ISSN 0026-2617, Microbiology, 2022, Vol. 91, No. 5, pp. 599–603. © Pleiades Publishing, Ltd., 2022.
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SHORT COMMUNICATIONS

The Effect of Lanthanum on the Composition of Methanotrophic Community of Sod-Podzolic Soil

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Received April 4, 2022; revised April 27, 2022; accepted May 20, 2022

Abstract—While lanthanum is known to regulate the metabolism of microorganisms using single-carbon compounds, there is no information about its effect on soil communities. This is the first report on response of methanotrophic communities to the introduction of lanthanum, determined using high-throughput sequencing of the 16S rRNA genes in experiments with soil microcosms. It was found that after one and two months after the introduction of lanthanum salts the proportion of *Methylobacter* in the total pool of sequences increased (up to 9 and 15%, respectively). At the same time, the content of methylotrophic *Methylotenera* increased up to 10 and 19%, respectively. Thus, lanthanum was found to stimulate the formation of *Methylobacter*–*Methylotenera* associations under elevated methane content in the soil, which may affect the contribution of agrosoils to the regulation of methane content in the atmosphere.

Keywords: agrosoils, microbial diversity, 16S rRNA gene high-throughput sequencing, *Methylobacter*, *Methylotenera*

DOI: 10.1134/S002626172260135X

Methane is an important greenhouse gas, and although its content in the atmosphere is less than 0.02%, its contribution to current global warming is estimated at 15% (Saunois et al., 2020). The only known biological way of absorbing methane from the Earth's atmosphere is its oxidation by microbial communities in aerobic soils; therefore, any changes in the intensity of this process can have global consequences. According to modern calculations, the intensity of methane absorption by soil microorganisms is 22.4 Tg yr^{-1}, of which half is in the soils of the temperate zone (Dutaur and Verchot, 2007).

The soils of agrocenoses are under the constant influence of various compounds supplied with atmospheric precipitation, fertilizers, emissions from enterprises and vehicles (Smith et al., 2016). Soil microbial communities are responsible for the implementation of the most important ecosystem processes, which causes an ever-increasing interest in studying their resilience and recovery after stressful impacts (Griffiths and Philippot, 2013). Data on changes in the composition of methanotrophic communities during the application of fertilizers are very limited, and nitrogen compounds are most often considered. Very little is known about the effect of other elements, such as lanthanides, on soil microbial communities.

Lanthanides are included in the group of rare earth elements (REE), which are widely used in modern technologies (Vodyanitskii and Rogova, 2016). The main part of the lanthanides that enter the soil during the decomposition of lithogenic minerals is contained in organometallic complexes and is inaccessible to plants and microorganisms. (Kotel'nikova et al., 2021). At the same time, lanthanides that enter the soil as a result of anthropogenic activity (mineral and organic fertilizers, microfertilizers) are in a biologically available form and can disrupt the REE cycle in the environment (Ramos et al., 2016). In the last decade, much attention has been paid to lanthanides, especially light ones, due to their stimulating effect on plant growth (Kotel'nikova et al., 2021), while information on the effect on soil microorganisms is very limited. As an example, systematic studies of the effect of lanthanides on nitrifying and ammonifying soil microorganisms in Buryatia have been carried out for a number of years (Chimitdorzhieva and Abasheeva, 2014).

The discovery of lanthanide-dependent methanol dehydrogenase (XoxF-MDH) (Chistoserdova, 2016) demonstrated the important role of rare earth elements in the metabolism of methylotrophs. Light lanthanides, especially lanthanum (La), can effectively

replace calcium in methanol dehydrogenase. As a result, the presence of lanthanides, even in nanomolar amounts, regulates the expression of genes for the synthesis of alternative methanol dehydrogenase and affects the formation of extracellular methanol by methanotrophs, which can lead to changes in the composition of satellite microorganisms (Krause et al., 2017). This opens up prospects for developing new approaches to regulating the composition and activity of soil methanotrophs and their associated microbiota. At the same time, the authors are not aware of works on the assessment of the effect of lanthanum or other lanthanides on methanotrophic soil communities.

The aim of this study was to evaluate the effect of lanthanum salts on the composition of methanotrophic communities in experiments with soil microcosms.

In November 2020, a series of experiments was laid with sod-podzolic medium loamy soil of the surface horizon A (0–20 cm) of a fallow forb-grass meadow. The object is located near the Poshekhonskaya poultry farm, Yaroslavl oblast (58°30′36′′ N and 39°08′32′′ E), and has been used in crop rotation with the introduction of high doses of organic fertilizer for more than 20 years. The soil is moderately acidic (pH 5.4), with high content of organic matter (C_{org} 21.7 g kg^{-1} according to Tyurin), high content of nitrates (261 mg kg^{-1}), and low content of ammonium nitrogen (<5 mg kg^{-1}).

The original soil (option K) was used to create microcosms, each of which was 10 g of soil of natural moisture placed in a 100-mL glass vial. The vials were placed in a desiccator with a gas mixture consisting of 10% of methane and 90% of air for incubation for two months at 25°C. Once a week, the gas phase was replaced and the soil moisture was controlled gravimetrically. The following options were investigated: M, no additives; ML1, addition of lanthanum chloride solution (5 μg La^{3+}), incubation for one month; ML2, introduction of a lanthanum chloride solution (5 μg La^{3+}), incubation for two months. Two separate samples per variant were used for DNA extraction and molecular analysis, a total of 8 samples.

Total DNA was isolated from 0.25 g of soil using the Power Soil DNA Isolation Kit (Qiagen, Carlsbad, CA, United States). The composition of prokaryotic soil communities was assessed by high-throughput sequencing of the variable V3–V4 region of 16S rRNA gene on a MiSeq sequencer (Illumina, United States), and the initial software processing of the obtained sequences was carried out at Biospark LLC. Two libraries were created for each DNA sample. Library analysis was performed by end-paired reads generating at least 10000 paired reads per sample using the following reagents: MiSeq Reagent Kit v2 nano and MiSeq v2 Reagent Kit. The sequencing data obtained were processed in a software written using the QIIME 1.9.1 algorithm, including the combination of forward and reverse reads, deletion of technical sequences, filtering of sequences with low reading reliability of individual nucleotides (quality less than Q30), filtering of chimeric sequences, alignment of reads to the reference 16S rRNA sequence. When calculating the diversity indexes, the data were normalized according to the sample with a minimum number of reads. The distribution of sequences by OTUs using the Silva database v. 132 and the calculation of diversity indexes were performed using the QIIME 2 software (Caporaso et al., 2010). The OTU classification algorithm with an open reference was used; the classification threshold was 97%.

The analysis performed showed that the share of methanotrophic 16S rRNA gene sequences in the original sod-podzolic soil was less than 0.1% of all identified sequences. Bacteria of the genera *Methylosinus* (0.05 ± 0.01%), *Methylocystis* (0.01 ± 0.006%), and *Methylomonas* (0.004 ± 0.002%) were detected.

The incubation of microcosms with methane, as well as methane and lanthanum salts, reduced the diversity of the microbial community (Table 1), but increased the share of methanotrophs and changed the structure of the methanotrophic community. After 30 days of incubation with methane, the amount of *Methylosinus* increased to 0.38 ± 0.08%. The community also contained *Methylobacter* (2 OTUs, 0.43 ± 0.11%) and single sequences of *Methylocella* (0.03 ± 0.01%) (Fig. 1), whose number in the initial soil was below the detection level. Thus, the number of methanotrophs after 1 month of incubation increased by an order of magnitude and amounted to about 1% of the total number of sequences.

The introduction of lanthanum fundamentally changed the number and composition of methanotrophs. One month later, the share of 16S rRNA gene sequences in methanotrophs reached 9.2%, of which *Methylobacter* constituted 8.97 ± 0.9% (3 OTUs) and *Methylosinus* 0.10 ± 0.08% (Fig. 1). The effect of lanthanum was prolonged, and after two months the share of *Methylobacter* gene sequences increased to 15.28 ± 3.2% (6 OTUs) and that of *Methylosinus* to 0.19 ± 0.1% (Fig. 1). All *Methylobacter* OTUs belonged to *M. tundripaludum* (98.12–99.06% identity of the 16S rRNA gene with the type strain), while *Methylosinus* OTUs belonged to *M. sporium* (99.24% identity) (Table 2).

The methylotrophs of the genus *Methylotenera* assigned to the class *Methylophilaceae* (Fig. 1) formed consortiums with methanotrophic bacteria in shallow methane seeps (Danilova et al., 2021) and lake sedi-

Table 1. Characteristics of the diversity of microbial communities in sod-podzolic soil and soils of the incubation experiment

Sample	Characteristics of libraries of the 16S rRNA gene fragments		Number of OTUs	Number of genera (phyla)	Diversity indexes				
	number of sequences	number of nucleotides in sequences			Chao 1	Shannon	Simpson	Pielou	Faith's PD
K	10715 ± 740	412 ± 12	1546	421 (21)	1794	9.09	0.99	0.90	5.9
M	10666 ± 486	417 ± 10	904	290 (16)	1183	8.08	0.99	0.89	5.6
ML1	5230 ± 620	409 ± 12	1046	203 (16)	1213	7.81	0.98	0.86	3.8
ML2	5966 ± 322	421 ± 8	906	218 (16)	1226	7.66	0.98	0.91	3.4

ments (van Grinsven et al., 2021). Two months later, the share of *Methylotenera* 16S rRNA gene sequences exceeded 19 ± 1.30% (Fig. 1). All of them belonged to *M. versatilis* (Table 2) showing 98.12–98.46% identity of 16S rRNA with the isolate from the bottom sediments of Lake Washington, United States (Lapidus et al., 2011). The methylotrophic bacteria *Hyphomicrobium facile* (2 OTUs, 0.4 ± 0.2%) were also found in the variants without the introduction of lanthanum.

Thus, it has been established for the first time that the introduction of lanthanum in the soil of the temperate climatic zone against the background of an increase in the methane content, which is typical of agricultural soils with the application of organic and mineral fertilizers, leads to the absolute dominance of methanotrophs of the genus *Methylobacter* and obligate methylotrophs of the genus *Methylotenera*. The coordinated response of *Methylobacter* and *Methylotenera* to the addition of lanthanum salt suggests

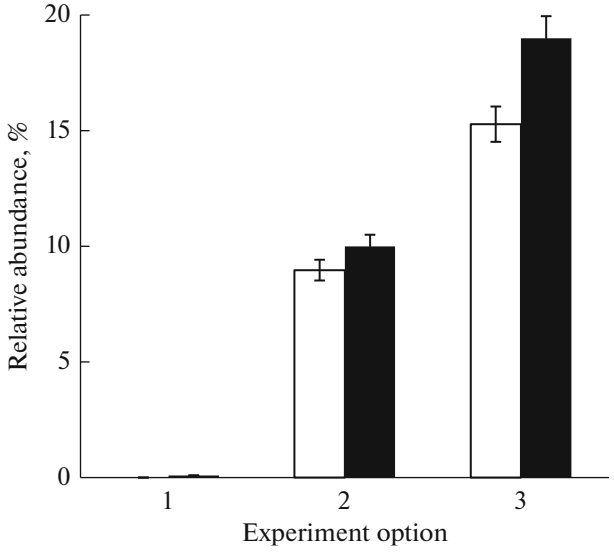

Fig. 1. Indicators of the relative abundance of the dominant methanotrophic bacteria *Methylobacter* (white columns) and methylotrophic bacteria *Methylotenera* (black columns) in the soil samples of the microcosm experiment. Designations of experiment options: 1, M; 2, ML1; 3, ML2.

Table 2. Characteristics of the main OTUs of methanotrophic and methylotrophic bacteria obtained as a result of molecular analysis of soil microbial communities in an incubation experiment

OTU	NCBI accession number	Representation in the community, %	Closest relative (NCBI number)	16S rRNA coverage, %	16S RNA identity, %
M—incubation with methane, 1 month					
S21-25	ON109140	0.23	Uncultured *Methylobacter* sp. clone (JX505341, AJ414655)	100.0	100.0
			Methylobacter tundripaludum SV96^T (NR_042107)	100.0	99.05
S21-21	ON109141	0.20	Uncultured *Methylobacter* sp. clone (JX505341, AJ414655)	100.0	99.05
			Methylobacter tundripaludum SV96^T (NR_042107)	100.0	98.12
S21-41	ON109142	0.38	Uncultured bacterium clone (JF135670)	100	100
			Methylosinus sporium (MT229167)	100	99.24
S21-979	ON109143	0.03	Uncultured *Methylocella* sp. clone OTU16 (MW143589)	100	98.48
			Methylocella tundrae isolate MTUNDRAET4	100	97.49
ML1—incubation with methane and lanthanum, 1 month					
S21-278	ON109144	5.31	Uncultured *Methylobacter* sp. clone (JX505341)	100	100
			Methylobacter tundripaludum SV96T (NR_042107)	100	99.06
S21-185	ON109145	3.54	Uncultured *Methylobacter* sp. clone (JX505341)	100	99.76
			Methylobacter tundripaludum SV96T (NR_042107)	100	98.82
S21-299	ON109146	5.71	Uncultured *Methylotenera* sp. clone 11(KX365915)	100	99.77
S21-173	ON109147	3.31	Uncultured *Methylophilaceae* bacterium clone 320 (MF042682)	100	99.76
S21-14	ON109148	0.27	*Hyphomicrobium* sp. strain TWH1 (MK124972)	100	100
S21-131	ON109149	0.13	Uncultured *Hyphomicrobiaceae* bacterium clone C (JX505000)	100	100
			Hyphomicrobium facile (Y14312)	100	99.25
ML2—incubation with methane and lanthanum, 2 months					
S21-507	ON109150	8.55	Uncultured *Methylobacter* sp. clone (JX505341)	100	100
			Methylobacter tundripaludum SV96T (NR_042107)	100	99.06
S21-359	ON109151	6.05	Uncultured *Methylobacter* sp. clone (JX505341)	100	99.76
			Methylobacter tundripaludum SV96T (NR_042107)	100	98.82
S21-472	ON109152	12.86	Uncultured *Methylotenera* sp. clone 11 (KX365915)	100	99.77
			Methylotenera versatilis isolate (MW010428)	100	98.12
S21-121	ON109153	2.04	*Methylotenera versatilis* isolate (MW010428)	100	98.46

their joint activity in the regulation of methane exchange between the soil and the atmosphere, which requires further study.

FUNDING

This study was supported by the Russian Science Foundation, project no. 22-24-00418.

COMPLIANCE WITH ETHICAL STANDARDS

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

Statement of the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

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Translated by E. Babchenko