

Vertical Methane Fluxes Driven by Methanogen in Riparian Buffers of Urban Wetlands

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Abstract

Abstract Background, Aims and Scope. Wetland soil is one of the largest natural contributors to methane emissions, which is a prevalent greenhouse gas. The vertical flux pattern of methane in soils is unclear. To investigate the relationship between methane vertical flux, soil total organic carbon (TOC) and methanogen, we were monitored in the riparian buffer of a wetland park from August 2018 to January 2020. The objectives of this study were to (1) analyze the vertical variation in methane fluxes within the riparian buffer and (2) investigate the vertical space relationships between methane fluxes, TOC and methanogens. Furthermore, the results of this study could provide better information for understanding the vertical linkage between methane, TOC and methanogens. **Methods.** The study area is the Living Water Garden (LWG), a wetland park in Chengdu, Sichuan Province, western China (30°40' N, 104°05' E), which is a city park using a constructed wetland system (CW) to treat polluted water from the Jin River. The sampling site is close to the park inlet, located on the bank of the Jin River, a flat riparian buffer with an area of about 100 m² (Fig. 1c, the red dashed box). Methane flux was measured once per month (in mid-month) using a portable greenhouse gas flux measurement system (WS-L1820, WEST Ltd., Italy). The sampling frequency is one time on a selected day and the sampling time was between 11:00 am and 12:00 am. There are 6 selected monitoring sites with 4 monitoring depths including surface, 5cm, 10cm and 15cm. Soil sampling and methane flux measurements are performed on the same days. After methane flux monitoring, soil samples were collected. Soil sampling sites were also same as the methane monitoring sites. At each sampling site, soil samples were excavated at depths of 0-5 cm, 5-10 cm and 10-15 cm. Gene copies of the methanogens (*mcrA*) were determined by q-PCR on the ABI 9700 Real-Time PCR system. Sequencing data was processed using the quantitative insights into the microbial ecology (QIIME) pipeline. SPSS software (version 21, SPSS Inc., USA) and the software package R (R Foundation for Statistical Computing, Austria) were used for statistical analyses and data graphing. Structural equation model (SEM) was conducted using the AMOS statistical software (version 21, IBM SPSS, USA). **Results.** During the study period, the average surface methane emission was 81.86 mg m⁻² h⁻¹ and ranged from 20.42 mg m⁻² h⁻¹ to 190.75 mg m⁻² h⁻¹. Cumulative methane emissions from studied area was 7.26 kg CO₂eq m⁻² year⁻¹ and the global warming potential (GWP) was at a moderate level. The results reveal that the Methanobacteriaceae, Methanosarcinaceae and Methanoregulaceae were the major methanogenic microorganisms in the study area. The mathematical regression of methane flux (*z*, mg m⁻² h⁻¹) with soil depth (*x*, cm) and TOC (*y*, g kg⁻¹) was as follows: $z = 52860.66 + 54.44x - 2.96x^2 - 26788.64y + 4487.80y^2 - 249.34y^3$ ($R^2=0.82$). It indicates that the relatio

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Methods. The study area is the Living Water Garden (LWG), a wetland park in Chengdu, Sichuan Province, western China (30°40' N, 104°05' E), which is a city park using a constructed wetland system (CW) to treat polluted water from the Jin River. The sampling site is close to the park inlet, located on the bank of the Jin River, a flat riparian buffer with an area of about 100 m² (Fig. 1c, the red dashed box). Methane flux was measured once per month (in mid-month) using a portable greenhouse gas flux measurement system (WS-L1820, WEST Ltd., Italy). The sampling frequency is one time on a selected day and the sampling time was between 11:00 am and 12:00 am. There are 6 selected monitoring sites with 4 monitoring depths including surface, 5cm, 10cm and 15cm. Soil sampling and methane flux measurements are performed on the same days. After methane flux monitoring, soil samples were collected. Soil sampling sites were also same as the methane monitoring sites. At each sampling site, soil samples were excavated at depths of 0-5 cm, 5-10 cm and 10-15 cm. Gene copies of the methanogens (*mcrA*) were determined by q-PCR on the ABI 9700 Real-Time PCR system. Sequencing data was processed using the quantitative insights into the microbial ecology (QIIME) pipeline. SPSS software (version 21, SPSS Inc., USA) and the software package R (R Foundation for Statistical Computing, Austria) were used for statistical analyses and data graphing. Structural equation model (SEM) was conducted using the AMOS statistical software (version 21, IBM SPSS, USA).

Results. During the study period, the average surface methane emission was 81.86 mg m⁻² h⁻¹ and ranged from 20.42 mg m⁻² h⁻¹ to 190.75 mg m⁻² h⁻¹. Cumulative methane emissions from studied area was 7.26 kg CO_{2eq} m⁻² year⁻¹ and the global warming potential (GWP) was at a moderate level. The results reveal that the Methanobacteriaceae, Methanosarcinaceae and Methanoregulaceae were the major methanogenic microorganisms in the study area. The

mathematical regression of methane flux (z , $\text{mg m}^{-2} \text{h}^{-1}$) with soil depth (x , cm) and TOC (y , g kg^{-1}) was as follows: $z = 52860.66 + 54.44x - 2.96x^2 - 26788.64y + 4487.80y^2 - 249.34y^3$ ($R^2=0.82$). It indicates that the relationship between methane flux, soil depth and TOC is non-linear and relatively high. Data analysis showed a significant vertical trend ($P < 0.05$) in methane emission fluxes at four soil depths (surface, 0-5 cm, 5-10 cm, 10-15 cm). Results of structural equation model (SEM) showed that vertical methane fluxes varied with soil depth and were mainly regulated by methanogenic community ($\lambda = 0.44$) and methanogenic diversity ($\lambda = -0.47$). Remarkably, the contribution of methanogenic gene abundance ($\lambda = -0.19$) to vertical methane fluxes was low. These results indicated that methanogenic community composition and diversity are strong predictors of vertical methane fluxes and modulate the relationship between TOC and vertical methane fluxes.

Conclusion.

In this study, we demonstrate the indirect effect of soil depth on methane fluxes. Soil depth influences vertical methane fluxes through TOC, methanogenic quantity, community structure and alpha diversity. The results of SEM show that there was a direct negative influence of soil depth, TOC, methanogens quantity and diversity on the variation of vertical methane fluxes, underlying 47%, 32%, 20% and 49% of the methane variance, respectively. And there was a direct positive effect of methanogenic community structure on methane variation, explaining 46% of the methane variation. The results further indicate that the differences in methane fluxes between soil depths are mainly due to carbon input and methanogenic community variation. Changes in soil depth could influence the structure of methanogenic community structures and methanogenesis pathways. Based on the results of this study, we claim that different soil depths have the potential to trigger profound effects on methane fluxes. The findings presented in this study add to our understanding of the vertical relationship between methane, TOC and methanogenic microcosms. In the future, it is particularly important to study in depth the relationship between methane fluxes and their abiotic and biotic influences in the vertical direction.

Key words: City Park; Methane fluxes; Methanogens; Soil Organic Carbon; Structural Equation Model.



Fig. 1 Location of riparian buffer and monitoring sites in the Living Water Garden (LWG). a Aerial photograph of the LWG (image: Google Earth, May 20, 2020). b Diagram of the monitoring sites. c Layout plan of riparian buffer (Arrows indicate the direction of water flow).

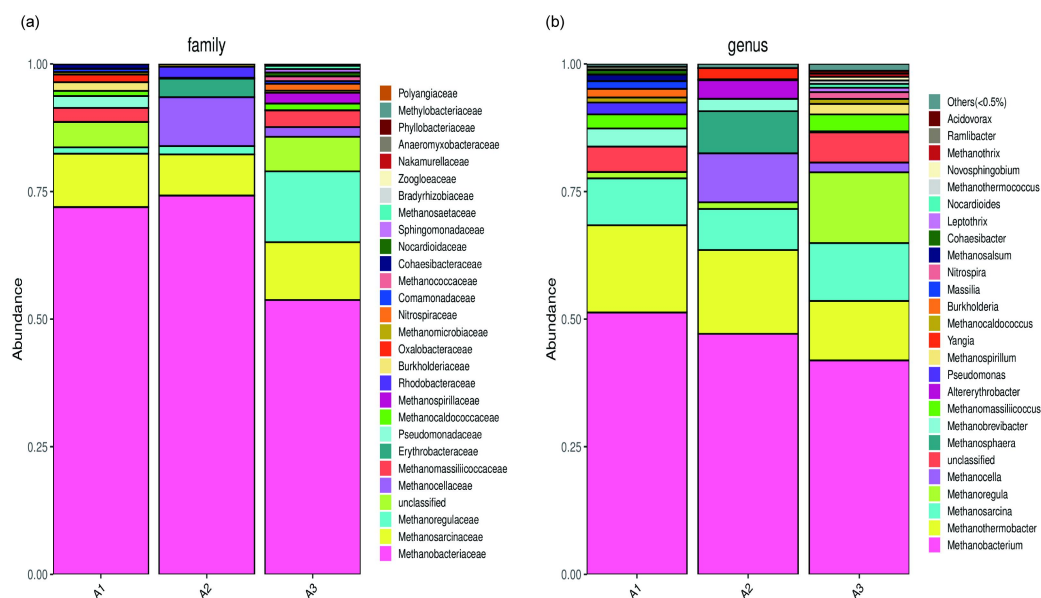


Fig. 2 Composition of the methanogenic community at different taxonomic levels (A1: 0-5cm soil depth, A2: 5-10cm soil depth, A3: 10-15cm soil depth). a family, b genus levels.

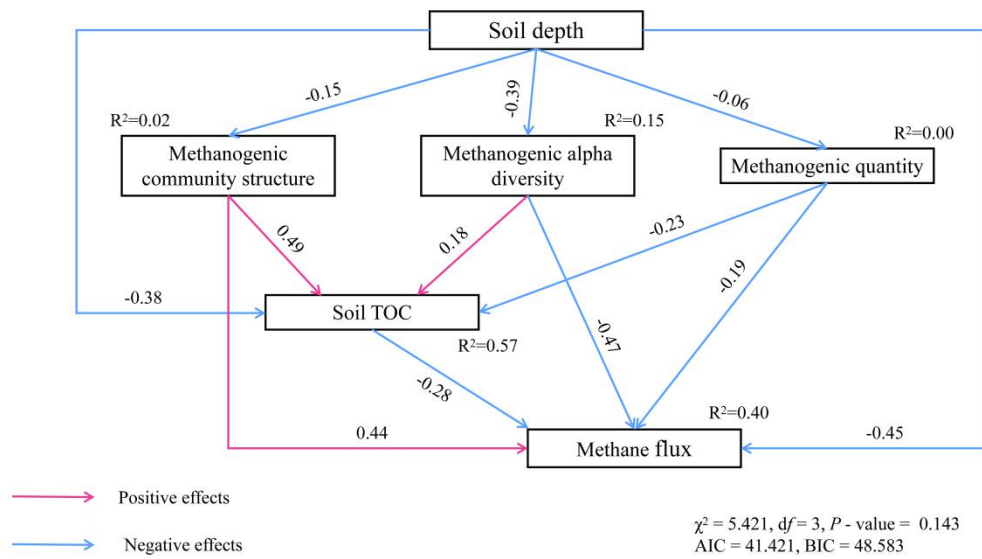


Fig. 3 Structural equation model showing the relative influence of soil factors on methane emission flux. Significant paths are shown in red if positive or in blue if negative. The amount of variance explained by the model (R^2) is shown for each response variable, and measures of overall model fit are shown in the lower right.