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BIODIVERSITY OF SHALLOW SOLONETS BACTERIAS, OCCUPIED LONGLY WITH CROP ROTATION WITH *BROMUS INERMIS* (POACEAE)

Research article

Abstract

Soil salinization is a growing global environmental problem today. It is possible to cultivate such soils by cultivating biomeliorant herbs. This aspect has been studied in Western Siberia (where 40% of the territory is occupied by saline soils) using the example of a shallow medium sodium solonetz, which has been occupied by the forage crop *Bromus inermis* Leyss for more than 30 years. *Bromus Inermis*, referring to grasses with high ecological plasticity and salt tolerance, has been sown in a crop rotation since 1987: field one is sown with millet, field two is sown with millet and bromegrass, and fields 3-6 are sown with bromegrass. Such long-term cultivation of *B. inermis* transformed the shallow solonetz and altered the metagenomic diversity of microorganisms. The taxonomic composition of the bacterial domain was determined in the 0-15 cm soil layer in comparison with virgin soil by the method of high-throughput sequencing of the 16S rRNA gene V3-V4 region. Total DNA was isolated from the samples using the DNeasy PowerSoil Kit (Qiagen), alpha and beta diversity was analyzed by Usearch v11.0.667. The cultivation of Bromus inermis in a forage crop rotation for 30 years led to the formation of a specific soil metagenome. It differs from the virgin solonetz shallow in taxonomic richness and uniformity. Under the influence of seeded cereals, the number of OTUs Gemmatimonadetes in the microbiome increased (they are considered indicators of dryness), the abundance of genera from the Proteobacteria class increased by 40%, and there were significantly fewer representatives of the main virgin phylum *Actinobacteria* (1.7 times). The first representatives indicate a decrease in moisture content of hydromorphic solonetz soil. The second representatives show an improvement in soil nitrogen exchange.

Keywords: solonetz, microbiome, phytomelioration, *Bromus inermis*, 16S r-RNA.

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БИОРАЗНООБРАЗИЕ БАКТЕРИЙ СОЛОНЦА МЕЛКОГО, ДЛИТЕЛЬНО ЗАНЯТОГО СЕВООБОРОТОМ С *BROMUS INERMIS* (POACEAE)

Научная статья

Аннотация

Засоление почв на сегодняшний день – растущая глобальная экологическая проблема. Окультурить такие почвы можно путем возделывания трав-биомелиорантов. Этот аспект изучен в Западной Сибири (где 40% территории занято засоленными почвами) на примере солонца мелкого средненатриевого, более 30 лет занятого кормовой культурой *Bromus inermis* Leyss. Кострец безостый, относящийся к травам с высокой экологической пластичностью и солеустойчивостью, с 1987 года высевается в севообороте: 1 поле – просо, 2 поле – просо и кострец, 3-6 поля – кострец. Такое длительное возделывание *B. inermis* трансформировало солонец мелкий и изменило метагеномное разнообразие микроорганизмов. Таксономический состав домена бактерий определен в слое почвы 0-15 см в сравнении с целиной методом высокопроизводительного секвенирования последовательностей участка V3-V4 гена 16S рРНК. Тотальная ДНК из образцов выделена с помощью набора DNeasy PowerSoil Kit (Qiagen), альфа- и бета-разнообразие проанализировано Usearch v11.0.667.

Возделывание *Bromus inermis* в кормовом севообороте в течение тридцати лет привело к формированию специфичного почвенного метагенома. От целинного солонца мелкого он отличается таксономическим богатством и выравненностью. Под влиянием сеяного злака в составе микробиома увеличилось число OTUs Gemmatimonadetes (их считают индикаторами сухости), на 40% возросло обилие родов из класса Proteobacteria и стало существенно меньше представителей основного филума целины *Actinobacteria* (в 1,7 раза). Первое указывает на снижение влажности гидроморфной солонцовой почвы, второе – на улучшение в почве азотного обмена.

Ключевые слова: солонцы, микробиом, фитомелиорация, *Bromus inermis,*16S р-РНК.

1. Introduction

Microflora is a key determinant of soil characteristics and biogeochemical functions of terrestrial ecosystems [1], [2]. Its contribution to the productivity of phytocenoses and the ecological functions of the soil depends on the biodiversity, abundance, and functional activity of soil microorganisms [3]. For the development of soil microflora of solonetz soils, according to the M. Beijerinck's ecological rules, two points are important: the impact of salts and the specificity of the plant community.

Solonetz soils often attract researchers in the field of soil microbiology. Sharp gradients of conditions make them convenient objects for studying the factors affecting the composition and structure of the microbiome. Salinity determines the dominance of prokaryotes of the phyla *Actinobacteria, Bacteroidetes,* and *Proteobacteria*, the representation of *Firmicutes* bacteria and archaea *Thaumarchaeota* [4], [5], [6], [7]. However, the role of vegetation cover is also important in the formation of soil microbiome features [9], [10], [11]. Changes in bacterial communities caused by the type of plant association can affect soil formation, the cycle of the main elements of carbon and nitrogen and have a desalinating effect on the soil [12], [13], [14]. In this paper, the cultivation of *Bromus inermis* Leyss., 1761 will be considered as the main ecological predictor of ameliorative effects. The purpose of the study is to reveal how the prokaryotic community of the shallow solonetz was transformed under the influence of thirty years fodder crop rotation with awnless brome.

2. Materials and methods of research

The diversity of microorganisms was studied in the Baraba steppe at the solonetz station of the Siberian Scientific Research Institute of Fodder Crops of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (55.389º N, 78.927º E). The object of the study was the shallow solonetz (high-columnar, medium-sodium soda-sulfate type of salinity of heavy granulometric composition) of virgin soil, as well as the solonetz transformed by crop rotation with awnless brome for 33 years. Soil tillage under crop rotation: milling to a depth of 8–10 cm in layers (or disking with heavy harrows) once per rotation and moldboard loosening to a depth of 30–35 cm. A sort of awnless brome - SIBNIISKhoz 189. Soil samples for analysis were taken on August 5, 2020, from a layer of 0-15 cm.

The taxonomic affiliation of bacteria was revealed by high-throughput sequencing of the sequences of the V3-V4 region of the 16S rRNA gene. The analysis was performed at the Center for Collective Use "Genomics" of the Institute of Chemical Biology and Fundamental Medicine SB RAS. Total DNA was isolated from the samples using the DNeasy PowerSoil Kit (Qiagen). Samples were sequenced on a MiSeq instrument (Illumina, USA), OTU (operational taxonomic units) sequences were assigned to taxa using SINTAX [15], α- and β-diversity was analyzed by Usearch v11.0.667. The results obtained were processed mathematically. Dispersion analysis was performed using the SNEDECOR computer program.

3. Results

The biological diversity of microorganisms in the solonetzic soil was rather strongly corrected by the sown perennial grass. In the virgin lands, the phyla Bacteria decreased in percentage representation in the following order: *Actinobacteria* (30.3%) > *Acidobacteria* (25.1%) > *Proteobacteria* (13.4%) > *unc_Bacteria* (10.5%) > *Verrucomicrobia* (7.2%) > *Gemmatimonadetes* (5.8%) > *Bacteroidetes* (4.4%) > *Chloroflexi* (1.6%) > *Armatimonadetes* (1.7%) > *Candidatus Saccharibacteria* (0.6%). In the shallow solonetz transformed by a perennial grass, the priority of the dominant phyla changed in comparison with the virgin soil (figure 1). Significant differences according to the Mann-Whitney U test (from $p < 0.05$) between this variant and virgin soil were observed for the phyla *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *unc_Bacteria*, *Gemmatimonadetes*.

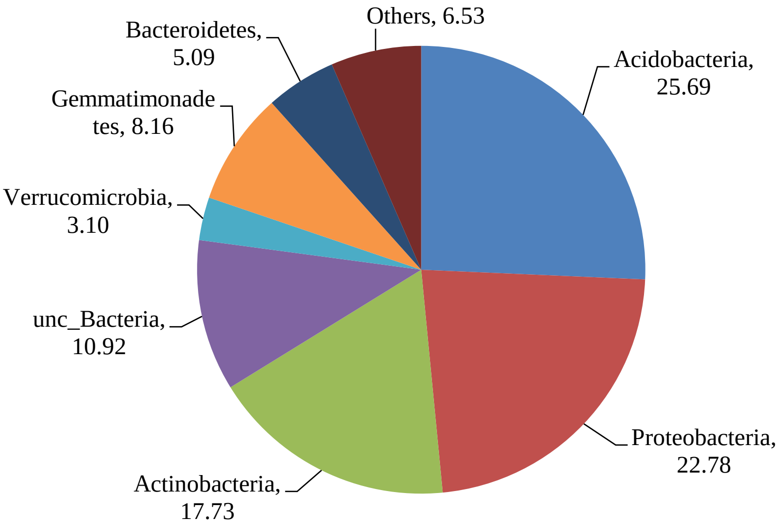

Fig.1 – Dominant phyla in the microbiome of shallow solonetz transformed by crop rotation with brome (0-15 cm layer)

Under the influence of brome, the proportion of the phylum *Proteobacteria* in the bacterial community increased by 40%. Its representatives are copiotrophs and are associated with improved soil fertility [16]. The content of active hydrolytics of the *Actinobacteria* phylum under the brome became 1.7 times lower. Fila *Verrucomicrobia* left the 5th place in the list of dominants, moving to 7th. Its representatives are mainly classified as oligotrophic flora, which strongly responds to an increase in soil organic matter [17], [18]. Therefore, a decrease in the abundance of these microorganisms indirectly indicates the enrichment of the soil with nitrogen. In the phytomeliorated soil, the seeding by the *Gemmatimonadetes* group has significantly increased. Due to the percentage increased to 8.2, it rose to the 5th place in the list of dominants. The latter indicates the partial drying of the solonetz under the phytomeliorant culture, as the gemmatimonadetes seed the soil more strongly in arid conditions [19]. The species representing the remaining phyla of the domain and designated in the figure as Others were revealed in the transformed solonetz by 2 times more than in the virgin soil.

The general differences in the state of the bacterial community in the reclaimed solonetz from the virgin solonetz are well illustrated by the indices of α-diversity (table 1). The Berger-Parker index, which shows the degree of dominance (and at the same time is insensitive to slight differences in biota) in the bacterial community changed by phytomelioration, increased by 2.5 times. The Chao 1 index, which estimates the total number of taxa in the community, increased by 1.2 times; the Shannon index, which describes the evenness of bacterial cenosis along with species richness, increased by 1.1 times. Based on this, it can be assumed that the community of prokaryotes formed in the solonetze by long-term cultivation of *B. inermis* became more complex than in the shallow virgin solonetz.

Table 1 – Characterization of the bacterial community of virgin solonetz and solonetz phytomeliorated with B. inermis through diversity indices

Diversity indicies	Shallow virgin solonetz	Solonetz phytomeliorated with B. inermis
Berger-Parker index	0,04 ±0,006	0,1 ±0,006
Chao 1 index	2360,4 ±264,9	2734,8 ±178,9
Shannon index	5,8 ±0.1	6,3 ±0,06

4. Conclusion

Cultivation of *Bromus inermis* in fodder crop rotation for thirty years led to the formation of a specific soil metagenome. It differs from fine shallow virgin solonetz in taxonomic richness and uniformity. Under the influence of seed cereals, the number of OTUs Gemmatimonadetes in the microbiome increased (they are considered indicators of dryness), the abundance of genera from the Proteobacteria class increased by 40%, and there were significantly fewer representatives of the main virgin phylum *Actinobacteria* (1.7 times). The first indicates a decrease in the moisture content of the hydromorphic solonetzic soil, the second indicates an improvement in nitrogen metabolism in the soil.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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