas chromatograph (GC), Shimadzu, Kyoto, Japan). The gas chromatograph was equipped with a flame-ionization detector for methane set at 200 °C and the carrier gas was 100% ultrapure N_2 with a flow rate of 25 mL min^{-1}. The column used for CH_4 was a 60/80 Carboxen 1000 (15 ft., 1/8 in.) set in an oven at 40 °C. Methane oxidation rates were estimated by fitting an exponential function to the concentration behavior in the incubation bottles over time: CH_4 concentration = N_0 $e^{(-\lambda t)}$. Where N_0 is the initial concentration and lambda is the decay constant. Methane oxidation was expressed as micromole per gram per hour.

DNA extraction and MiSeq sequencing

Soil from the upper layer (1 g) was taken from incubation bottles 1 h before and 48 h after methane addition. The DNA was extracted using Power Soil extraction kit (MOBIO Inc., Carlsbad, USA) according to the manufacturer's instructions. Quantification and quality control of the extracted DNA was checked using NanoDrop 2000 spectrophotometer (Thermo Scientific Inc., USA), diluted to 10 ng/μL and stored at − 20 °C for downstream analysis. PCR amplification was conducted using the universal bacterial 16S rRNA gene primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) with 12 bp unique barcodes at 5′-end of 515F to amplify the V4–V5 hypervariable region of 16S rRNA gene (Yao et al. 2014). The PCR mixture (25 μL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.4 μM deoxynucleoside triphosphate, 1.0 μM primers and 0.5 U ExTaq polymerase (TaKaRa, Dalian), and 10 ng of soil genomic DNA. The PCR amplification program included the following steps: initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min (Li et al. 2014). The PCR replicates were pooled, separated on 1.5% agarose gel using electrophoresis, and purified using Sangon Gel Extraction Kit (Sangon Biotech, Shanghai, China). All PCR products were pooled at equimolar amounts and used for paired-end sequencing (2 × 250 bp) using Illumina MiSeq sequencer at environmental sequencing platform of Chengdu Institute of Biology, Chinese Academy of Sciences.

Sequence data analysis

The QIIME Pipeline Version 1.7.0 was used to process the sequencing data (Caporaso et al. 2010). All sequencing reads were trimmed and demultiplexed according unique barcode sequences. High-quality sequences (length > 300 bp, without ambiguous base "*N*," and average base quality score > 30) were used to process downstream analysis. Chimera check was conducted using Uchime algorithm (Edgar et al. 2011). All samples were randomly rarefied to the minimum library size (7600 reads). Sequences were clustered according to taxonomic units (OTUs) at 97% identity using Uparse algorith (Edgar 2013). OTUs were taxonomically classified using QIIME's implementation of a Naïve Bayesian classifier against Ribosomal Database Project database 1.1.1. (Wang et al. 2007). Rare OTUs occurring with less than three reads

per sample were excluded to reduce computation efforts. Phylogenetic maximum likelihood–approximation trees were reconstructed using the generalized time-reversible model in FastTree 2.1.1 (Price et al. 2010). The original sequence data are stored at European Nucleotide Archive (https://www.ebi.ac.uk/ena/data/view/PRJEB25417).

Processes shaping prokaryotic community

The mean nearest taxon index (mean NTI) and the mean nearest relatedness index (mean NRI) were calculated for all samples per treatment on average using "mntd," "mpd," "ses.mntd," and "ses.mpd" in the "picante" package in R (Kembel et al. 2011). The mean NRI calculates the mean pairwise distance between all species in each community. Similarly, the mean NTI calculates the mean nearest taxon distance, the mean distance separating each species in the community from its closest relative. To evaluate the degree of non-random phylogenetic community structure, OTUs and their relative abundances were randomized across the tips of phylogeny (null.model = "|taxa.labels" in "ses.mntd," "ses.mpd") (Stegen et al. 2012). The mean NTI and NRI, taken across all treatments that were significantly different from zero indicated clustering (|NTI|, |NRI| > 2; $p < 0.05$) or overdispersion (|NTI|, |NRI| < 2; $p > 0.05$), on average (Kembel et al. 2011; Stegen et al. 2012, 2013). The p value describes the differences between phylogenetic distances in the observed communities versus null communities generated with randomization method (Kembel et al. 2011; Stegen et al. 2012). Closely related organisms are expected to be phylogenetically structured in the same or similar niche set of conditions, shaped by deterministic factors (Tilman 2004; Stegen et al. 2012). On the other hand, less closely related organisms are considered as phylogenetically overdispersed, more controlled by stochastic factors (Kembel et al. 2011; Stegen et al. 2012).

Statistical analysis

The effects of pH and methane addition on relative abundances of prokaryotic taxa were calculated using hierarchical (nested) ANOVA followed by Tukey's test (Simecek and Simeckova 2013). Alpha diversity indices, distance-based (Bray–Curtis) redundancy analyses (dbRDA), and PerMANOVA were performed using "phyloseq" package (McMurdie and Holmes 2012, 2013). Effect of pH gradient on methane oxidation rates was analyzed using linear regression model. To identify characteristic organisms for each pH and methane treatment, we performed an indicator species analysis (Timling et al. 2014). We used indicator value analysis with the function "indval" from the R package labdsv to find the significant indicators in our dataset (Timling et al. 2014). Then a heatmap based on those significant indicator values was computed using the "heatmap.2" function of the gplots package. Co-occurrence neural networks were used to reveal co-occurrence patterns of soil microbial communities before and after methane addition. Co-occurrence networks were calculated from correlation matrix of main phyla and classes using "cor_auto" (all correlations) and "FDR network" (significant correlation only) functions in package "qgraph." False discovery rate (FDR) correction was done using the Bonferroni correction to adjust p value of multiple correlations (Smith et al. 2006). All statistics and graphics were performed in R program (www.r-project.org).

Results

Effects of increased methane concentration on the diversity of soil prokaryotes along artificial pH gradient

A total of 880,000 chimera-free sequences were obtained using Miseq sequencing of 16S rRNA gene amplicons from 40 soil samples with at least 7600 sequences per sample. In total, 2875 OTUs with relative abundances of more than 0.01% were obtained from all 40 samples. OTUs richness, Chao1, Simpson index, and Simpson evenness did not show any significant differences in soils after methane addition. In addition, none of alpha diversity indices significant changed along pH gradient (Table 1).

Composition of soil prokaryotic communities in soils after methane addition clearly separate from those in soils before methane addition based on PerMANOVA with Bray–Curtis distance (Fig. 1). Furthermore, we investigated the changes in soil prokaryotic compositions in soils at various pH (Fig. 1). In contrast, prokaryotic community composition before and after methane addition was similar within individual pH treatments (no interaction; Fig. 1).

The p value showing percentage of null model expectation of the mean NTI indicated strong phylogenetic relatedness and thus strong effect of deterministic factors on soil prokaryotic community in soils before and after methane addition along the artificial pH gradient (mean NTI > 0; $p < 0.05$, Table S2). In contrast, neither artificial pH gradient nor methane addition showed any significant differences between mean NTI (Table 1). Conversely, null model expectation of NRI showed weak phylogenetic relatedness (dominant effect of stochastic factors) of soil prokaryotic communities in soils with neutral pH (mean NRI < 0; $p > 0.05$) while soils treated pH 4.5, 5.5, 7.5, and 8.5 showed strong phylogenetic relatedness (mean NRI > 0; $p < 0.05$, Table 1). In addition, null model expectation of mean NRI indicated strong phylogenetic relatedness of soil prokaryotic communities in soils before and after methane treatment. Finally, mean NRI did not show

Table 1 The effects of increased methane addition on the alpha diversity indices of soil prokaryotic communities along artificial pH gradient (two-way ANOVA followed by Tukey's test)

pH	OTUs richness ± SD	Simpson index ± SD	Simpson evenness ± SD	Chao1 index ± SD
4.5	789 ± 154 ns	402 ± 54 ns	0.522 ± 0.06 ns	1435 ± 139 ns
5.5	785 ± 132 ns	365 ± 54 ns	0.487 ± 0.05 ns	1435 ± 88 ns
6.8	720 ± 118 ns	320 ± 116 ns	0.422 ± 0.14 ns	1355 ± 395 ns
7.5	790 ± 114 ns	359 ± 89 ns	0.466 ± 0.1 ns	1453 ± 122 ns
8.5	801 ± 158 ns	356 ± 105 ns	0.467 ± 0.13 ns	1419 ± 115 ns
Methane addition				
Ambient	798 ± 134 ns	346 ± 116 ns	0.46 ± 0.14 ns	1363 ± 268 ns
Methane	811 ± 205 ns	376 ± 42 ns	0.487 ± 0.05 ns	1476 ± 91 ns
Two-way ANOVA				
pH	$F = 1.402$; $p = 0.256$	$F = 2.402$; $p = 0.127$	$F = 2.834$; $p = 0.09$	$F = 0.044$; $p = 0.835$
Methane addition	$F = 2.307$; $p = 0.189$	$F = 2.316$; $p = 0.134$	$F = 1.444$; $p = 0.231$	$F = 4.356$; $p = 0.07$
pH*methane addition	$F = 0.906$; $p = 0.458$	$F = 4.074$; $p = 0.059$	$F = 3.724$; $p = 0.059$	$F = 0.309$; $p = 0.58$

ns means non-significant at $p < 0.05$ for the values in the same column

any significant difference between soils before and after methane addition as well as among pH treatments.

Effects of increased methane concentration on the relative abundances of soil prokaryotes taxa along artificial pH gradient

The relative abundances of Crenarchaeota, Chloroflexi, Bacteroidetes, and Planctomycetes increased after methane addition while the relative abundances of Actinobacteria and Gemmatimonadetes did not significantly change between soils before and after methane treatment (Table 2). In contrast, relative abundance of Actinobacteria and Gemmatimonadetes varied within individual pH treatments before and after methane addition (significant interaction; Table 2). Our results also showed significant effects of artificial pH gradient on the relative abundances of Chloroflexi and Gemmatimonadetes. The relative abundance of Chloroflexi increased in acidic pH. In contrast, the relative abundance of Gemmatimonadetes showed higher abundance in soils with alkaline pH (Table 2).

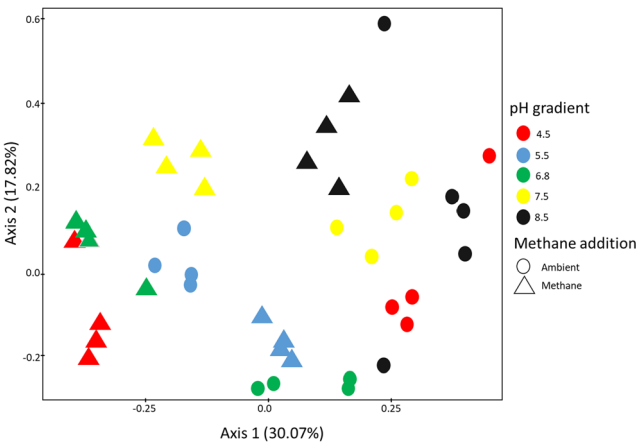

Fig. 1 Distance-based redundancy analysis (dbRDA) based on Bray–Curtis distance showing the effects of increased methane concentration on the structure of soil prokaryotic community along artificial pH gradient. PerMANOVA (pH $R^2 = 0.114$, $p = 0.001$; methane addition $R^2 = 0.051$, $p = 0.001$; pH × methane addition $R^2 = 0.085$, $p = 0.078$)

Effects of artificial pH gradient on methane oxidizing Proteobacteria

Our results showed significant effects of artificial pH gradient on the relative abundance of Alphaproteobacteria. Soils treated by acidic pH showed lower relative abundance of Alphaproteobacteria than soils with alkaline pH. The relative abundances of Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria did not significantly change along our artificial pH gradient (Table 3). However, relative abundance of Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria varied within individual pH treatments before and after methane addition (significant interaction; Tables 3 and 4). Furthermore, the relative abundances of Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria did not indicate any significant differences between soils before and after methane addition.

Species indicators of increased methane concentration in soils treated by various pH

Linear regression model revealed significant decreasing of methane oxidation along elevated pH gradient (Fig. 2). Soils treated by acidic pH showed significantly higher methane oxidation than those soils treated by alkaline pH (Fig. 2). Species indicator analysis revealed 18 OTUs (at genera level) associated with various pH and methane concentration (Fig. 3). *Staphylococcus*, *Devosia*, *Propionibacterium*, *Finegoldia*, and *Frankia* affiliated OTUs showed higher indicator value (fidelity)

Table 2 The effects of increased methane concentration on the phylogenetic structures of soil prokaryotes along artificial pH gradient (two-way ANOVA followed by Tukey's test)

pH	NTI ± SD	$P_{\text{Null model expectation}}$	NRI ± SD	$P_{\text{Null model expectation}}$
4.5	7.712 ± 1.6 ns	0.01	3.3 ± 1.4 ns	0.01
5.5	7.87 ± 1.3 ns	0.01	3.91 ± 1.7 ns	0.01
6.8	7.22 ± 1.6 ns	0.01	2.97 ± 1.5 ns	0.07
7.5	8.2 ± 1.4 ns	0.01	3.51 ± 0.8 ns	0.01
8.5	7.87 ± 1.2 ns	0.01	3.15 ± 0.7 ns	0.01
Methane addition				
Ambient	7.45 ± 1.6 ns	0.01	3.67 ± 1.5 ns	0.01
Methane	8.06 ± 1.2 ns	0.01	3.02 ± 0.9 ns	0.01
Two-way ANOVA				
pH	$F = 0.612$; $p = 0.112$		$F = 1.481$; $p = 0.09$	
Methane addition	$F = 3.123$; $p = 0.07$		$F = 0.923$; $p = 0.17$	
pH*methane addition	$F = 1.221$; $p = 0.562$		$F = 1.426$; $p = 0.212$	

ns means non-significant at $p < 0.05$ for the values in the same column

in more acidic soils under ambient (normal) methane concentration. In contrast, *Niastella* and *Synechococcus* showed higher indicator values in more alkaline soils under ambient methane concentration. Genera Candidatus *Nitrososphaera*, Candidatus *Solibacter*, Candidatus *Koribacter*, *Nitrospira*, *Pirellula*, *Planctomyces*, *Hyphomicrobium*, and *Burkholderia* showed higher indicator values in soils treated with various pH under increased methane concentration. In contrast, genera *Opitutus* and *Rubrivivax* showed higher indicator value in more acidic soils under increased methane concentration.

Co-occurrence patterns of soil prokaryotic communities

In total, artificial network connected 34 taxa at phylum and 4 at class level, respectively (Fig. 4). Taxa with close distance showed high co-occurrence, while taxa with far distance showed weak co-occurrence pattern. Network analysis revealed different co-occurrence patterns among various prokaryotic phyla (Fig. 4). Phyla Thermotogae, Thermi and Aquificae strongly co-occur before and after methane addition, while phyla AD3, OD1, and WS3 did not show any co-occurrence pattern before and after methane addition.

In contrast, network analysis revealed increased number of negative interactions (based on negative Pearson's correlation coefficient) among various prokaryotic phyla after methane addition (Fig. 4). Network also uncovered different correlation patterns among various prokaryotic taxa before and after methane addition. More detailed analyses after FDR corrections showed positive correlations of OTUs' abundances among Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria in soils incubated before methane addition. Conversely, false correlation network showed negative correlation of OTUs' abundances of Alphaproteobacteria,

Table 3 The effects of increased methane concentration on the relative abundances of soil prokaryotic phyla along the artificial pH gradient (two-way ANOVA followed by Tukey's test)

pH	Actinobacteria ± SD	Crenarchaeota ± SD	Chloroflexi ± SD	Bacteroidetes ± SD	Gemmatimonadetes ± SD	Planctomycetes ± SD
4.5	0.13 ± 0.04 ns	0.043 ± 0.02 ns	0.054 ± 0.01a	0.05 ± 0.01 ns	0.026 ± 0.007a	0.033 ± 0.008 ns
5.5	0.15 ± 0.03 ns	0.06 ± 0.03 ns	0.048 ± 0.009b	0.04 ± 0.02 ns	0.032 ± 0.01b	0.028 ± 0.008 ns
6.8	0.13 ± 0.05 ns	0.06 ± 0.04 ns	0.044 ± 0.01ab	0.05 ± 0.02 ns	0.026 ± 0.009a	0.023 ± 0.01 ns
7.5	0.14 ± 0.03 ns	0.05 ± 0.02 ns	0.046 ± 0.009b	0.04 ± 0.01 ns	0.035 ± 0.009b	0.031 ± 0.008 ns
8.5	0.14 ± 0.02 ns	0.05 ± 0.02 ns	0.045 ± 0.009b	0.05 ± 0.02 ns	0.039 ± 0.009b	0.032 ± 0.008 ns
Methane addition						
Ambient	0.13 ± 0.04 ns	0.04 ± 0.03a	0.043 ± 0.01a	0.04 ± 0.02a	0.03 ± 0.01 ns	0.024 ± 0.008a
Methane	0.13 ± 0.03 ns	0.06 ± 0.02b	0.053 ± 0.009b	0.05 ± 0.01b	0.03 ± 0.01 ns	0.035 ± 0.007b
Two-way ANOVA						
pH	$F = 0.121$; $p = 0.73$	$F = 0.892$; $p = 0.349$	$F = 4.607$; $p = 0.03$	$F = 1.188$; $p = 0.28$	$F = 8.543$; $p = 0.01$	$F = 0.182$; $p = 0.671$
Methane addition	$F = 0.077$; $p = 0.78$	$F = 9.951$; $p = 0.008$	$F = 15.137$; $p = 0.01$	$F = 5.698$; $p = 0.02$	$F = 0.522$; $p = 0.471$	$F = 36.242$; $p = 0.01$
pH*methane addition	$F = 17.422$; $p = 0.01$	$F = 0.541$; $p = 0.465$	$F = 2.437$; $p = 0.125$	$F = 1.761$; $p = 0.19$	$F = 10.975$; $p = 0.001$	$F = 1.629$; $p = 0.208$

Different letters in a column means significant difference at $p < 0.05$

Table 4 The effects of increased methane concentration on the relative abundances of most frequent proteobacterial sub-phyla along artificial pH gradient (two-way ANOVA followed by Tukey's test)

pH	Alphaproteobacteria ± SD	Betaproteobacteria ± SD	Gammaproteobacteria ± SD	Deltaproteobacteria ± SD
4.5	0.094 ± 0.04a	0.052 ± 0.02 ns	0.028 ± 0.01 ns	0.047 ± 0.02 ns
5.5	0.1 ± 0.01ab	0.046 ± 0.007 ns	0.036 ± 0.009 ns	0.047 ± 0.01 ns
6.8	0.111 ± 0.01b	0.046 ± 0.007 ns	0.03 ± 0.005 ns	0.042 ± 0.005 ns
7.5	0.112 ± 0.02b	0.049 ± 0.007 ns	0.03 ± 0.008 ns	0.044 ± 0.008 ns
8.5	0.123 ± 0.02b	0.052 ± 0.005 ns	0.035 ± 0.01 ns	0.034 ± 0.007 ns
Methane addition				
Ambient	0.11 ± 0.01 ns	0.05 ± 0.009 ns	0.03 ± 0.007 ns	0.05 ± 0.02 ns
Methane	0.11 ± 0.03 ns	0.047 ± 0.01 ns	0.03 ± 0.01 ns	0.04 ± 0.01 ns
Two-way ANOVA				
pH	$F = 6.578$; $p = 0.013$	$F = 0.001$; $p = 0.977$	$F = 2.061$; $p = 0.157$	$F = 3.740$; $p = 0.06$
Methane addition	$F = 1.345$; $p = 0.251$	$F = 1.617$; $p = 0.209$	$F = 0.939$; $p = 0.337$	$F = 2.487$; $p = 0.121$
pH*methane addition	$F = 1.316$; $p = 0.257$	$F = 8.295$; $p = 0.01$	$F = 4.360$; $p = 0.042$	$F = 16.380$; $p = 0.01$

Different letters in a column means significant difference at $p < 0.05$

Betaproteobacteria, and Deltaproteobacteria in soils incubated after methane addition.

Discussion

Many studies show that energy sources have great effects on the diversity and abundance of soil biota (Eisenhauer et al. 2012; Liu et al. 2016). We did not observe any significant changes in alpha diversity of prokaryotes after the addition of methane using lab incubation experiments. This implicated that methane pulse for short time (48 h in this study) probably did not have significant effects on the species richness of soil methanotrophs. However, methane addition affected the structure of soil prokaryotic community. These changes in the structure of soil prokaryotes were closely related to the changes in the relative abundances of dominating prokaryotic taxa.

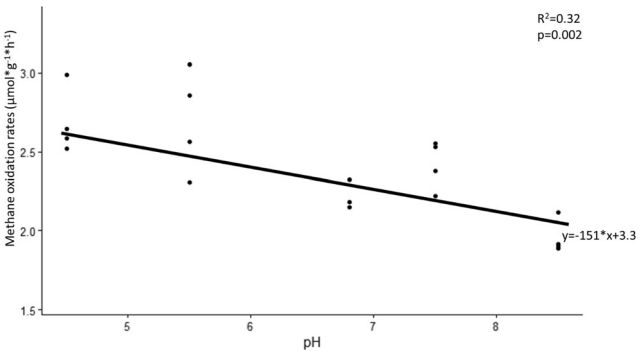

Fig. 2 The effect of soil pH changes on methane oxidation rates (linear regression model)

The relative abundances of Crenarchaeota, Chloroflexi, Bacteroidetes, and *Planctomycetes* increased in soils under increased methane concentration. We suggested that the abundances increase in these phyla could reflect cross feeding interactions with methanotrophs, or even methanotrophic capacity of some members in these phyla. Previous reports indicate the potential for methane consumption of Crenarchaeota (Vaksmaa et al. 2017) and other bacterial phyla such as Chloroflexi, Bacteroidetes and Planctomycetes (Héry et al. 2008; Nunoura et al. 2012). This study also emphasized the possible roles of Chloroflexi, Bacteroidetes, and Planctomycetes in methane oxidation, yet more evidences are needed. In contrast, as an abundant phylum in soil, Proteobacteria was not sensitive to methane addition and did not respond significantly to elevated methane.

We used a single soil for incubation experiments at various pH levels under laboratory conditions. Soil pH has significant effects on bacterial diversity at larger geographical scale (Lauber et al. 2009) and increase species richness along artificial pH gradient in a long-term limed soil (Rousk et al. 2010). The bacterial diversity is higher in neutral soil than that in acidic soil (Wu et al. 2017). However, in our short-term incubation experiment, species richness of soil prokaryotes did not change significantly. For the bacterial composition, the relative abundance of Chloroflexi decreased and the relative abundance of Gemmatimonadetes increased along artificial pH gradient. In contrast, another study (Lauber et al. 2009) shows that compositional changes in soil microbial communities with pH are largely driven by the changes in the relative abundances of Acidobacteria, Actinobacteria, and Bacteroidetes.

The relative abundance of Alphaproteobacteria was lower in acidic pH than that in alkaline pH. Some studies show

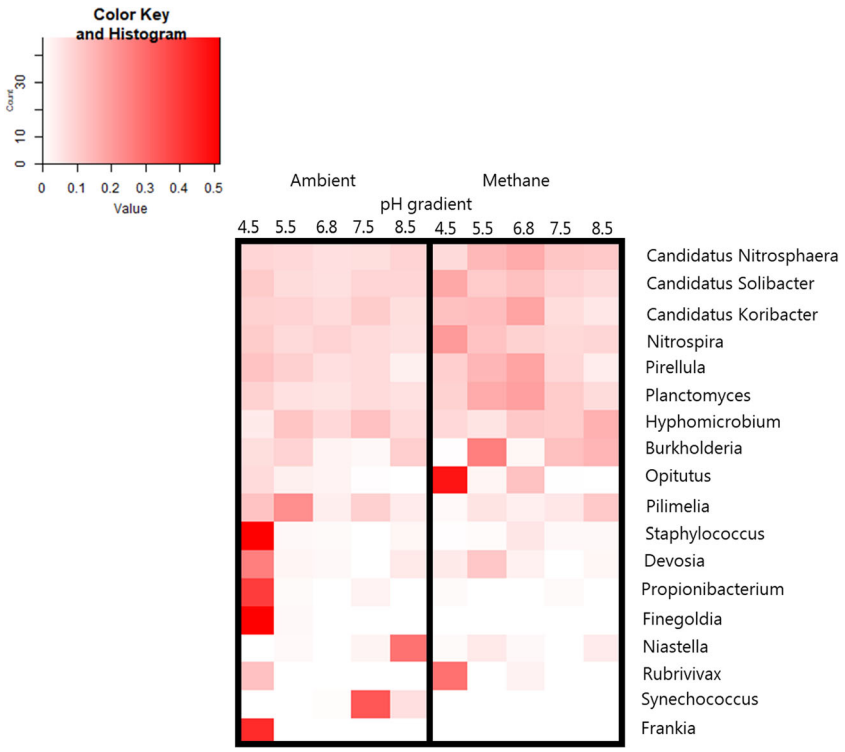

Fig. 3 Heatmap of species indicator values for OTUs affiliated to prokaryotic genera

opposite response of Alphaproteobacteria to pH (Rousk et al. 2010; Zhang et al. 2015). These discrepancies implicated that various responses of specific taxonomic group/subgroups to pH were likely influenced by other integrative factors, such as carbon sources and soil properties. Our results provided an evidence that short-term methane elevation did not alter the responses of prokaryote communities to pH gradient. Our incubation system is a closed incubation system without input of C from plant. It may also influence the sensitivity of prokaryotes to pH changes.

Percentage of null model expectation of the mean NTI indicated strong phylogenetic relatedness of soil prokaryotic community in soils before and after methane addition along artificial pH gradient. Our results indicated soil pH as a strong environmental filter in species assembly of soil prokaryotic community. This indicates that disturbances, e.g., altering pH, promotes the dominance of deterministic processes (Lin et al. 2017; Yao et al. 2017). In contrast, study of Ho et al. (2017) hypothesize that microorganisms belong to the same phylogenetic hierarchy at family or genus level possess distinct physiological traits.

Microbial communities in soil are characterized by complex networks forming intricate relationships of synergistic, antagonistic, and/or neutral nature (Ho et al. 2016). Network analysis revealed different correlation patterns among various prokaryotic taxa before and after methane addition. Increased methane concentration may provide energy resource for methanotrophs and their abundance; therefore, we speculate that increased the abundances of methanotrophs can substantially affect other members of soil prokaryotes non-sensitive to methane concentration (Knief 2015) through secondary metabolites of methanotrophs or cross feedings.

For example, obligate gammaproteobacterial methanotroph *Methylomicrobium alcaliphilum* may directly excrete carbon-based compounds (e.g., acetate) which can be used by other microbes (Kalyuzhnaya et al. 2013). Conversely, specific heterotrophs such as *Rhizobium* spp. likely provide essential nutrients important for the growth of alphaproteobacterial methanotroph *Methylovulum miyakonense* (Iguchi et al. 2011). These phenomena implicate the important roles of biotic interactions in shaping co-occurrence patterns of soil prokaryotic communities (Ho et al. 2016).

Linear regression model revealed significant decrease in methane oxidation along elevated pH gradient. Our results corroborates with previous study (Dedysh and Panikov 1997) showing acidic pH as an optimum for higher methane oxidation rates than more alkaline pH. Negative relationship between soil pH and methane oxidation rates is observed in soils from various grasslands across different geographical regions (Kou et al. 2017). The effects of pH on methane oxidation rates are possibly realized through the stimulation of bacterial growth or activity as well as substantial shifts in the methanotroph community structure. We focused on methane oxidizing prokaryotes in topsoil because that upper layer is expected

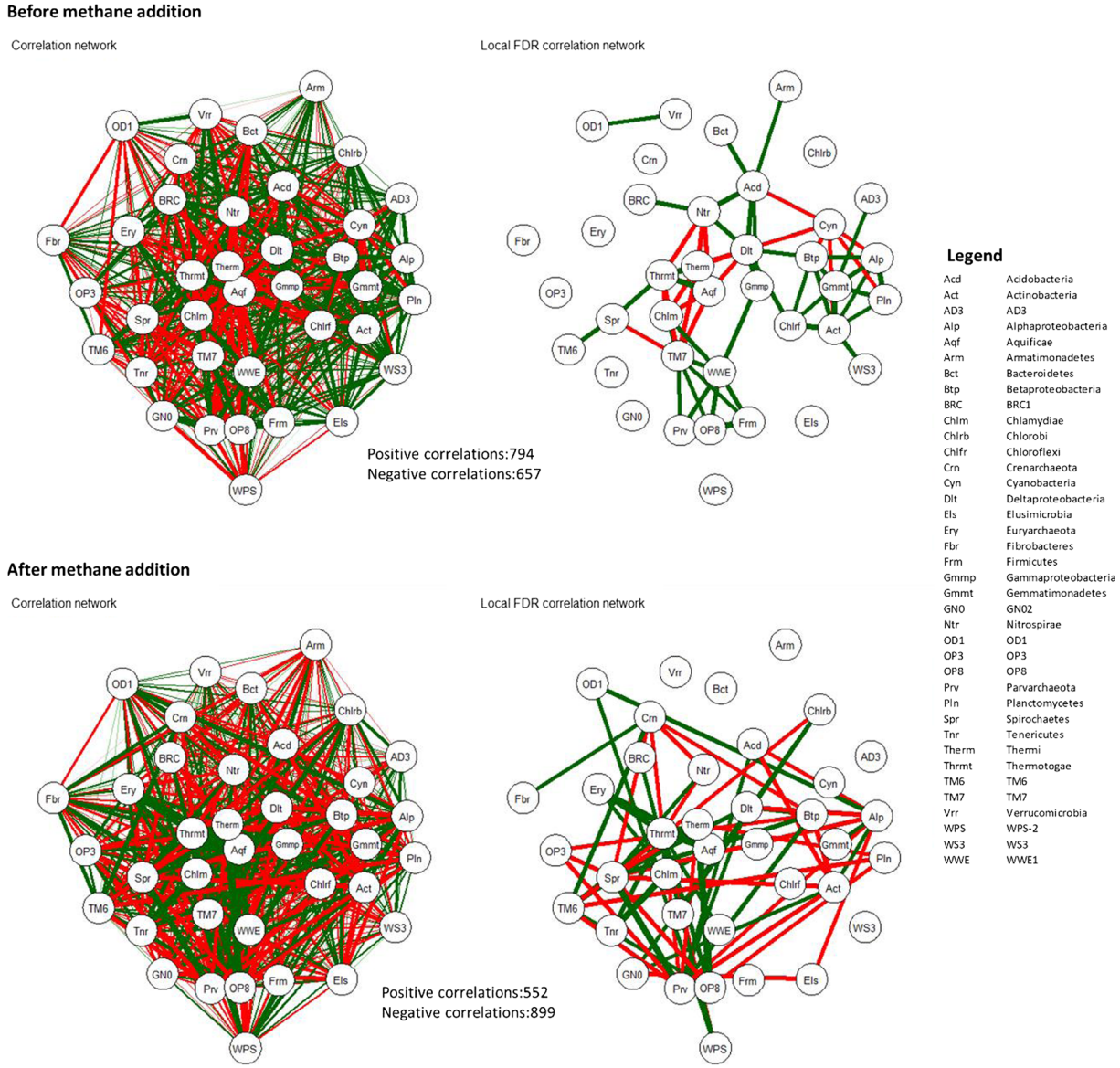

Fig. 4 Correlation networks and local false discovery rates network analysis of partial correlation matrix before and after methane addition. Lines indicate significant Pearson's correlation coefficient. Green and red colors indicate positive and negative correlations, respectively. Local FDR network depicts significant correlation only ($p < 0.05$)

be more "aerated" and thus more active in methane oxidizing. However, topsoil layer can by colonized by anaerobic methanothrophs II type localized in soil aggregates with extremely low oxygenic concentration (Angel et al. 2012; Fierer 2017). These anaerobic prokaryotes can also increase methane oxidation rates in soil with lower pH and increased nutrient depletion.

Conclusions

Our results revealed changes of the relative abundances of main prokaryotic taxa in soil after addition of methane. Structure of prokaryotic community based on Bray–Curtis distance was strongly affected by artificial pH gradient and methane concentration. Analysis of phylogenetic relatedness indicated that microbial community was mainly shaped by deterministic factors. Therefore, soil pH constituted a strong environmental filter in species assembly of soil prokaryotic community. In addition, methane oxidation rates decreased significantly with elevated pH. The interactive effects of elevated methane concentration and pH are therefore promising topic for future research.

Acknowledgements We thank Ondřej Mudrák for helpful advice with the statistical analysis and Adrienne Godschalx for help with English grammar and spelling.

Funding The work was supported by the National Natural Science Foundation of China (41771293, 31670503, 41630751), Key Laboratory of Sichuan Province (KLCAS-2017-3), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB15010303), 13th five-year information plan of Chinese Academy of Sciences (XXH13503-03-106), and Ministry of Education, Youth and Sports of the Czech Republic - MEYS (projects LM2015075, EF16_013/0001782).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals No human or animal participants were involved in this study.

Informed consent Informed consent rules were not applicable to this research because no human participants were involved.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Angel R, Soares MIM, Ungar ED, Gillor O (2010) Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME J 4: 553–563. https://doi.org/10.1038/ismej.2009.136

Angel R, Claus P, Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6:847–862. https://doi.org/10.1038/ismej.2011.141

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) Correspondence QIIME allows analysis of high- throughput community sequencing data intensity normalization improves color calling in SOLiD sequencing. Nat Publ Gr 7:335–336. https://doi.org/10.1038/nmeth0510-335

Conrad R (2007) Microbial ecology of methanogens and methanotrophs. Adv Agron 96:1–63. https://doi.org/10.1016/S0065-2113(07)96005-8

Dedysh SN, Panikov NS (1997) Effect of pH, temperature, and concentration of salts on methane oxidation kinetics in Sphagnum peat. Microbiology 66:476–479

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998. https://doi.org/10.1038/nmeth.2604

Edgar RC, Haas BJ, Clemente JC et al (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Eisenhauer N, Cesarz S, Koller R et al (2012) Global change belowground: impacts of elevated CO 2, nitrogen, and summer drought on soil food webs and biodiversity. Glob Chang Biol 18:435–447. https://doi.org/10.1111/j.1365-2486.2011.02555.x

Evans SE, Wallenstein MD, Burke IC (2014) Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? Ecology 95:110–122

Fang H, Cheng S, Yu G et al (2011) Responses of CO2 efflux from an alpine meadow soil on the Qinghai Tibetan Plateau to multi-form and low-level N addition. Plant Soil 351:177–190. https://doi.org/10.1007/s11104-011-0942-4

Fierer N (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro.2017.87

Gao J, Zhang X, Lei G, Wang G (2013) Soil organic carbon and its fractions in relation to degradation and restoration of wetlands on the Zoigê Plateau, China. Wetlands 34:235–241. https://doi.org/10.1007/s13157-013-0487-9

Gilbert JA, Steele JA, Caporaso JG et al (2012) Defining seasonal marine microbial community dynamics. ISME J 6:298–308. https://doi.org/10.1038/ismej.2011.107

Heděnec P, Rui J, Yao M et al (2018) Temporal response of soil prokaryotic communities to acidification and alkalization under laboratory conditions. Eur J Soil Biol 86:63–71. https://doi.org/10.1016/j.ejsobi.2018.03.005

Henckel T, Jäckel U, Schnell S, Conrad R (2000) Molecular analyses of novel methanotrophic communities in forest soil that oxidize atmospheric methane. Appl Environ Microbiol 66:1801–1808. https://doi.org/10.1128/AEM.66.5.1801-1808.2000

Héry M, Singer AC, Kumaresan D et al (2008) Effect of earthworms on the community structure of active methanotrophic bacteria in a landfill cover soil. ISME J 2:92–104. https://doi.org/10.1038/ismej.2007.66

Ho A, Angel R, Veraart AJ et al (2016) Biotic interactions in microbial communities as modulators of biogeochemical processes: methanotrophy as a model system. Front Microbiol 7:1–11. https://doi.org/10.3389/fmicb.2016.01285

Ho A, Di Lonardo DP, Bodelier PLE (2017) Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol Ecol 93:1–14. https://doi.org/10.1093/femsec/fix006

Hubbell SP, Ahumada JA, Condit R, Foster RB (2001) Local neighborhood effects on long-term survival of individual trees in a neotropical forest. Ecol Res 16:859–875. https://doi.org/10.1046/j.1440-1703.2001.00445.x

Iguchi H, Yurimoto H, Sakai Y (2011) Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl Environ Microbiol 77:8509–8515. https://doi.org/10.1128/AEM.05834-11

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil—V: a method for measuring soil biomass. Soil Biol Biochem 8:209–213. https://doi.org/10.1016/0038-0717(76)90005-5

Kalyuzhnaya MG, Yang S, Rozova ON, et al (2013) Highly efficient methane biocatalysis revealed in a methanotrophic bacterium. Nat Commun 4. https://doi.org/10.1038/ncomms3785

Kembel SW, Eisen JA, Pollard KS, Green JL (2011) The phylogenetic diversity of metagenomes. PLoS One 6:e23214. https://doi.org/10.1371/journal.pone.0023214

Kemmitt SJ, Wright D, Goulding KWT, Jones DL (2006) pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol Biochem 38:898–911. https://doi.org/10.1016/j.soilbio.2005.08.006

Knief C (2015) Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.01346

Kögel-Knabner I, Amelung W, Cao Z et al (2010) Biogeochemistry of paddy soils. Geoderma 157:1–14. https://doi.org/10.1016/j.geoderma.2010.03.009

Kou Y, Li J, Wang Y et al (2017) Scale-dependent key drivers controlling methane oxidation potential in Chinese grassland soils. Soil Biol Biochem 111:104–114. https://doi.org/10.1016/j.soilbio.2017.04.005

Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75: 5111–5120. https://doi.org/10.1128/AEM.00335-09

Li X, Rui J, Mao Y et al (2014) Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biol Biochem 68:392–401. https://doi.org/10.1016/j.soilbio.2013.10.017

Lin Q, De Vrieze J, Li C et al (2017) Temperature regulates deterministic processes and the succession of microbial interactions in anaerobic digestion process. Water Res 123:134–143. https://doi.org/10.1016/j.watres.2017.06.051

Liu J, Sun F, Wang L et al (2014) Molecular characterization of a microbial consortium involved in methane oxidation coupled to denitrification under micro-aerobic conditions. Microb Biotechnol 7:64–76. https://doi.org/10.1111/1751-7915.12097

Liu D, Tago K, Hayatsu M et al (2016) Effect of elevated CO₂ concentration, elevated temperature and no nitrogen fertilization on methanogenic archaeal and methane-oxidizing bacterial community structures in paddy soil. Microbes Environ 31:349–356. https://doi.org/10.1264/jsme2.ME16066

Lue C, Tian H (2007) Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data. J Geophys Res 112. https://doi.org/10.1029/2006JD007990

McMurdie PJ, Holmes S (2012) Phyloseq: a bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. Pac Symp Biocomput 235–46

McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8. https://doi.org/10.1371/journal.pone.0061217

Nelson PN, Su N (2010) Soil pH buffering capacity: a descriptive function and its application to some acidic tropical soils. Aust J Soil Res 48:201–207. https://doi.org/10.1071/SR09150

Nemergut DR, Schmidt SK, Fukami T et al (2013) Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev 77:342–356. https://doi.org/10.1128/MMBR.00051-12

Nunoura T, Takaki Y, Kazama H et al (2012) Microbial diversity in deep-sea methane seep sediments presented by SSU rRNA gene tag sequencing. Microbes Environ 27:382–390. https://doi.org/10.1264/jsme2.ME12032

Pandey VC, Singh JS, Singh DP, Singh RP (2014) Methanotrophs: promising bacteria for environmental remediation. Int J Environ Sci Technol 11:241–250. https://doi.org/10.1007/s13762-013-0387-9

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 - approximately maximum-likelihood trees for large alignments. PLoS One 5. https://doi.org/10.1371/journal.pone.0009490

Putkinen A, Larmola T, Tuomivirta T et al (2014) Peatland succession induces a shift in the community composition of Sphagnum-associated active methanotrophs. FEMS Microbiol Ecol 88:596–611. https://doi.org/10.1111/1574-6941.12327

Rousk J, Baath E, Brookes PC et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351. https://doi.org/10.1038/ismej.2010.58

Simecek P, Simeckova M (2013) Modification of Tukey's additivity test. J Stat Plan Inference 143:197–201. https://doi.org/10.1016/j.jspi.2012.07.002

Singh BK, Tate KR, Kolipaka G et al (2007) Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. Appl Environ Microbiol 73:5153–5161. https://doi.org/10.1128/AEM.00620-07

Smith VA, Yu J, Smulders TV et al (2006) Computational inference of neural information flow networks. PLoS Comput Biol 2:1436–1449. https://doi.org/10.1371/journal.pcbi.0020161

Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J 6:1653–1664. https://doi.org/10.1038/ismej.2012.22

Stegen JC, Lin X, Fredrickson JK et al (2013) Quantifying community assembly processes and identifying features that impose them. ISME J 7:2069–2079. https://doi.org/10.1038/ismej.2013.93

Tilman D (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proc Natl Acad Sci U S A 101:10854–10861. https://doi.org/10.1073/pnas.0403458101

Timling I, Walker DA, Nusbaum C et al (2014) Rich and cold: diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Mol Ecol 23:3258–3272. https://doi.org/10.1111/mec.12743

Vaksmaa A, van Alen TA, Ettwig KF et al (2017) Stratification of diversity and activity of methanogenic and methanotrophic microorganisms in a nitrogen-fertilized Italian paddy soil. Front Microbiol 8:1–15. https://doi.org/10.3389/fmicb.2017.02127

Vellend M (2010) Conceptual synthesis in community ecology. Q Rev Biol 85:183–206

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07

Ward SE, Ostle NJ, Oakley S et al (2013) Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. Ecol Lett 16:1285–1293. https://doi.org/10.1111/ele.12167

Wu Y, Zeng J, Zhu Q et al (2017) pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. Sci Rep 7:40093. https://doi.org/10.1038/srep40093

Yao, M. Yao M, Rui J, Li J, Dai Y, Bai Y, Heděnec P et al (2014) Rate-specific responses of prokaryotic diversity and structure to nitrogen deposition in the Leymus chinensis steppe. Soil Biol Biochem 79:81–90. https://doi.org/10.1016/j.soilbio.2014.09.009

Yao M, Rui J, Niu H, et al (2017) The differentiation of soil bacterial communities along a precipitation and temperature gradient in the eastern Inner Mongolia steppe. Catena 152. https://doi.org/10.1016/j.catena.2017.01.007

Yun J, Zhuang G, Ma A et al (2012) Community structure, abundance, and activity of methanotrophs in the Zoige wetland of the Tibetan Plateau. Microb Ecol 63:835–843. https://doi.org/10.1007/s00248-011-9981-x

Yvon-Durocher G, Allen AP, Bastviken D et al (2014) Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. Nature 507:488–491. https://doi.org/10.1038/nature13164

Zhang X, Liu W, Zhang G et al (2015) Soil Biology & Biochemistry Mechanisms of soil acidi fi cation reducing bacterial diversity. Soil Biol Biochem 81:275–281. https://doi.org/10.1016/j.soilbio.2014.11.004

Zhang X, Liu S, Li X et al (2016) Changes of soil prokaryotic communities after clear-cutting in a karst forest: evidences for cutting-based disturbance promoting deterministic processes. FEMS Microbiol Ecol 92:1–12. https://doi.org/10.1093/femsec/fiw026

Zhou J, Deng Y, Shen L et al (2016) Temperature mediates continental-scale diversity of microbes in forest soils. Nat Commun 7:12083. https://doi.org/10.1038/ncomms12083