

Influence of topsoil manure mix on affects germination in Pea and Onion seeds

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Abstract. Nutrient-rich southern chernozems have lost a significant part of their fertility during their exploitation in agrocenoses. The decrease in the fertility of the surface layer of soils in the steppe landscapes of the Orenburg Cis-Urals contributed to a decrease in the yield of grain crops and an increase in the volume of applied mineral fertilizers. The use of reverse technology of mechanical tillage contributed to the weathering of the surface fertile soil layer and its degradation. Plowing of the Orenburg steppes for agrocenoses led to a decrease in the number of wild ungulates and their adaptation to new living conditions in agrocenoses. Our work was devoted to modeling the process of the influence of the soil-manure mixture of the surface layer of southern chernozem and the manure of large phytophages on the germination of pea and onion seeds in laboratory conditions. Changes in the composition of bacterial communities during the preparation of the soil-manure mixture are one of the factors influencing the germination and morphometric growth parameters of onion and pea seeds. There is no doubt that the nutritional composition of the manure of large phytophages is of decisive importance for stimulating the growth of onions and peas. However, the differences we have shown in the bacterial composition of soil-manure mixtures of two large phytophages raises the problem of increasing or decreasing the nutritional value of the soil-manure mixture depending on the composition of the microbial communities of the manure of large phytophages.

1 Introduction

The fertile Orenburg steppes were plowed during the development of virgin lands in the 60s of the 20th century. The southern chernozems, rich in nutrients, have lost a large amount of fertility accumulated over decades during their exploitation in agrocenoses. The decrease in the fertility of the surface layer (horizon A) of the soils of the steppes of the Orenburg Cis-Urals has led to the fact that today a good harvest of grain crops depends on the use of various mineral fertilizers. In addition, the use of reverse technology during mechanical tillage, in conditions of winds and low humidity, led to the degradation of the surface fertile soil layer. The scale of development of large steppe spaces for agrocenoses has led to a

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decrease in the number of wild ungulates and their adaptation to new living conditions in agrocenoses. Despite the fact that the introduction of large steppe phytophages has important environmental significance, predictive models of the impact of the introduction of large phytophages on the steppe ecosystem, affecting individual trophic levels, have not yet been developed. Finally, the introduction of large phytophages into steppe ecosystems will help enrich the soil with organic matter and increase fertility. Succession of microbial communities of the surface soil layer, with an increase in organic load, can not only contribute to an increase in the biodiversity of soil microbial communities, but also lead to the activation of steppe phytocenoses. However, we are not talking about restoring the previous steppe ecosystem, but about restoring trophic connections between all components of the ecosystem after the loss of such an important component as large ungulates. All these facts contribute to the fact that the introduction of large phytophages is often perceived as one of the components of the sustainability of steppe ecosystems (<http://rewildingeuropa.com>). The appearance of large phytophages in the steppe affects not only the carbon cycle, but also the plant and animal biodiversity of the steppe. The manure of wild phytophages has different nutritional value (Irshad et al., 2013), which is associated not only with the plant preferences of a given animal species, but also with the ability of the animal's intestinal microbial community to ferment the consumed plant matter. To predict the situation with an increase in organic load on the surface layer of soil, we conducted a long-term experiment to assess the possible succession of the soil bacterial community and the influence of the soil-manure mixture of large phytophages on the growth rate of onion and pea seeds [1 - 5].

Therefore, the purpose of our study was to assess the influence of a soil-manure mixture of Bactrian camel (*Camelus bactrianus*) and Tibetan Yak (*Bos mutus*) on the germination and morphometric properties of onion and pea seeds

2 Statement of the problem

The surface layer of soil or the horizon A is the most fertile layer of soil. However, as a result of plowing steppe soils and using them for agrocenoses, it led to depletion of the surface layer of soil. One of the ways to enrich the surface layer of soil is to enrich it with manure of large phytophages, which occurs under natural grazing conditions. The work is devoted to the analysis of the influence of the soil-manure mixture of large phytophages introduced into the steppe ecosystem on the germination of onion and pea seeds.

3 Solution method

Using soil-manure mixture as a substrate for growing crops of onions and peas. Assessing changes in the composition of microbial communities and the dominant groups of microorganisms in the soil-manure mixture will contribute to the analysis of the profitability of introducing large phytophages into the steppe ecosystem to increase the fertility of the surface soil layer.

4 Object of study

The object of the study is the surface layer of soil (southern chernozem) as a soil-manure mixture from the manure of large phytophages of Bactrian camel (*Camelus bactrianus*) and Tibetan Yak (*Bos mutus*). As a control, we used the surface (150 mm) layer of southern chernozem, taken at a distance of 150 mm from the test sample.

5 Materials and Methods

5.1 Soil

The surface layer of soil was collected on the territory of the «Orenburg Tarpaniya» station. The soil was represented by southern chernozem. To take soil samples, sterile (1.5 atm; 30 min) aluminum samplers with a diameter of 25 mm and a length of 150 mm were used.

5.2 Manure of large steppe phytophages

The manure of large phytophages (*Bos mutus* and *Camelus bactrianus*) was collected from animal pens, dried and crushed. In the experiment it was used in the form of a fine-grained powder.

5.3 Growing onion and pea seeds

The soil-manure mixture was prepared in a ratio of 60:40. Sprinkled into holes (50 mg) into which pea or onion seeds were sown. For planting, we used seeds of onion (*Allium cere*) of the «White Feather» variety and peas (*Pisum sativum*) of the «Premium» variety. The cultivation of onion and pea seeds was carried out in laboratory conditions at a temperature of 22-26°C, watering regime of 20 ml after 48 hours, in natural light for 20 days.

5.4 Isolation of total DNA and bioinformation processing

The soil and soil-manure mixture were homogenized, diluted with sterile water (1 g/10 ml) and filtered through 0.22 µm membrane filters. The filters were stored in the refrigerator at -20°C for 24 hours until samples were sequenced. Total DNA was isolated from the filters using a combined method, which included mechanical homogenization in combination with the enzymatic lysis method (Belkova, 2009). 400 µL of Tris-buffered saline (20 mmol/L EDTA, 750 mmol/L NaCl, 100 mmol/L Tris-HCl; pH 8.0) was added to the samples and homogenized using a TissueLyser LT homogenizer (QIAGEN, Germany) using a lysis agent. matrix E («MP Biomedicals», USA) for 1 min at a frequency of 50 Hz. After this, 50 µl of Tris-buffered saline with lysozyme (50 mg/ml) was added and incubated for 60 min at 37°C. Then a 10% sodium dodecyl sulfate solution was added to the mixture to a final concentration of 1% and 2 µl of a proteinase K solution (10 mg/ml) and incubated for 60 min at 60°C. After extraction with a mixture of phenol-chloroform-isoamyl alcohol (25:24:1) and subsequent extraction with a solvent system of chloroform-isoamyl alcohol (24:1). DNA from the aqueous phase was precipitated with a threefold volume of absolute ethanol with the addition of 10 M ammonium acetate (1:10) at -20°C for 8 hours. After centrifugation and double washing with 80% ethanol, the DNA was dried and dissolved in 30 µl of deionized water. To exclude possible contamination from the results of high-throughput sequencing at the sample preparation stage, a negative control was used, for the preparation of which 100 µl of deionized autoclaved water was treated using the method described above. DNA purity was monitored by electrophoresis in 1% agarose gel and photometry on a NanoDrop 8000 device (Termo Fisher Scientific Inc., USA). The DNA concentration was determined on a Quantus fluorometer (Promega, USA) using the Quanti Fluor dsDNA kit (Promega, USA). High-throughput sequencing. DNA libraries for sequencing were created using the Illumina protocol (<http://support.illumina.com/documents/documentation/chemistrydocumentation/16s/16sme-tagenomic-library-prep-guide-15044223-b.pdf>), with primers for the V3 variable region –

V4 of the 16S rRNA gene S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Klindworth et al., 2013). Sequencing was carried out on the MiSeq platform (Illumina, USA) using the MiSeq Reagent Kit V3 PE6DD reagents (Illumina, USA) at the Center for Shared Use of Scientific Equipment “Persistence of Microorganisms” of the Institute of Cellular and Intracellular Symbiosis, Ural Branch of the Russian Academy of Sciences. Read quality assessment was performed using MiSeq Reporter Generate FASTQ Workflow to generate FASTQ files for subsequent analysis. Using MiSeq Reporter (MSR) software, sequencing data were processed according to 16S rRNA gene amplicons V3–V4 using the Greengenes database (<http://greengenes.lbl.gov/>). Taxonomic classification of OTUs was performed using the Greengenes database (<http://greengenes.lbl.gov/>).

6 Results

Succession of microbial communities in the surface soil layer is one of the fundamental responses to changes in physicochemical conditions under the influence of various stress factors. Therefore, at the first stage of the study, we compared the microbial composition of the surface layer of southern chernozem and its changes with the addition of manure from large phytophages (Fig. 1, 2).

Thus, in the surface layer (15 cm) of southern chernozem, representatives of *Actinomycetota* dominated, and representatives of *Pseudomonadota* dominated in the soil-manure mixture. However, there were even greater differences at the family level (Fig. 1). Thus, representatives of the families *Chthoniobacteraceae* (7.1%) and *Sphingomonadaceae* (5.2%) dominated in the surface layer of southern chernozem. Representatives of the families *Pseudomonadaceae* (15.8%) and *Enterobacteriaceae* (11.5%) dominated in the soil-manure mixture with *Bos mutus* manure, and representatives of the *Bacillaceae* family (8.2%) dominated in the soil-manure mixture with *Camelus bactrianus* manure.

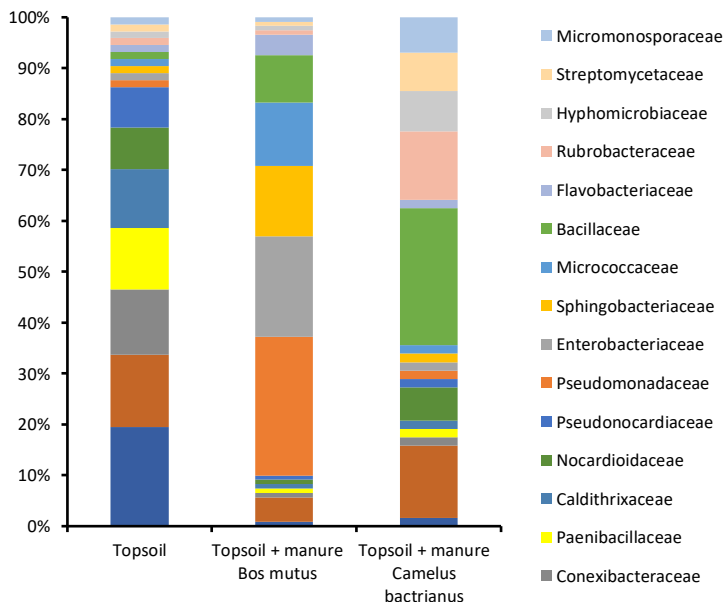


Fig. 1. Composition of microbial communities (families) in a soil-manure mixture consisting of soil and manure from *Bos mutus* and *Camelus bactrianus*; $\geq 1\%$ of reads. (Compiled by the authors)

When analyzing the differences in the soil-manure mixture at the genus level, the dominance of representatives of the genera *Chthoniobacter* (7.1%) and *Conexibacter* (4.7%) was noted in the surface layer of soil in southern chernozem. Representatives of *Pseudomonas* (15.8%) and *Arthrobacter* (6.7%) dominated in the soil-manure mixture with *Bos mutus* manure, and representatives of the genus *Bacillus* (6.1%) dominated in the soil-manure mixture with *Camelus bactrianus* manure.

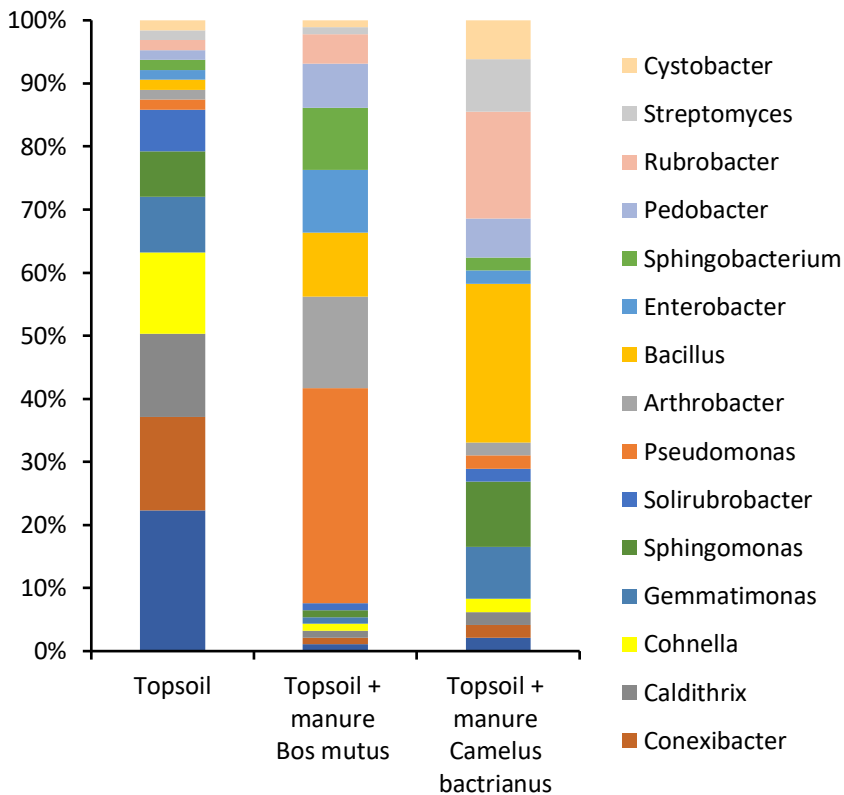


Fig. 2. Composition of microbial communities (genus) in a soil-manure mixture consisting of soil and manure from *Bos mutus* and *Camelus bactrianus* ($\geq 1\%$ reads). (Compiled by the authors)

At the next stage of the study, we assessed the time of appearance of the first shoots and changes in morphometric growth indicators of peas (Tab. 1) and onions (Tab. 2). The first to appear were pea seedlings in cells with a mixture of southern chernozem and *Camelus bactrianus* manure on the 7th day of growth. However, in the control samples of the surface layer of the southern chernozem there were no seedlings, so this was not recorded. The recording of the results began from the 9th day of growth (Tab. 1), when sprouts appeared on the surface of the southern chernozem.

Thus, the length of pea sprouts on the 9th day of growth was greatest in cells with *Camelus bactrianus* soil-manure mixture. They exceeded the growth by more than 2 times in comparison with the surface layer of southern chernozem. On the 14th day of growth, the length of pea sprouts in *Camelus bactrianus* soil-manure mixture differed from the control by 3 times. The growth of peas in *Bos mutus* soil-manure mixture also exceeded the growth in a cell with a surface layer of southern chernozem. The length of the pea root on the 9th

day of growth did not differ significantly in all cells with a soil-manure mixture. On day 14, the length of the pea root was greater in cells with a soil-manure mixture of *Camelus bactrianus* and *Bos mutus*. However, by the 20th day of growth, the average root length did not differ in all samples of the soil-manure mixture from southern chernozem.

Tab. 1 Changes in morphometric indicators of pea growth in topsoil manure mix (60:40)*

	Sprout, cm			Root, cm		
	9 day	14 day	20 day	9 day	14 day	20 day
Topsoil	2.9±0.1	5.8±0.1	12.0±0.6	3.4±0.3	3.7±0.9	5.5±0.3
Topsoil + manure <i>Bos mutus</i>	7.2±0.8	16.3±0.7	18.1±1.2	3.5±0.5	5.2±0.4	5.3±0.9
Topsoil + manure <i>Camelus bactrianus</i>	8.2±1.0	17.7±1.1	17.9±0.2	3.8±0.7	5.2±0.8	5.4±0.3

*Compiled by the authors

The first onion shoots appeared on the 12th day of growth, but assessment of morphometric growth indicators, due to the low value of the onion root length, began to be measured only from the 14th day of growth (Tab. 2).

Tab. 2 Changes in morphometric parameters of onion when growing in topsoil manure mix (60:40)*

	Sprout, cm			Root, cm		
	14 day	17 day	20 day	14 day	17 day	day 20
Topsoil	6.4±0.7	7.5±0.9	10.2±0.4	2.0±0.8	2.1±0.1	2.2±0.1
Topsoil + manure <i>Bos mutus</i>	6.6±0.1	8.5±0.5	18.3±0.3	2.0±0.6	2.6±0.4	2.8±0.3
Topsoil + manure <i>Camelus bactrianus</i>	6.9±0.7	8.7±0.2	16.0±0.5	1.8±0.6	2.1±0.3	2.4±0.4

*Compiled by the authors

The rate of increase in the length of the onion sprout and root was the greatest when the onion grew in *Bos mutus* soil-manure mixture. However, the relative value of the increase in the length of the sprout and roots was much lower than that of pea seeds.

7 Discussion

As a result of our study, it was shown that changes in bacterial communities in the soil-manure mixture of the surface layer of soil with *Bos mutus* and *Camelus bactrianus* manure leads to the dominance of representatives of *Pseudomonadota*. These changes are reflected at lower taxonomic levels (family, genus). Thus, in the soil-manure mixture prepared from the surface layer of southern chernozem and Tibetan Yak manure (*Bos mutus*), bacteria representatives of the families *Pseudomonadaceae* and *Enterobacteriaceae* dominated, and in the soil-manure mixture prepared from the manure of the Bactrian camel (*Camelus bactrianus*), bacteria representatives of the family *Bacillaceae* dominated. In samples of the surface layer of southern chernozem, representatives of these families of bacteria were absent ($\geq 1\%$ of reads). Stimulation of pea growth was more characteristic when cultivating Bactrian camel (*Camelus bactrianus*) in a soil-manure mixture, and onion in Tibetan Yak (*Bos mutus*) soil-manure mixture. The dominance of *Pseudomonadota* representatives in

soils is often associated with increased soil moisture (Chirak et al., 2013), but in our study we used a dry soil-manure mixture, which most likely indicates the dominance of *Pseudomonadota* representatives in the intestinal contents of large phytophages. In this connection, the question arises about the duration of sowing of *Pseudomonadota* representatives and their influence on the dynamics of plant growth.

8 Conclusion

Enrichment of the surface layer of soil with manure of wild steppe phytophages will promote “temporary succession” of bacterial communities with a change in the dominant groups of bacteria. Despite the fact that we have not studied the duration of such an effect, we can confidently say that even a temporary change in the dominant groups of bacteria can affect trophic relationships in bacterial communities. In addition, in the future it is possible to enrich soil with a certain group of bacteria to stimulate plant growth.

References

1. WWF. Living Planet Report 2016: Summary. Gland, Switzerland (2016)
2. N. Belkova, Diversity of microbial communities of inland water bodies of Russia: Study guide (Yaroslavl: Printhouse LLC publishing house, 2002)
3. A. Klindworth, et al., Nucl. Acids Res. **41**, 11 (2013)
4. M. Irshad, et al., Journal of Soil Science and Plant Nutrition **13**, 1 (2013)
5. E. Chirak, et al., Agricultural biology. **48**, 3 (2013)