Soil Biology and Biochemistry 135 (2019) 10–12

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Short Communication

Ethylene rather than acetylene inhibits soil methane oxidation rates in a subtropical evergreen forest

Xuelei Bu[a,1], Sascha M.B. Krause[b,1], Xinyun Gu[a], Jianqing Tian[c], Xiaoqi Zhou[a,*]

[a] Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Center for Global Change and Ecological Forecasting, Shanghai Key Lab for Urban Ecological Processes and Eco-restoration, School of Ecological and Environmental Sciences, East China Normal University, Shanghai, 200241, China
[b] Department of Chemical Engineering and Department of Microbiology, University of Washington, Seattle, WA, 98195, USA
[c] Institute of Microbiology, Chinese Academy of Sciences, Beijing, 10080, China

ARTICLE INFO

Keywords:
Microbial processes
Inhibition
Metalloenzymes
Methane
Soils

ABSTRACT

Ethylene and acetylene have been widely applied as inhibitors for microbial processes such as denitrification, nitrification, methanogenesis, and methane oxidation. Here, we tested the inhibition effects of these two trace gases on methane oxidation rates in subtropical evergreen forest soils. One-week laboratory incubations showed that aerobic soil methane oxidation rates at ambient or 50 parts per million (ppm) methane concentration were not affected by the addition of 100 or 100,000 ppm acetylene. In contrast, increasing amounts of ethylene markedly reduced soil methane oxidation rates. Our results suggest the selective inhibition of soil methane oxidation rates between ethylene and acetylene in these subtropical evergreen forest soils, and highlight the importance of verifying the efficiency of commonly-applied inhibitors before investigating microbial processes in underrepresented environments.

Atmospheric methane (CH_4) is a key driver of global warming with a 34 × higher warming potential than carbon dioxide (CO_2) (IPCC, 2013). Atmospheric CH_4 concentrations are a result of production and oxidation processes in various environments such as wetlands, rice fields, landfills, and forest soils (Aronson et al., 2013). In particular, forest soils have been identified as contributing significantly to an estimated 2.5–7.5% global CH_4 sink (Kolb, 2009; Aronson et al., 2013).

Aerobic CH_4 oxidation is mediated by CH_4-oxidizing bacteria (methanotrophs) that use CH_4 as their main carbon and energy source. They are found within the Verrucomicrobia, NC10, Gammaproteobacteria (Type I methanotrophs), and Alphaproteobacteria (Type II methanotrophs) (Knief, 2015). All aerobic methanotrophs oxidize CH_4 via methanol and formaldehyde to CO_2 (Hanson and Hanson, 1996; Bowman, 2006; McDonald et al., 2008; Trotsenko and Murrell, 2008). The first step in this pathway is catalyzed by methane monooxygenases (MMO) (McDonald et al., 2008; Knief, 2015).

In forest soils, methanotrophic activity has been linked to high-affinity methanotrophs that can grow at low atmospheric CH_4 concentrations (Bender and Conrad, 1992; Pratscher et al., 2018). Recently Pratscher et al. (2018) used fluorescence-labeled acetylene (C_2H_2) to demonstrate a link between the high-affinity activity of CH_4 oxidation and specific methanotrophic cells *in situ*. It is well known that both C_2H_2 and ethylene (C_2H_4) have been commonly used to inhibit soil CH_4 oxidation (Prior and Dalton, 1985; Bender and Conrad, 1992; Chan and Parkin, 2000; Jäckel et al., 2004; Crombie and Murrell, 2014; Pratscher et al., 2018). However, few studies have focused on the effect of these two inhibitors on soil CH_4 oxidation in forest ecosystems (Chan and Parkin, 2000). Here, we selected soil from an evergreen subtropical forest to test the inhibition potential of C_2H_2 and C_2H_4 on CH_4 oxidation. We hypothesized that C_2H_2 has stronger inhibition effects on soil CH_4 oxidation than C_2H_4, as the molecular weight of the former is smaller and more similar to CH_4, the primary substrate of MMO.

The soil was collected from a subtropical evergreen forest in Tiantong National Forest Park, Eastern China (29°52′N, 121°39′E, 200 m above sea level). The region is dominated by a typical subtropical monsoon climate, with a mean annual temperature and precipitation of 16.2 °C and 1374.7 mm, respectively (Bu et al., 2018). We established three treatments in July 2013 (Fig. S1) (Bu et al., 2018): an ambient treatment (control), a 70% rainfall reduction treatment (drought), and a shade treatment (disturbance) to investigate the effects of drought on soil CH_4 oxidation. Soil samples from the different treatments were collected in August 2016, December 2016, and February 2017 and incubated in 1-L sealed flasks with 0, 100, or

* Corresponding author.
E-mail address: xqzhou@des.ecnu.edu.cn (X. Zhou).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.soilbio.2019.04.001
Received 6 July 2018; Received in revised form 2 March 2019; Accepted 1 April 2019
Available online 06 April 2019
0038-0717/

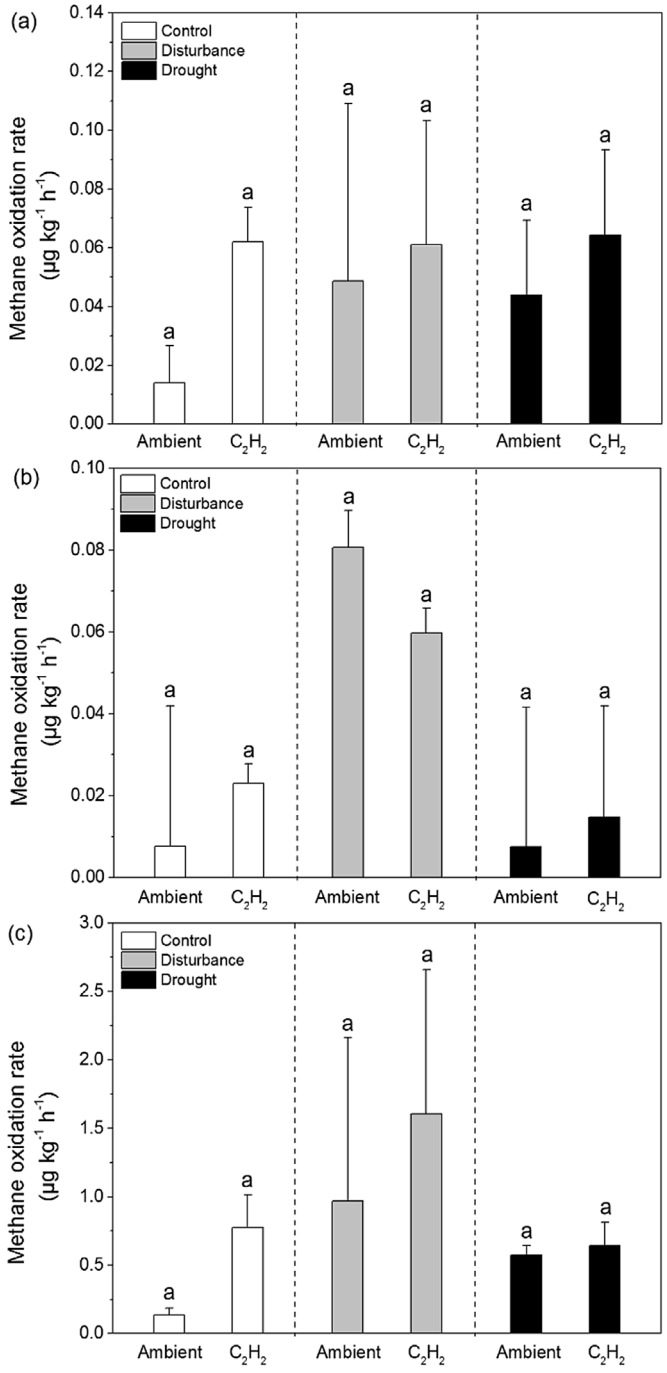

Fig. 1. Variations in the methane oxidation rate in the ambient atmosphere with or without 100 ppm C_2H_2 after 7 days of incubation in soils collected in August 2016 (a), December 2016 (b), and February 2017 (c) from a subtropical evergreen forest. Positive values indicate the soil methane oxidation rate (n = 3).

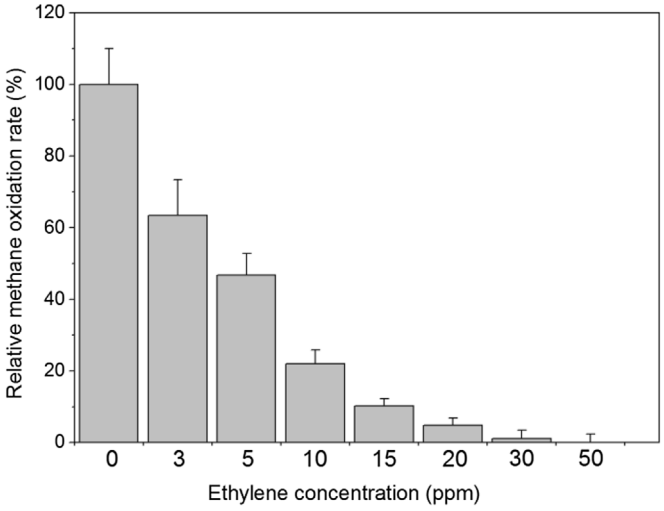

Fig. 2. Inhibition of methane oxidation by increasing the concentration of ethylene from 0 ppm to 50 ppm in soils of the control plots collected in February 2017 from a subtropical evergreen forest (n = 3).

100,000 ppm C_2H_2 and ambient air in the headspace (containing ~2 ppm CH_4) or 50 ppm CH_4 in the dark at 22 °C for 5 h or 7 days. In a second experiment, we incubated soil samples from the control sites collected in February 2017 with 3, 5, 10, 15, 20, 30, and 50 ppm C_2H_4 and ambient air in the headspace at 22 °C for 7 days to compare the effect of C_2H_4 on soil CH_4 oxidation rates. To further compare the effect of C_2H_4 and C_2H_2 on soil CH_4 oxidation rates, we also tested soil samples collected in February 2017 with 5 ppm C_2H_4 and 100 ppm C_2H_2 and ambient air in the headspace at 22 °C in 1-day incubations. Microbial community compositions were measured via *16S* rRNA high-throughput sequencing from the soil samples collected in August and December 2016. More detailed information about the experimental site and the process of data analysis are given in the Supplementary Materials.

We observed large variations in soil CH_4 oxidation rates at different sampling times during the 7-day incubations (Fig. 1). In addition, CH_4 oxidation rates were highest (approximately 10-fold) in soils collected in February 2017 (Fig. 1c). Irrespective of these changes, no significant differences in CH_4 oxidation rates were detected across all soil samples (treatments and sampling times) with 100 ppm C_2H_2 (Fig. 1). To verify these initial findings we performed additional incubations with samples from February 2017. Although the 5-h and 7-day incubations with 100,000 ppm C_2H_2 and 50 ppm CH_4 depicted similar results (Fig. S2), we found that the addition of 100 ppm C_2H_2 significantly decreased the soil CH_4 oxidation rates by 59% only at the control site in the 1-day incubations (Fig. S3).

In contrast, the addition of C_2H_4 had a remarkable inhibitory effect on soil methanotrophic activities in the 7-day incubations (Fig. 2). With 3 ppm C_2H_4 in the headspace, the soil CH_4 oxidation rate was inhibited by nearly 40%; at 10 ppm, it was inhibited by nearly 80%; it was completely inhibited at 50 ppm C_2H_4 (Fig. 2). We also observed that the addition of 5 ppm C_2H_4 produced consistent inhibition trends on soil CH_4 oxidation rates across the treatments in the 1-day incubations (Fig. S3) but with significantly lower soil CH_4 oxidation rates at the control site only (Fig. S3).

Many studies have shown that C_2H_2 can inhibit soil CH_4 oxidation rates (Prior and Dalton, 1985; Bender and Conrad, 1992; Crombie and Murrell, 2014; Pratscher et al., 2018). For instance, Prior and Dalton (1985) reported that C_2H_2 inhibited CH_4-oxidizing activity because C_2H_2 binds to the MMO at the active site. Chan and Parkin (2000) also showed that C_2H_2 had a strong inhibitory effect on the first-order rate constants of CH_4 oxidation and that 0.01% (100 ppm) C_2H_2 can completely inhibit CH_4 oxidation. We noticed that most of the previous studies examined the inhibition effects of C_2H_2 on soil CH_4 oxidation rates in 30-h incubations. Our results on the inhibition effects of soil CH_4 oxidation rates in 1-day incubations partially support these previous results, but we only observed significant decreases in soil CH_4 oxidation rates with the addition of C_2H_2 at the control site (Fig. S3). Our results indicate that the inhibition of CH_4 oxidation follows different dynamics in these soils than in those used in previous studies.

We did not find any evidence that C_2H_2 was toxic to cells because no differences in soil microbial activity were seen across the treatments and sampling times in the 1-week incubations (Fig. S4). Another reason

for the observed responses might be attributed to the binding stability between C_2H_2 and MMO. We suspect that C_2H_2 may initially have bound closely to the active site of MMO, which then resulted in significant decreases in soil CH_4 oxidation rates at the control sites in the 1-day incubations. However, the binding between C_2H_2 and MMO might become unstable over time, resulting in no marked inhibition effects of C_2H_2 on soil CH_4 oxidation rates in the 7-day incubations (Fig. 1). We did not find any other study reporting that C_2H_2 does not inhibit soil CH_4 oxidation. However, Schmidt and colleagues (2001) showed that ammonia monooxygenase (AMO), a MMO homolog, significantly reduced the C_2H_2 inhibition of ammonia oxidation activity for cells incubated in the presence of nitric oxide. They suggested that nitric oxide and C_2H_2 compete for the same binding site on the AMO. Hence, an unknown mechanism for MMO may be responsible for the observed results.

The soil microbial community that contributes to methane consumption was dominated by members of the genus *Methylobacter* (a Type I methanotroph) and the genus *Methylocella* (a Type II methanotroph) (Fig. S5). In particular, the genus *Methylocella* and its metabolic flexibility may provide a mechanism that explains the reduced inhibition potential of C_2H_2 in this subtropical evergreen forest soil. First, these organisms possess only the so-called soluble MMO (sMMO), which have been shown to be able to degrade other compounds or pollutants than the more widely distributed particulate MMO (Dedysh et al., 2005; Semrau, 2011). More importantly, Crombie and Murrell (2014) identified an additional soluble di-iron center monooxygenase (SDIMO) in the strain *Methylocella silvestris* that is involved in short-chain alkane (i.e. propane) metabolism. They demonstrated that growth on propane was completely inhibited in the presence of 2% C_2H_2. These results also suggest that the representatives of the genus *Methylcella* found in this study may contain this additional SDIMO. As a result, this enzyme may have bound C_2H_2 in our experiments, thereby allowing the sMMO to keep oxidizing CH_4. However, this mechanism still remains speculative and needs to be verified in further studies.

The observed inhibition of soil CH_4 oxidation rates by C_2H_4 in the 7-day incubations was in line with previous studies. For instance, Jäckel et al. (2004) showed that soil CH_4 uptake was nearly completely inhibited at a concentration of 10 ppm C_2H_4 in deciduous forest soil. Our previous studies confirmed that C_2H_4 acted as an inhibitor of CH_4 oxidation *in situ*, as this level of C_2H_4 concentration can be easily reached following a plant stress event (Zhou et al., 2013, 2018). We noticed that addition of 5 ppm C_2H_4 did not significantly inhibit soil CH_4 oxidation rates at the disturbance and drought sites. We observed higher *in situ* C_2H_4 concentrations at the drought sites (2.04 ± 0.75 ppm) than at the control sites (0.22 ± 0.09 ppm) (data not shown), which might suggest the resistance of soil CH_4 oxidation. We did not observe significant differences in *in situ* C_2H_4 concentrations between the disturbance and the ambient control sites, which suggests that short-term inhibition effects might occur.

In conclusion, our study showed that C_2H_2, a commonly applied inhibitor for metalloenzymes such as nitrogenase, AMO, and MMO (Hyman and Wood, 1985) did not always inhibit CH_4 oxidation rates in a subtropical evergreen forest soil. This was unexpected but highlights the importance of verifying common research methodologies in novel and underrepresented environments. It further suggests that the metabolic potential of specific methanotrophs (e.g. *Methylocella*) may be underestimated in environments where CH_4 and other plant-derived hydrocarbons may co-occur.

Declarations of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 31870497 and No. 31600406), the Shanghai Science and Technology Innovation Fund (No. 18391902300), and the Fundamental Research Funds for the Central Universities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.04.001.

References

Aronson, E.L., Allison, S.D., Helliker, B.R., 2013. Environmental impacts on the diversity of methane-cycling microbes and their resultant function. Front. Microbiol. 4, 183–189.
Bender, M., Conrad, R., 1992. Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. FEMS Microbiol. Lett. 101, 261–270.
Bowman, J., 2006. The methanotrophs—the families Methylococcaceae and Methylocystaceae. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, pp. 266–289.
Bu, X.L., Gu, X.Y., Zhou, X.Q., Zhang, M.Y., Guo, Z.Y., Zhang, J., Chen, X.Y., Wang, X.H., 2018. Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest. For. Ecol. Manag. 429, 18–27.
Chan, A.S.K., Parkin, T.B., 2000. Evaluation of potential inhibitors of methanogenesis and methane oxidation in a landfill cover soil. Soil Biol. Biochem. 32, 1581–1590.
Crombie, A.T., Murrell, J.C., 2014. Trace-gas metabolic versatility of the facultative methanotroph *Methylocella silvestris*. Nature 510, 148–151.
Dedysh, S.N., Knief, C., Dunfield, P.F., 2005. Methylocella species are facultatively methanotrophic. J. Bacteriol. 187, 4665–4670.
Hanson, R.S., Hanson, T.E., 1996. Methanotrophic bacteria. Microbiol. Rev. 60, 439–471.
Hyman, M.R., Wood, P.M., 1985. Suicidal inactivation and labelling of ammonia monooxygenase by acetylene. Biochemical Journal 227, 719–725.
IPCC, 2013. Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
Jäckel, U., Schnell, S., Conrad, R., 2004. Microbial ethylene production and inhibition of methanotrophic activity in a deciduous forest soil. Soil Biol. Biochem. 36, 835–840.
Knief, C., 2015. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on *pmoA* as molecular marker. Front. Microbiol. 6, 1346.
Kolb, S., 2009. The quest for atmospheric methane oxidizers in forest soils. Environ. Microbiol. Rep. 1, 336–346.
McDonald, I.R., Bodrossy, L., Chen, Y., Murrell, J.C., 2008. Molecular ecology techniques for the study of aerobic methanotrophs. Appl. Environ. Microbiol. 74, 1305–1315.
Pratscher, J., Vollmers, J., Wiegand, S., Dumont, M.G., Kaster, A.-K., 2018. Unravelling the identity, metabolic potential and global biogeography of the atmospheric methane-oxidizing upland soil cluster α. Environmental Microbiology 20, 1016–1029.
Prior, S.D., Dalton, H., 1985. Acetylene as a suicide substrate and active site probe for methane monooxygenase from *Methylococcus capsulatus* (Bath). FEMS Microbiol. Lett. 29, 105–109.
Schmidt, I., Bock, E., Jetten, M.S., 2001. Ammonia oxidation by *Nitrosomonas eutropha* with NO_2 as oxidant is not inhibited by acetylene. Microbiology 147, 2247–2253.
Semrau, J.D., 2011. Bioremediation via methanotrophy: overview of recent findings and suggestions for future research. Frontiers in Microbiology 2, 209.
Trotsenko, Y.A., Murrell, J.C., 2008. Metabolic aspects of aerobic obligate methanotrophy. Advances in Applied Microbiology 63, 183–229.
Zhou, X.Q., Smaill, S.J., Clinton, P.W., 2013. Methane oxidation needs less stressed plants. Trends Plant Sci. 18, 657–659.
Zhou, X.Q., Xu, C.Y., Bai, S.H., Xu, Z.H., Smaill, S.J., Clinton, P.W., Chen, C.R., 2018. Manipulating interactions between plant stress responses and soil methane oxidation rates. Biogeosciences 15, 4125–4129.