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EFFECT OF LANTHANUM ON NITRIFYING ACTIVITY AND COMPOSITION OF MICROBIAL COMMUNITIES OF SOD-PODZOLIC AGROSOIL

Research article

Abstract

Microbial communities of soils carry out the most important ecosystem processes of the cycle of biogenic elements, which causes an ever-growing interest in the study of their activity and diversity. One of these processes is nitrification, as a result of which plants receive nitrogen in an accessible form. In laboratory experiments with soil microcosms of sod-podzolic agrosoil, the stimulating effect of lanthanum on the nitrification process was established. The addition of lanthanum salt stimulated the potential nitrifying activity, and after 19–24 days the nitrate nitrogen content was 24–30% higher compared to the lanthanum-free variant. For the first time, using high throughput 16S rRNA sequencing, it was found that the introduction of lanthanum salts reduces the taxonomic diversity of the bacterial community of the soil and leads to significant changes in its structure. A decrease in the relative content of gram-positive bacteria of Actinobacteria and Firmicutes was noted in the microbial community of soils, while the proportion of Proteobacteria increased. The results obtained require further studies of the mechanisms of the influence of lanthanum on the microbial processes of ammonium oxidation in the soil and ways of their regulation. The data can be used to determine effective and safe doses of ammonium fertilizers and lanthanum-containing micronutrients in the conduct of modern environmentally oriented agriculture in central Russia.

Keywords: nitrification, lanthanum, microbial communities of soils.

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ВЛИЯНИЕ ЛАНТАНА НА НИТРИФИЦИРУЮЩУЮ АКТИВНОСТЬ И СОСТАВ МИКРОБНЫХ СООБЩЕСТВ ДЕРНОВО-ПОДЗОЛИСТОЙ АГРОПОЧВЫ

Научная статья

Аннотация

Микробные сообщества почв осуществляют важнейшие экосистемные процессы круговорота биогенных элементов, что вызывает постоянно растущий интерес к изучению их активности и разнообразия. Одним из таких процессов является нитрификация, в результате которой растения получают азот в доступной форме. В лабораторных экспериментах с почвенными микрокосмами дерново-подзолистой агропочвы установлено стимулирующее воздействие лантана на процесс нитрификации. Внесение соли лантана стимулировало потенциальную нитрифицирующую активность, и через 19–24 суток содержание нитратного азота было выше на 24–30% по сравнению с вариантом без лантана. Впервые методом высокопроизводительного секвенирования 16S рРНК установлено, что внесение солей лантана снижает таксономическое разнообразие бактериального сообщества почвы и приводит к значительным изменениям в его структуре. Отмечено снижение относительного содержания грамположительных бактерий филумов Actinobacteria и Firmicutes в микробном сообществе почв, в то время как увеличивается доля Proteobacteria. Полученные результаты требуют проведения дальнейших исследований механизмов влияния лантана на микробные процессы окисления аммония в почве и способов их регуляции. Данные могут быть использованы для определения эффективных и безопасных доз аммонийных удобрений и содержащих

лантан микроудобрений при ведении современного экологически - ориентированного сельского хозяйства в средней полосе России.

Ключевые слова: нитрификация, лантан, микробные сообщества почв.

1. Introduction

Lanthanides (Ln) include 15 elements with atomic numbers from 57 (lanthanum, La) to 71 (lutetium, Lu), possessing great similarity in chemical and physical properties, which is explained by the close structure of the outer electronic levels of their atoms. Lanthanides are divided by atomic mass into light (La, Ce, Pr, Nd, Sm, Eu) and heavy (Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) [1].

In the last decade, much attention has been paid to lanthanides due to their stimulating effect on plant growth and participation in the metabolism of microorganisms. The entry of lanthanides into the soil is associated with the destruction of lithogeny minerals during soil formation, as well as using phosphorous flour as a fertilizer. However, it should be noted they are mainly in an inaccessible form − as part of the mineral phase and complexes with humus compounds. In a number of countries, water-soluble salts of lanthanides are used as micro-fertilizers to increase the yield and quality of agricultural crops [2]. Developments in this direction are also being carried out in our country, in particular in Buryatia and encouraging results have been obtained [3].

The total content of rare-earth elements in soils ranges from 30 to 700 micrograms/g, which is comparable with the content of such common heavy metals as cadmium, copper or lead [4]. The soils of the forest regions of Russia, developing on loamy deposits in a humid climate, are characterized by a weak participation of lanthanides in soil-forming processes and the absence of their accumulation in the upper horizons of soils. This is facilitated by the acidic reaction of the soil solution (pH 4,5-5,5), the washing regime and the predominance of fulvic acids in the humus.

Information on the effect of lanthanides on soil microorganisms is very limited. For the soil of tea plantations, it has been shown that high doses of lanthanum (>600 mg/kg) reduce the content of C and P in the soil microbial biomass and enzymatic activity but increase soil acidity [5]. High doses of lanthanum salts (1000 mg/kg) inhibited the dehydrogenase and respiratory activity of soil microorganisms [6]. At the same time a positive effect on the number of ammonifying and nitrifying bacteria was shown for the Buryatia soils when the moderate doses of lanthanum sulfate (3 and 6 mg La/kg) were applied [3].

Lanthanides have recently been proven to be involved in biological processes when it was first shown that they act as a coenzyme in methanol dehydrogenase of methylotrophs [7]. Currently, intensive studies are being conducted on the biological role of lanthanum and the participation of lanthanum-containing enzymes in the metabolism of multi-carbon compounds [8].

The aim of the study was to evaluate the effect of lanthanum salts on the nitrifying activity and diversity of microbial communities in sod-podzolic soil.

2. Methods

For experiments with microcosms, we used sod-podzolic medium loamy soil in accordance with the classification of soils of Russia (horizon A, 0–20 cm) from a fallow grass meadow near Poshekhonskaya poultry farm, Yaroslavl region. A mixed sample was taken from the surface horizon in June 2019. The soil-forming rock is represented by moraine deposits. The soil is moderately acidic (pH 5,4), with a high content of organic matter (S_{org}. according to Tyurin, 19,3 – 21,7 g/kg), high nitrate content (261 mg/kg) and low ammonium nitrogen content (<0,05 mg/l of aqueous extract). Prior to the experiments, the soil was stored at 4°C.

Microcosm experiment was started in the November 2019. 10 grams soil samples with a moisture content of 25% were placed in 100 ml vials. The following variants were used in the experiment: *control* – without additives; *NH_4^+* -with ammonium sulfate (100 μg N/g), *La*- with ammonium sulfate and lanthanum chloride (100 μg N/g + 5 μg La/g). Soil samples were incubated for a month at 28°C, with the maintenance of constant soil moisture. On a regular basis, a part of the samples was taken and measured for ammonium and nitrate content on the pX-150.1MI ionomer with ion-selective electrodes. Soil DNA was isolated at the beginning and the end of the experiment to determine the taxonomic structure of the bacterial community using Illumina high-performance sequencing of the 16S rRNA gene (LLC BIOSPARK, Troitsk, Moscow region). With the help of QIIME 2 software packages we have combining nucleotide sequences in OTE with a similarity level of 97% and calculated the diversity indices. Alpha diversity was assessed by the Shannon index, the alignment of OTE in the community − according to the Pielou index. As an indicator of phylogenetic diversity, the Feit index PD was used.

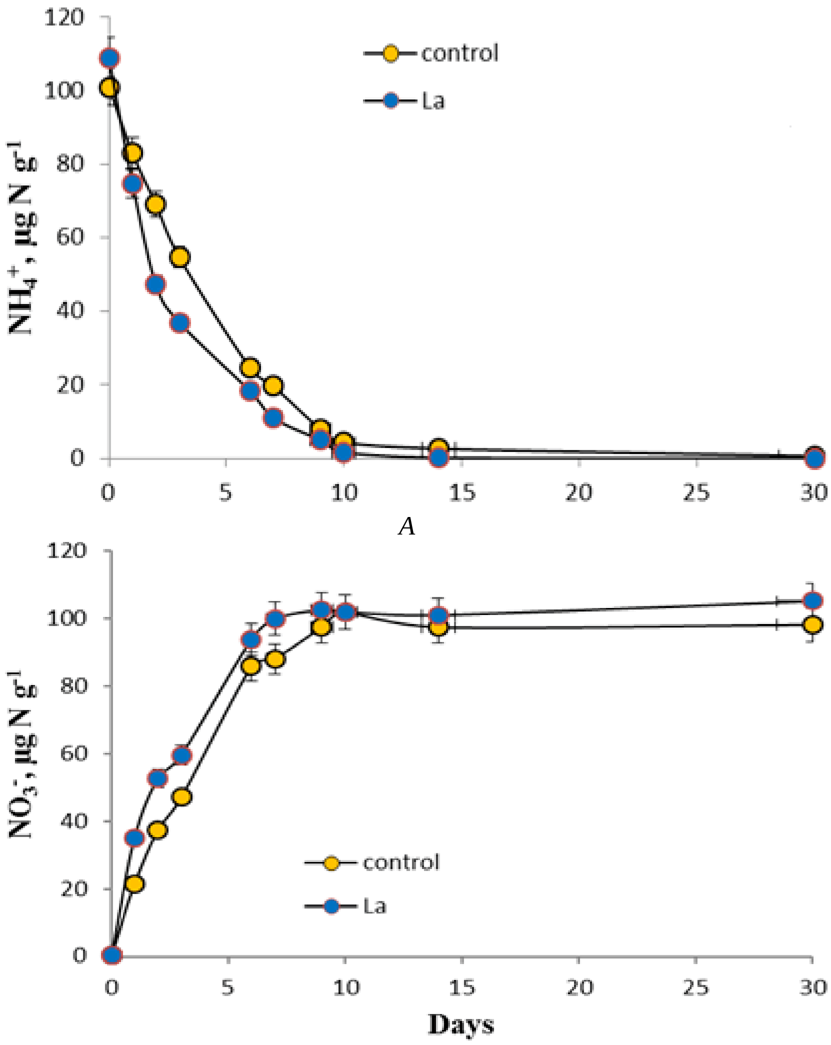
The data are presented as averages over three samples. In all studies, the observed effects of treatment were considered statistically significant at p<0.05. The statistical analysis was carried out using Excel (Microsoft Office Excel 2011).

3. Results and discussion

The sod-podzolic soil had a moderately acidic reaction of the medium (5,4 pH). The data of ammonium and nitrate ions content in the soil solution of soil microcosms are shown in Figure 1. The initial nitrate content (261 μg/g) was twice as high as the maximum permissible concentration of nitrates in the soil (130 μg/g), which is probably due to the prolonged use of high doses of organic fertilizer in the fields of the Poshekhonsky poultry farm.

In the first week of incubation, an increase in soil acidity was observed (data are not provided), since the introduced compounds are physiologically acidic salts. During the first week of incubation, the pH decreased from 5,4 to 4,9 in the soil with ammonium sulfate and to 4,8 in the soil with ammonium sulfate and lanthanum chloride, while in the control, the pH value of the water dropped only to 5,2. This was probably due to the inhibition of the nitrification process in the first days of the experiment. Because of the buffer properties of soil e, by the 19th day, the acidity level returned to the initial level and during further incubation was within the values of 5,2–5,5.

With an increase in the pH of the soil solution, an increase in the activity of the nitrification process was activated (Fig.1). In the soil with ammonium, the nitrate content increased significantly: on the 19th day, the concentration was 49 µg N-NO_3^-/g in the soil with ammonium sulfate and 56 µg N-NO_3^-/g in the soil with ammonium and lanthanum. The stimulating effect was observed until the end of incubation, when 105 µg N-NO_3^-/g were determined in the soil with lanthanum chloride, and 96 µg without it.



B

Fig. 1 – Dynamics of the ammonium decrease (*A*) and accumulation of nitrate (*B*) in experiments to assess potential nitrifying activity

Thus, in the presence of lanthanum the accumulation of nitrates increased by approximately 24%. It is interesting to note that in some soil microcosms the content of nitrate nitrogen exceeded the amount that should have been obtained by oxidation of the introduced ammonium. Perhaps this is a manifestation of the so called "priming effect", in which a large amount of ammonium readily available for oxidation stimulated the nitrifying activity of microorganisms and allowed them to also consume the nitrogen of soil organic matter.

Thus, lanthanum stimulates the processes of ammonium oxidation and nitrate accumulation; however, it must be borne in mind that when applying lanthanum chloride, soil acidification is possible, which inhibits nitrification processes for a period depending on the buffer properties of the soil. For sod-podzolic soil, the inhibition period was about 7 days

The relative content of the bacterial phyla in the soil before and after incubation is shown in Figure 2. In the control variant the dominants were Proteobacteria (48%) and gram-positive bacteria Actinobacteria (20%) and Firmicutes (8%). In addition, Acidobacteria (7%), Bacteroidetes (5%), Chloroflexi (5%), Gemmatimonadetes (3%), and Verrucomicrobia (2%) also made a significant contribution (Figure 1 Control). The contribution of less than 1% was made by representatives of Chlorobi, Cyanobacteria, Elusimicrobia, Fibrobacteres, Latescibacteria, Nitrospirae, Planctomycetes and Saccharibacteria.

When salts were added, Proteobacteria also dominated in soil bacterial communities, but their proportion increased: in soil with lanthanum, their relative content was 66% and with ammonium was 55%. Acidobacteria (9–13%) and Bacteroidetes (8–11%) were in the second and third places in terms of relative abundance in these communities, respectively. Compared with the control soil, the contribution to the community made by the bacteria of the phylum Actinobacteria (4–5%) and Firmicutes (2–4%) significantly decreased. The proportion of Chloroflexi decreased to a lesser extent (1%) and the proportion of Gemmatimonadetes (5–7%) and Verrucomicrobia (2–4%) increased. Minor components were represented by Armatimonadetes, Chlorobi, Cyanobacteria, Elusimicrobia, Latescibacteria, Nitrospirae, Parcubacteria, Planctomycetes, Saccharibacteria, Spirochaetae, Tenericutes.

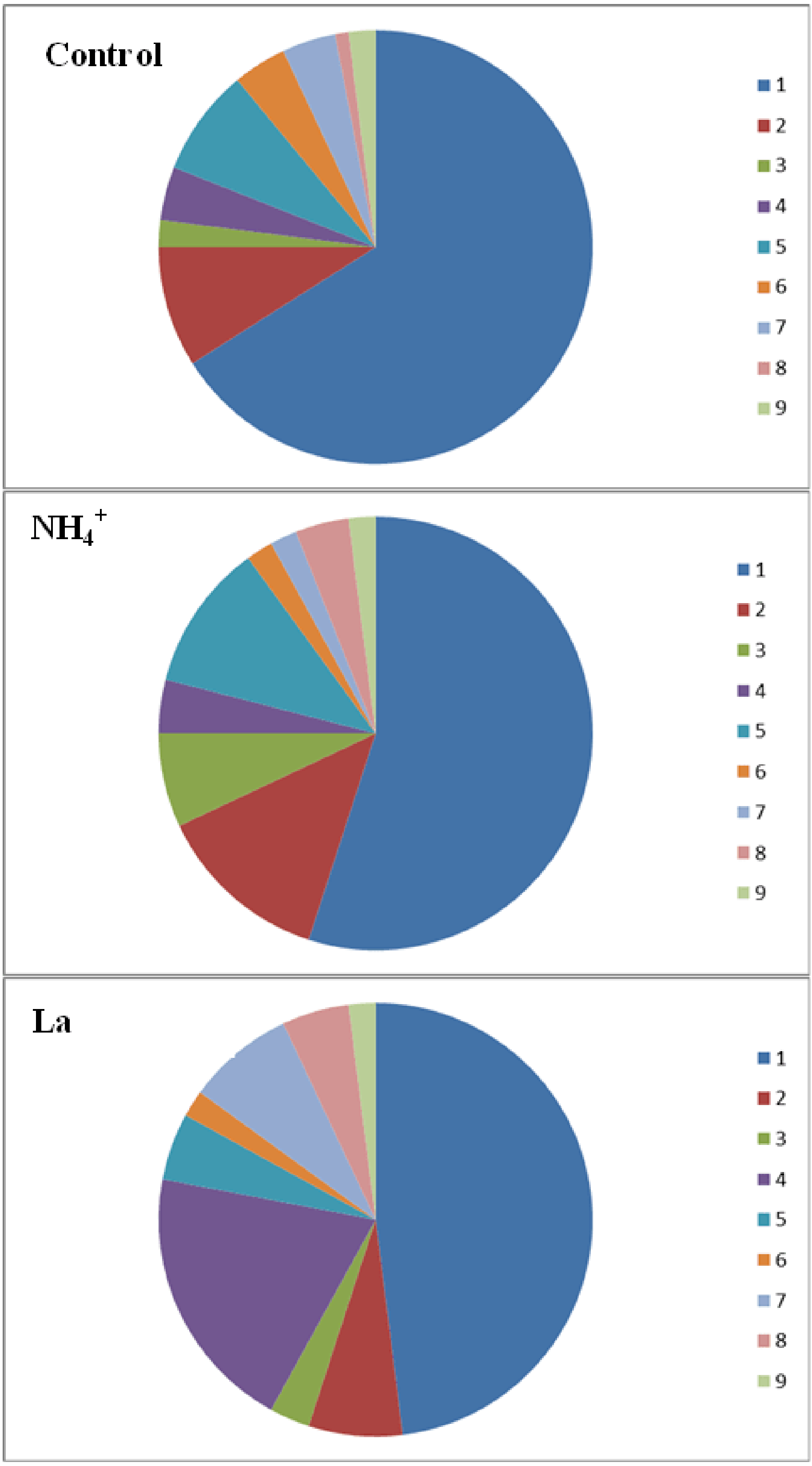


Fig. 2 – The structure of soil microbial communities at the phylum level with a representation of more than 1% in the microbial community:
1 – Proteobacteria; *2* – Acidobacteria; *3* – Gemmatimonadetes; *4* – Actinobacteria; *5* – Bacteroidetes; *6* – Verrucomicrobia; *7* – Firmicutes; *8* – Chloroflexi; *9* – Others

To determine the effect of ammonium and lanthanum salts on the alpha diversity of the bacterial community of sod-podzolic soil, we compared the number of phyla and OTE defined before the genus. In the QIIME 2 program, the indices of Pielu alignment, Shannon diversity and phylogenetic diversity of Feit PD were calculated for OTE with a similarity level of 97% (Table 1).

In the community of the control soil, 290 genera from 16 phyla were identified, which is significantly less than in the native soil (421 genera, 21 phyla). The addition of lanthanum chloride further reduced diversity: a month after the introduction, only 203 genera of the 16 phyla was identified. The negative effect of lanthanum salt on diversity was confirmed by the values of the Shannon diversity indices (4-4,2) and PD (3,4-3,8), which were significantly lower relative to the same values in the control soil (Shannon index = 4,9; PD = 5,6). In addition, the value of taxon alignment in the community decreased from 0,89 to 0,86 during the month. When incubated with ammonium sulfate, the diversity also decreased, but to a lesser extent. In the bacterial community, 228 genera from 17 phyla were identified; the diversity according to the Shannon index (4,8) was at the same level as in the control (4,9). The value of the alignment indicator even increased from 0,89 to 0,93. At the same time, the phylogenetic diversity index of Feit decreased from 5,6 to 4,2, which indicates that a larger number of taxa began to be relatively close to each other on the phylogenetic tree. In this case, the main diversity of taxa was concentrated within the phylum Proteobacteria, whose contribution to the community has grown greatly. The results obtained are in line with the other studies in which a decrease in the diversity of the bacterial community was found when applying mineral fertilizers [6], pesticides [9] and in general when cultivating the soil [10]. Apparently, at the same time, the established combination of ecological niches is violated, and the relative abundance of specific groups of microorganisms grows due to those who have not adapted to the new conditions.

Table 1 – Characteristics of the microbial community’s diversity in soddy-podzolic soil and soils of the laboratory microcosm experiment

Parameter	Control	NH_4	La
Number of genera (phyla)	290 (16)	228 (17)	203 (16)
Pielou index	0,89	0,93	0,86
Shannon index	4,9	4,8	4,0
Faith’s PD index	5,6	4,2	3,8

4. Conclusion

Based on the conducted experiments, it can be concluded that ammonium sulfate and lanthanum chloride, which enter the soil with nitrogen (mineral and organic), phosphorous (phosphorites) and lanthanum-containing fertilizers, have a significant impact on the soil microbial community.

The introduction of ammonium sulfate into the soil in an amount corresponding to the dose widely used in agriculture (100 μg of $N{-}NH_4^+$/g = 300 kg of $N{-}NH_4^+$/ha) leads to an increase in the proportion of Proteobacteria in the bacterial community up to 55%. A similar but stronger effect was shown with the addition of lanthanum chloride, when the Proteobacteria community share was 66%. The results obtained require further research aimed at studying the possible mechanisms of the influence of lanthanum on the processes of ammonium oxidation in the soil; determining the factors influencing the dominance of certain microorganisms in the community in natural conditions; investigation of the dependence of the number of bacteria in ecosystems on their function. The results of the work can contribute to the development of modern approaches for determining safe doses of ammonium fertilizers and lanthanum-containing micronutrients in agriculture in central Russia. The characteristics of microbial communities (activity, diversity) can be used as informative indicators of the response of soil biota to anthropogenic impact. The insufficient amount and discussion of the available information on the effect of ammonium and lanthanum salts on microbial communities of soils requires further research in model experiments with soil microcosms simulating the natural habitat. Such studies with a further transition to field experiments will help to eliminate gaps in existing knowledge.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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