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METHANE OXIDATION AND BIOGEOCHEMICAL PARAMETERS OF GRAY FOREST SOIL AS INFLUENCES BY FALLOW-TO-WOOD SUCCESSION

Research article

Abstract

The succession of microbial community structure and function is a central ecological topic, as microbes drive the Earth's biogeochemical cycles and are responsible for the regulation of the gaseous composition of the atmosphere, the processes of soils formation and their resistance to natural and anthropogenic factors. To elucidate the response and mechanistic underpinnings of soil methane-oxidizing microbial community structure and metabolic potential relevant to natural forest succession on gray forest soils of Moscow region from farming through the fallows of different ages (5-25 years) to a forest biocenosis was studied. Highest methane oxidation was observed from older restored sites it's recovery with restoration age. Molecular analyses indicate the presence of aerobic type I and type II groups of methanotrophs in arable and meadow sites and only type II methanotophs in forested sites. Collectively, these data indicate shifts in microbial populations associated with methane oxidation in the context of soil restoration age, and provide significant insights into the response of these microbial populations to severe disturbance and recovery.

Keywords: natural reforestation, bacterial diversity, community composition, methane oxidation, methanotrophs.

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ВЛИЯНИЕ ЛЕСОВОССТАНОВИТЕЛЬНОЙ СУКЦЕССИИ НА ОКИСЛЕНИЕ МЕТАНА И БИОГЕОХИМИЧЕСКИЕ ПАРАМЕТРЫ СЕРЫХ ЛЕСНЫХ ПОЧВ

Аннотация

Изучение сукцессионных изменений структуры и функции микробных сообществ является центральной темой экологии, так как микробы осуществляют биогеохимические циклы Земли и отвечают за регулирование газового состава атмосферы, процессов формирования почв и их устойчивости к природным и антропогенным факторам. Для того, чтобы выяснить чувствительность и основные механизмы регуляции состава и метаболического потенциала метанокисляющего микробного сообщества, был изучен сукцессионный ряд объектов серой лесной почвы Московской области, включающий агрокультуру, залежи различного возраста (5-25 лет) и лесной биоценоз. Наибольшее окисление метана наблюдали на участках поздних стадий сукцессии. Молекулярные исследования выявили наличие аэробных метанотрофов I и II типа в пахотных и луговых участках и только метанотрофов II типа в лесном участке. В совокупности эти данные демонстрируют значительные изменения микробных популяций, отвечающих за окисление метана, в зависимости от срока восстановления почвы, и являются ценной информацией для оценки реакции этих микроорганизмов на серьезные экологические нарушения и восстановление активности.

Ключевые слова: естественное лесовосстановление, бактериальное разнообразие, видовой состав, окисление метана, метанотрофы.

1. Introduction

Ecological succession is one of the fundamental topics of ecology, and it can be initiated from two different conditions: primary and secondary [1]. Secondary succession starts after disturbances, which do not completely destroy the organic substrate and after which succession can initiate from the remaining vegetation, soil, seed and microbial bank. Microorganisms are the main biological drivers of many soil functions and properties and their metrics has been suggested be used as indicators of change in ecosystems [2]. For example, the effect of land use changes can be assessed using bacterial community abundance and composition [3], enzymatic activity [4], uptake and formation of greenhouse gases [5]. Measurements based on evaluation of microbial function activity may be the most appropriate to determine the status of the terrestrial ecosystems. Because of sensitivity to changes in land status, as well as their role in soil biogeochemical processes bacterial communities, their diversity and functional activity, have been found to be useful as environmental bioindicators [6]. But here has not been such a study that demonstrates the utility of diversity and functional status of soil microbial communities as indicators of the reforestation.

Methane (CH_4) is important greenhouse gas, and its atmospheric concentration is rising due to natural and anthropogenic disturbances such as forest clearing, land-use changes and farming practices. CH_4 oxidation by forest soils has been studied extensively because these soils represent a major part of the soil sink in the global CH_4 budget [7]. It is well documented that forest soils are the more active for methane consumption, followed by grasslands and cultivated soils and various factors associated with agriculture have been shown to inhibit atmospheric CH_4 oxidation, including soil compaction, acidification and fertilization [8].

It may be proposed that the restoration of degraded forest ecosystems would significantly contribute to the recovery of methanotrophic activity in the soil and the soil CH_4 sinks potential. There is clear evidence that reforestation could be the potential strategy to mitigate the excess CH_4 emissions. Based on data from the previous studies, abandonment of agriculture can lead to at least partial recovery of methanotroph populations and atmospheric CH_4 uptake [9].The reforestation or restoration of degraded forests can be an effective tool of environmental management, which addresses the global concern for rising methane concentrations in the atmosphere due to enhanced methanotrophic bacteria activity.There is a need to formulate an integrated approach to study the response of temperate ecosystems, with a special emphasis on soil methanotrophs dynamics.

Our objective was to characterize activity and composition of methane oxidizing communities and biogeochemical parameters in the sites from the same soil type but with different stages of natural reforestation. It was hypothesized that the soil bacterial communities would be ecologically distinct in their diversity and functional activity.

2. Methods

The sampling sites were located about 100 km South of Moscow, Russia, near Pushchino town (54.8ºN , 37.6ºE) on the right site of the Oka river. The soil type is Gray Forest Soil, a loamy-clay soil (GleyicPhaeozems). Soil samples were collected in August 2016 from nine plots. Soil sampling and preparation for analysis were done as described in [10]. Samples were analyzed for pH with a Mettler TOLEDO pH meter (Seven Easy pH, Switzerland). For soil organic carbon (SOC) and nitrogen (SON) , air-dried soils were analyzed using the dry combustion method in a Vario EL III CHNS Elemental Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). NO_3 -N and NH_4 -N were measured by photocolorimetric method with phenolate hypochlorite reaction.

The content of active (potentially mineralizable) soil organic matter (AOM) at the initial moment of incubation was evaluated in incubation experiments by technique described in [11]. The biokinetic parameters C_0 and k were calculated by the nonlinear estimation method using Statistica 6.0 software. The content of total organic carbon (C_{org}) in the soil was determined with a Leco 932 CNHS analyzer. The biological stability index (BSI) of OM (BSI = (C_{org}– C_0)/C_0) was calculated.

Potential methane oxidation activity was evaluated in bath incubation experiments according to a previously described protocol [10]. Detection of methanotrophs in soil samples was done via analysis of the *pmoA* gene, which encodes a ß- subunit of the particulate methane monooxygenase (pMMO), using primer sets A189/A682, A189/mb661 and A189/A650[12], 2017). In situ hybridization with fluorochrome labeled oligonucleotide probes (FISH) was done as described in [13].

3. Results and discussion

Geochemical data showed remarkable variability among individual sites. Soil pH among the land types showed significance differences between wood soil (W) and agromodified soils ($p < 0.01$), and lowest pH was evaluated in wood site (Table 1). Significant differences were also observed in soil carbon content, and it was more than three times higher in wood soil as compare with arable soil (A) (Table 1). W site not only showed the highest carbon content, but also the highest N-NH_4+ and lowest P_2O_5 content as well. Soils in later succession sites share similar related organic matter, nitrate, and ammonium contents (Table 1) than sites at early stages of recovery.

Table 1 – Characteristics of the field sites and physicochemical soil properties

Soil ID	Site, coordinates	Land management, vegetation	pH (H_2O)	C_{tot}, (%)	N_{tot}, (%)	C_{org}, mg/kg SOC	N-NH_4^+, mg\kg	N-NO_3^-, mg\kg	NO_3 + NH_4, mg\kg	P_2O_5, mg\kg	K_2O , mg\kg,	Ca^{2+}, mmol\ kg	Mg^{2+}, mmol\ kg
W	N 54°49'05.1" E 37°35'39.3"	Wood, secondary forest	5.29	2.48	0.16	153.5	7.6	7.2	14.80	46.2	141.0	224.0	6.0
F10	N 54°49'06.4" E 37°35'38.8"	Fallow, re-forested	5.99	2.42	0.16	84.5	2.9	4.1	7.00	196.0	117.0	293.0	7.0
F7	N 54°49'05.4" E 37°35'44.4"	Fallow, meadow	6.05	2.56	0.17	104.3	3.4	4.7	8.10	226.7	124.6	276.0	5.0
A1	N 54°49'05.4" E 37°35'44.4"	Agrocenosis, vetch-oat mixture	6.58	2.53	0.16	46.9	2.6	4.4	7.00	241.7	112.5	301.0	5.0
F5_1	N 54°49'52.0" E 7°30'22.3"	Fallow, meadow	5.82	2.36	0.15	59.1	2.5	12.0	14.50	154.0	77.5	292.0	11.0
A2	N 54°49'29.5" E 37°33'45.7"	Agrocenosis, vetch-oat mixture	6.4	2.49	0.16	55.7	1.8	6.80	8.60	246.2	207.5	354.0	6.0
F15	N 54°49'38.6" E 37°33'57.2"	Fallow, re-forested	5.68	2.25	0.14	70.7	1.9	2.2	4.10	143.6	170.5	246.0	10.0
F25	N 54°49'39.2" E 37°33'58.2"	Fallow, re-forested	5.62	2.38	0.15	94.1	4.3	3.5	7.80	87.2	137.0	220.0	6.0
F5_2	N 54°49'43.1" E 37°34'03.0"	Fallow, meadow	6.08	2.18	0.13	84.3	2.1	6.7	8.80	58.6	139.0	260.0	8.0

Arable soils generally contain less AOM than uncultivated soils, which agrees with earlier data. As a result, the supply of arable soils with AOM is lower than that of their uncultivated analogues and corresponds to the low (75–35 mg C/100 g) level. A strict linear relationship between the contents of total (C_{org}) and active OM is revealed. This shows that AOM is the transformable component of soil organic matter (SOM), and the decrease of AOM is an indicator of upcoming humus depletion. The ability of SOM to be mineralized by microorganisms is illustrated by three parameters: the share of AOM in total SOM (C_0, % of C_{org}), the biological stability index (BSI), and the constant of SOM mineralization rate (k, days^{-1}). In the studied soil series, 2.7–5.19% of C_{org} can be mineralized with mineralization constants of 0.0675 to 0.0874 days^{-}1 (Table 2).

Table 2 – Active organic matter in soils of ecosystems from the different stages of postagrogenic succession

No	Site	Cumulative C-CO_2 production, mr C/100 g for 150 days	Soil organic matter		Active soil organic matter		*k*, days^{-1}	BSI**
			C_{opr}, %	C_{opr}, мg /100 g	C_{opr}, %	C_{org}, мg/100 g		
1	W	139.78±0.44*	2.53±0.07*	2528	5.19	131.29±0.21*	0.0675±0.0003*	18
2	F10	90.91±1.45	2.42±0.00	2418	3.55	85.72±1.42	0.0751±0.0011	27
3	F7	102.77±0.96	2.57±0.02	2568	3.75	96.23±0.81	0.0874±0.0008	26
4	A1	73.58±0.48	2.55±0.03	2548	2.70	68.73±0.34	0.0765±0.0015	36
5	F5_1	71.97±1.04	2.34±0.03	2335	2.92	68.23±1.12	0.0735±0.0023	33
6	A2	57.76 ± 1.28	2.63 ± 0.20	2628	2.05	53.86 ± 1.29	0.0705 ± 0.0022	48
7	F15	73.84±0.60	2.32±0.10	2315	3.00	69.51±0.59	0.0729±0.0010	32
8	F25	101.43±0.82	2.40±0.04	2403	3.97	95.30±0.81	0.0721±0.0011	24
9	F5_2	68.86±1.52	2.18±0.01	2180	2.98	64.91±1.82	0.0804±0.0053	33

Notes: *Mean± standard deviation. ** Biological stability index.

In contrast to the share of potentially mineralizable carbon in C_{org}, the mineralization constant is a less variable parameter of SOM mineralization capacity We determined the BSIs of SOM, which show what fold the content of resistant carbon exceeds that of potentially mineralizable carbon and their values were from 18 to 48 (Table 2).

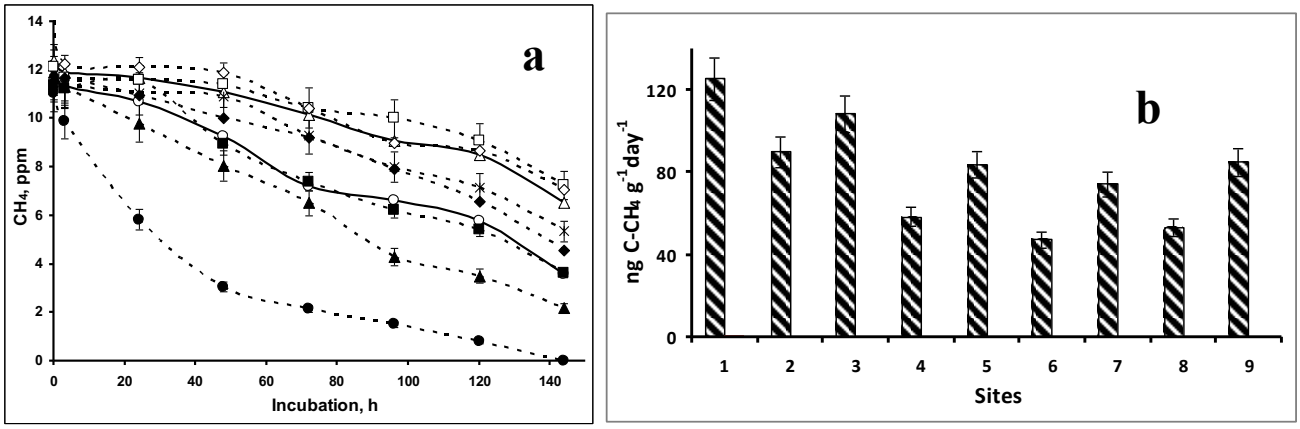

Figure 1 – Dynamics of methane uptake (a) and methane oxidation activity (b) in different soil samples. For site ID see Table 2. The results are the means of three independent replicates, and error bars on each point represent the standard errors. Abbreviations: ●-1; ○-2; ▲-3; Δ-4; ■-5; □-6; ♦-7; ◊-8; *-9.

As microorganisms play key roles in nutrient cycling and other important functions in soils, the shifts in microbial communities caused by land-use change might directly affect the functioning of ecosystems, such as biogeochemical cycles.

We have chosen the atmospheric methane oxidation as an example of an important biospheric cycle involving microbial processes, and investigated the activity and abundance of methane-oxidizing bacteria in soil. When soils samples were incubated with 12 ppm CH_4, oxidation started immediately, but the rate of increase in oxidation was low initially. The most rapid oxidation was observed in soils from W site, which was significantly different from all other soil samples and after 144 h of incubation, almost all methane was consumed (Fig 1 a). In other samples from 20 to 80% methane was oxidized. The potential methane oxidation activity was the highest in W sample and lowest in A sample – 5.2 and 2.4 ng C-CH_4 g^{-1} h^{-1}, correspondently (Fig.1b).

PCR analysis revealed the presence of *pmoA*- possessing methanotrophs in studies soils (Fig 2). Amplification with A189/A650 primer system targets usual *pmoA* and A189/mmb661R is known to target not conventional, including uncultured and *amoA* was successful. No product of the correct size was observed with mmoX206-882R primers encoding soluble methane monooxygenase (sMMO) as far as USCα, USCγ or other clusters of uncultured methanotrophs (A189-689R) were not detected (Fig.2). Thus it was concluded that sMMO possessing and previously described groups of uncultured methanotrophs are likely to provide the minor contribution to atmospheric methane oxidation in the studied soils.

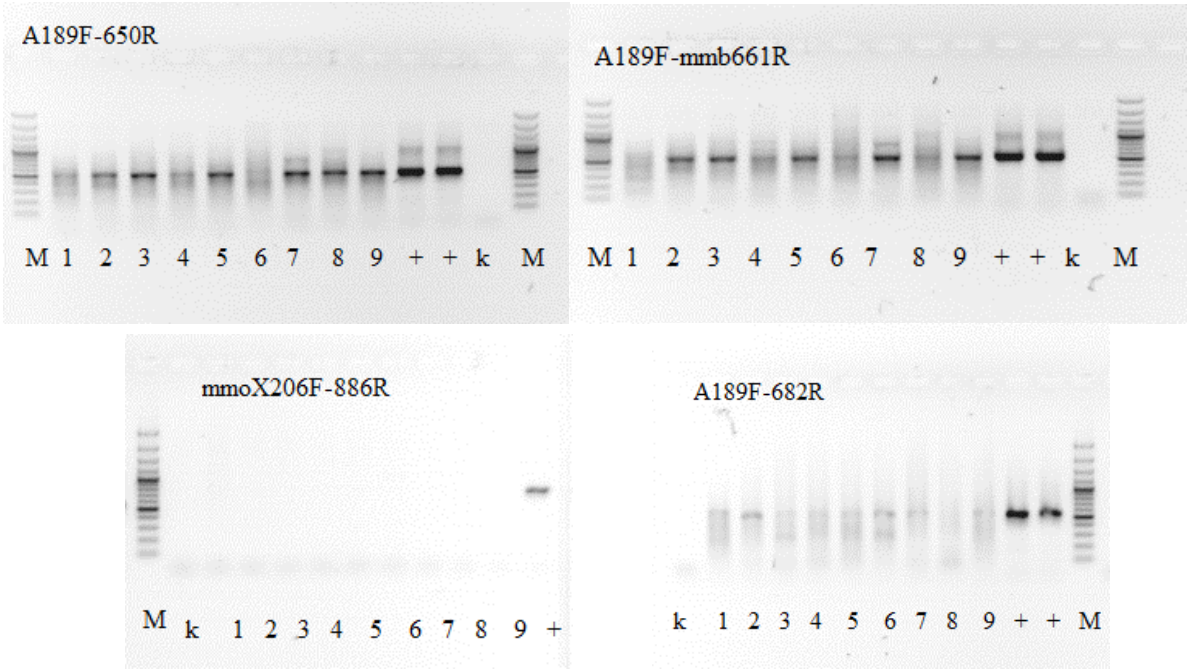

Figure 2 – PCR detection of methanotrophic bacteria in soil samples. Abbreviations: k- negative control (without DNA), + - positive control, *Methylocystis* sp., 1-9 as described in Table 2.

Quantitative real-time PCR (qPCR) and fluorescence *in situ* hybridization (FISH), has been applied to study native microbial populations in soils. In W soil, the copy numbers of bacterial 16S rRNA and *pmoA* genes were found to be 8×10^8 and 32×10^6g soil $^{-1}$, correspondingly. In agrimodified soils numbers of ribosomal genes were significantly lower ($P < 0.05$), and the number of *pmoA* genes was much lower than those in wood soil (Table 3). The number types I and II active methanotrophs obtained by

FISH varied from 36 to 98 × 10^5 cells g^{-1} of soil and methanotrophs of type II dominated over type I (Table 3). Methanotrophs proportions in relation to total active bacterial cells are 0.2-03%.

Table 3 – Quantitative analysis of microbial communities in soil from experimental plots determined by quantitative PCR and direct microscopy methods

No	Soil sample ID	No. of gene copy (10^5 g-^{1} soil)				No. of cells (10^5 g $^{-1}$ soil)			
		16s rRNA-specific qPCR			*pmoA*-specific qPCR	DAPI-staining	FISH		
		All Bacteria	Type I methano-trophs	Type II methano-trophs			EUB 338mix	M-84 +M705	M-405
1	W	8000	32	320	320	9400	89	1	30
2	F10	8000	24	160	160	9100	95	3.3	13
3	F7	4000	16	120	200	5700	84	3.7	14
4	A1	8000	16	120	160	7800	44	3.4	9.6
5	F5_1	3600	12	80	120	4200	96	1.7	5
6	A2	8000	28	120	160	8100	58	8	14
7	F15	8000	16	160	80	9300	59	11	13
8	F25	12000	40	280	240	10400	54	14	11
9	F5_2	8000	40	nd	160	8200	31	5	15

4. Conclusion

In this work we showed that succession changes have a primary effect on the abundance, structure and activity of soil methanotrophic communities. Although the soil type is the same for the nine land use systems, they are clearly distinct in physicochemical parameters, vegetation cover and historical use. We suggest that the differences found in microbial communities are result of variability in land-use caused by management practices, which affect soils. This work is of major contribution to understand how changes in soil properties due to secondary forest succession have altered the activity and diversity of methane-oxidizing communities and promoted a better view on how these communities respond to anthropogenic disturbances.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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