

Biostimulator for Arid Pastures in the South of Kazakhstan

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ABSTRACT

Long-term unsystematic use of near-settlement pastures in the desert zone of South Kazakhstan had a negative impact on the physic-chemical parameters of *Calcisol*. Excessive grazing leads to trampling of soil and reduction of soil fertility. The aim of the study was to develop a technology to improve the productivity of degraded near-settlement pastures. The tasks of the research were to determine the effect application of environmental safe biostimulator and biofertilizer on microbial communities, the content of carbon and phosphorus in the soil, the effectiveness of biostimulator application on degraded pastures in order to increase green mass. For this purpose, geobotanical, bacteriological and helminthological methods of analysis were used. The result of the used proposed technology is the activation of soil microorganisms, which leads to the prevention of degradation of arid pastures. The results of the conducted research contribute to the introduction of a better technology to increase the productivity of arid near-settlement pastures. Statistical analysis of the experiments showed that all manipulations on soil microorganisms increase the number and green mass of arid plants.

Keywords: biofertilizer; biostimulator; degradation; soil microorganisms; village pastures.

INTRODUCTION

The land fund capacity of the world is 134 million kilometers, which is 26.3% of the entire territory of the planet. Land resources have a certain structure: 11% are arable land, meadows, and orchards, i.e. the cultivated land. About 23% of the land is pastures. Anthropogenic landscapes account for 3% in common. There are also unproductive lands, which account for about 33% of land. Non-systematic grazing in arid pastures by small and large cattle destroys fruits, seeds, seedlings, young shoots, branches, and leaves. During seasonal cultivation of crops such as legumes and cereals, plant residues are burned (Luna et al. 2008; Dmytrash-Vatseba et al. 2020). This results in the loss of nutrients vital to the soil microbiota. The traditional method of determining soil physical and chemical properties is used to assess soil biocenosis productivity. Soil microbial communities

are much more effective for indicating soil impact, because they more clearly reflect all possible soil changes compared to chemical analyses. Their main advantage is that they are safer than chemical exposures. The problem of preservation and improvement of ecological state of pastures, rational use, increase of their productivity now has a legal status in the Republic of Kazakhstan.

The results of the study conducted in light chestnut soils of the West Kazakhstan region showed that soil density on virgin land in the soil layer 0–30 cm was 1220 kg/m³, with insignificant grazing the soil density increased by 4.91% and become 1280 kg/m³. With overgrazing, it reached 1.38 g/cm³. It was also found that haphazard grazing had the highest content of weeds and noxious plants in the summer period at 9%. In the composition of phytocenoses used in unsystematic use, an increase in the proportion of wormwood to 15%, motley grasses (little eaten) to –21% was

noted (Nasiev et al. 2021). Arid pastures are an integral part of sustainable agricultural production (Parzhanov et al. 2020). If we cultivated pastures with intercrops are created, in addition to providing fresh feed for livestock, they provide an opportunity to strengthen the integration between crop and livestock production, which in turn effectively increases the ecological performance of the area, the productivity of farms and agricultural land (Bell et al. 2014). Perennial pastures create a special ecosystem to restore soil microflora, and thereby increase soil fertility, (Mpai et al. 2022) which results in reduced soil degradation (Hayes et al. 2021; Hayes et al. 2018). In addition, incorporating pasture into cropping systems would isolate more organic carbon (C) in the soil in the long term (Hayes et al. 2021), which could offset the negative effects of nitrous oxide (N₂O) emissions during the cropping phase of mixed cropping systems.

An analysis of 350 observations to examine improved pasture management practices on carbon content showed that pasture could act as a significant carbon sink when improved management is implemented (Shi et al. 2022; Eze et al. 2018; Conant et al. 2001). In addition, it is necessary to determine the average vegetation cover ratio, the number of all grasses, legumes and other families (Seydoşoğlu et al. 2019). It is also necessary to group plants by cluster: reducing, enhancing and capturing, and in relations of valuable nutritive qualities of plant species for farm animals. In addition, followed by reductions in the number of weed and poisonous plants not eaten by grazing livestock. Because the lack of a nutritious food unit initiates the process of trampling of the soil by the hooves of livestock, which is the beginning of the degradation of the soil of the pasture areas.

Pasture plants and plants in general are mistakenly considered as independent objects. They contain a huge world of microscopic living bacteria and fungi that envelop the plant in the endosphere and rhizosphere. They are actively involved in plant life in the selection of nutrition and sustainable development under direct as well as indirect stresses. This relationship between the plant and microorganisms is not yet fully understood. To understand their action, it is necessary to divide them hierarchically into clusters according to microbiota life activity (Vandenkoornhuyse et al. 2015; Liu et al. 2020; Corcoz et al. 2021; Zhang et al. 2017). One solution to this problem is seeded pastures with the application of plant growth biostimulants,

which have a positive effect on the soil microbial communities (Coolon et al. 2013). The soil microbiota represents an important labile source of nutrients (Leff et al. 2015), mainly C, N, P (Oelmann et al. 2021; Zani et al. 2023; Li et al. 2023; Bardgett et al. 2003; Kuzyakov et al. 2013; Bai et al. 2022) and S, being a direct sink of these nutrients and an important agent of organic matter transformation (humic compounds), but still most farmers use different types of fertilizers such as mineral fertilizers, organic fertilizers and biostimulants for plant growth (Cassman et al. 2016; Vâtcă et al. 2020).

For the sustainable development of agriculture, different kinds of techniques have been applied to create a favorable environment for plants from having abiotic and biotic stresses. These technological solutions were best solutions at that time (Ali et al. 2019; Moon et al. 2022). However, the world does not stand still. New anthropogenic influences on the environment are changing everything in a circle. Chemical and synthetic fertilizers are not completely assimilated by plants and are used in larger quantities. Thus, they damage the ecosystem by killing soil microorganisms. All pesticides and the pesticides that have been used only a small part (about 1%) affect the target organisms. Most of them contaminate nature (Kaur et al. 2014; Nusillard et al. 2023; Okagu et al. 2023). In recent years, the scientific community and farmers have turned to natural biostimulants because the use of agro-technological means alone has proven insufficient to address sustainable agriculture. The solution to this shortcoming is the use of “natural tools of restoration and fertility” – microbial plant biostimulants. Which are based on plant, manure composts, single and multi-component biostimulants with microorganisms (Orozco-Mosqueda et al. 2023; Nascimento et al. 2019).

Microbial plant biostimulants are used to transform plant and agricultural wastes into nutrients for the target higher plants. There is a positive growth effect in the biostimulant market in the near future. According to statistics, the market for commercial biostimulants will grow about 10% by 2025. Biostimulants include mycorrhizal and non-mycorrhizal fungi, symbiotic bacteria, and all other microorganisms that stimulate, indirectly or directly treat, activate the protective properties of target higher plants (Hamid et al. 2021; Damodaran et al. 2023; Vlajkov et al. 2023; Shahrajabian et al. 2023). Microbial biomass is more visualized as a permanent catalyst in the short term than over an annual cycle because of its

seasonal fluctuations (Q. Sun et al. 2023), and can be very useful for impact assessment and management, soil recovery and productivity. Microbial carbon, in particular, has been used to determine biomass because it makes up an average of 47% of the cell structure. In the microbial cell, C, N, P, and S (Van Der Heijden et al. 2008) maintain an unstable 250:40:9:2.6 ratio. The soil carbon balance, although not showing availability to the microbiota (Soong et al. 2020; Zhou et al. 2022), is consistent with the energy requirements of this living pool. This has been very useful in studying nutrient biocycling and crop cultivation practices as well as biostimulant applications in many agroecosystems, temperate and degraded soils.

Thus far, no work has been done to study the state of the soil cover of near-settlement forage lands and no treatment with micro- and macroelements has been carried out. Communities of soil microorganisms positively influence the productivity of rangelands (Van Der Heijden et al. 2006; Badger Hanson et al. 2023). Application of biostimulant with manure is a source of valuable organic nutrients, increasing the fodder unit of arid near-settlement pastures (Burkle et al. 2015). The conducted studies allowed determining the most effective concentrations of biostimulant (Castiglione et al. 2021; Bera et al. 2022) and proper composting of cattle manure (Cattle), which gave an opportunity to obtain more above-ground eatable part of arid plants. For the systematic restoration of degraded near-settlement pastures, the optimum is the application of biostimulant on the basis of microorganisms in the complex with cattle manure. The aim of the research was to develop a technology for increasing the productivity of degraded village pastures. The objective of the research was to determine the effect of biostimulator and biofertilizer on microbial communities, increase of carbon (C) and phosphorus (P) content in soil, as well as selection of optimal conditions for increasing green mass of plants.

MATERIALS AND METHODS OF RESEARCH

Facilities

Biostimulant – MERS on the basis of plant extract with soil microorganisms. Biofertilizer – based on vermicompost with addition of MERS biostimulator (The method of biofertilizer

preparation is given in paragraph Preparation of biofertilizer)

Geobotanical inventory

Soil is light gray sandy loamy soil with lumpy sands anchored by plants such as *Artemisia paniculata*, *Ceratocarpus arenarius*, *Stipa hohenackeriana*, *Peganum harmala*, *Kochia prostrata*, *Astragalus*, *Halimodendron halodendron*. The eastern part of the site is represented by wormwood-ephemeral herbage. Among the herbage the dominant species is wormwood (*Artemisia diffusa*), also there is camel's thorn – *Alhagi pseudalhagi*, sand acacia – *Ammodendron aphyllum*. Dried ephemera and motley grass are not found in the herbage, as they are eaten, and the wormwood herbage is weeded. *Cousinia dipinnata*, *Cousinia syrdariensis*, *Cousinia erectispina*, *Artemisia leucodes*, *Calligonum aphyllum* and sand acacia *Ammodendron aphyllum* are rampant here. Of the eating plants preserved *Chrozophora sandy* – *Chrozophora sabulosa*, wormwood – *Artemisia diffusa* is very rare.

EXPERIMENTS

Experimental field

Arid pastures of 5 villages – Kozhatogai, Shogirli, Baytogai, Shoshkabalak and Darbaza – were selected for conducting experiments. The climate of the area is harsh continental, with cold winters and dry summers. Because of this, the ephemeral vegetation of pastures and the soil microbiome wake up early, and their activity decreases or stops completely in summer. Therefore, soil samples (in February, May, August, and November) 2020–2022 were collected to assess microbial diversity and soil microbiome dynamics (randomly selected). Four samples were collected from 4 pastures with a repeatability of 4 times per year; the total number of soil samples obtained over 3 years was 192 pieces. The obtained samples were stored in a climatic chamber at -60°C for further microbiological study.

Kozhatogai village is located 150 km west of Shymkent, 60 km from Arys city and is located on the right coast of the Syrdarya river. The village of Shogirli (42.104285, 68.245335/42.087995, 68.256854) is 15 km to the north, the village of Baytogai (41.92086 с.ш. 68.15052 в.д.

/ 41.885313, 68.138175) 18 km to the south, the village of Shoshkabalak (41.956170, 68.498643 / 41.976592, 68.468645) 50 km to the south-west, and the village of Darbaza (42.020318, 68.666656 / 41.970243, 68.597048) 45 km to the east of the village of Kozhatogai (42.087210, 68.235522 / 42.068178, 68.236552).

Setting up the experiment

Two experiments were simultaneously conducted on the experimental plot of scientific laboratory “Industrial Biotechnology” and the experimental field of South-West Research Institute of Animal Husbandry and Crop Production. Soil composition was tested according to International State Standard SS 17.4.4.02-84. “Nature Protection. Soil. Methods of sampling and preparation of samples for chemical, bacteriological, helminthological analysis”, moisture absorption and soil analyses were carried out on lysimeter design («International State Standard SS 17.4.4.02-84. „Nature Protection. Soil. Methods of sampling and preparation of samples for chemical, bacteriological, helminthological analysis“»).

Each pasture was divided into 4 identical parts for assessment and condition monitoring. Samples were randomly selected from each part to determine the physicochemical properties and microbiological composition of the soil. In addition, the soil cover, plant density of *Artemisia diffusa*, *Kochia arenaria* and *Eurotia ewersmanniana* were investigated at each part of 60 m². Information was collected 4 times a year during 2020-2022: in February, May, September, and November (Yuan et al. 2022).

Preparation of microbial suspension

To prepare a microbial suspension, a nodule strain of the bacteria genus *Bradyrhizobium spp. vigna* was extracted from large nodules of *Vigna radiata* plants during its full maturation. The strain was provided by the laboratory “Industrial Biotechnology” and stored in the collection.

Preparation of biofertilizer

MERS biostimulator is obtained by dilution with distilled water in the ratio of 1:30. The obtained solution is added to the vermicompost prepared in the ratio of 1:50 of the vermicompost mass beforehand. The prepared suspension is sprayed on

the compost in layers and left for 7 days. Then, a microbial suspension is added with a titer of $4-8 \cdot 10^9$ CFU/ml on the basis of nodule bacteria *Bradyrhizobium spp. vigna*, previously diluted in 10 liters of distilled water and again left for 72 hours.

Statistical analysis

All data obtained during the study were processed by statistical analysis using Minitab statistical programming (version 21.1.1). The effect difference was tested by one-factor analysis of variance (ANOVA), only when the other factors were background factors for all samples, if the level of significance for all samples corresponded to $P < 0.05$. However, when the level of reliability differed between the chosen method, we used the method of grouping the samples with the least significant difference by Fisher’s criterion. $P < 0.05$ is the usual level of statistical significance. For clarity, they were traditionally marked with one asterisk $P < 0.01$ – a high level of significance. Traditionally, they were designated with two asterisks.

RESULTS AND DISCUSSION

Soil horizon

Light grey soils (*Calcisol*) are characterized by low natural fertility. Humus content in 2022 in 0–10 cm horizon of light gray loamy soils amounted to 0.55%, which decreased by 0.27% compared to 1980 (0.82%). In horizon 10–20 cm it amounted to 0.32%, almost at the level of 1980 (0.38%). Humus content in the horizon 0–10 cm of light-grey loamy soils in 2022 amounted to 0.71% and compared to 1986 (0.79) decreased insignificantly 0.08%. In the horizon 10–20 cm the decrease in humus content in this interval 1980–2022 amounted to 0.20%.

The absence of summer-autumn vegetative plant species in *Artemisia*-ephemeral pastures led to an increase in the content of mobile nitrogen compared to herbaceous-shrub pastures in the 0–10 cm soil layer by 19.6 mg/kg, phosphorus by 7 mg/kg, potassium by 240 mg/kg. From the obtained data it follows that in *Artemisia*-ephemeral pastures the resources of nutrients are quite sufficient, but they are not fully utilized. Uneven decrease of humus content in 0–10 cm layer of light-sulfur loamy soils is explained by soil deflation as a result of overgrazing. It is known that the most

fertile soil particles are blown away from the soil surface, the process is intensified when aggregates of 1 mm size in the surface layer decrease up to 26%. Aggregates of 3±1 mm are absent at all. The content of aggregates 1.0±0.25 mm in 1980 in light-gray loamy soils was 7.7–7.8%, in light-gray loamy soils 5.1–6.5%, in 2022, respectively 28.494–26.085% and 37.35–41.68%. High content of 1–0.25 mm aggregates in light-sulfur soils is associated with the protective role of the above-mentioned plants, which reduce the deflation process. The content of agronomically valuable aggregates of 0.25±0.05 mm in soil was 43.795–53.428% and decreased by 12.072–19.695% compared to 1980 (62.1–65.5%). Table 1 shows the granulometric and microaggregate composition of soil.

After treatment according to the recommended technology granulometric and microaggregate composition of soil improved by 17±2%, agrochemical composition by 11±3%, alkalinity decreased and pH of medium normalized by 9±2%. This can be explained by the dynamics of the number of soil microorganisms. The greater the increase in soil microaggregate composition, the greater the increase in soil fertility. For example, soil microorganisms create colonies in their vital activity. Colonies organize large clumps of soil. All the results are summarized in Tables 1, 2 and 3. The results of the research showed (Tables 1-3) that the effect of microbial biostimulant had a

beneficial effect on granulometric and microaggregate composition, agrochemical index increased, alkalinity decreased by 12±2% and soil pH normalized. The gross and mobile forms of mineral elements in the soil in percentage terms increased by a reliable 11±3%. This is an indicator that microbial associations jointly started to regulate their micro-ecobiota.

Population dynamics of soil microorganisms

The study of the dynamics of the microbial complex at a depth below 10±5 cm, showed that the number of colonies of phosphate-mobilizing bacteria is relatively average in the summer and autumn periods, winter and spring are active. Their activity is related to plant activity. Soil microorganisms are not evenly distributed in the soil horizon. It still depends on the number and diversity of plants. In this connection, bacteria and fungi differed in seasonal activity (Wang et al. 2022). A colony of soil microorganisms determines the level of assimilated phosphorus in the soil, their vitality and the species composition of the microbial community directly depends on the transformation of organic matter into minerals and free mineral nitrogen compounds (Aydin et al. 2005). The dynamics of activation and population of soil microorganisms is the main factor linking cycles of P, N, S, C and other nutrients (Wang et

Table 1. Granulometric and microaggregate composition of light-grey soils

Soil type	Soil layer, cm	before treatment	Fraction content in % per absolute dry soil						
			Fraction sizes, mm						
			Sand		Dust			Silt	Threefold screening
			1.0±0.25	0.25±0.05	0.05±0.01	0.01±0.005	0.005±0.001	<0.001	Fractions <0.01
Light loamy grey soils	0-10		28.494	43.795	15.663	2.811	3.614	5.622	12.048
	11-20		26.085	45.378	16.479	2.010	4.019	6.029	12.058
Light sandy loamy grey soils	0-10		41.68	45.088	5.213	0.401	1.604	6.014	8.019
	11-20		37.35	53.428	4.411	3.208	0.802	0.802	4.812
Soil type	Soil layer, cm	after treatment	Fractional content in % per absolute dry soil						
			Fraction sizes, mm						
			Sand		Dust			Silt	Threefold screening
			1.0±0.25	0.25±0.05	0.05±0.01	0.01±0.005	0.005±0.001	<0.001	Fractions <0.01
Light loamy grey soils	0-10		33.338	51.240	18.326	3.289	4.228	6.578	14.096
	11-20		30.519	53.092	19.280	2.352	4.702	7.054	14.108
Light sandy loamy grey soils	0-10		48.766	52.753	6.099	0.469	1.877	7.036	9.382
	11-20		43.700	62.511	5.161	3.753	0.938	0.938	5.630

Table 2. Results of agrochemical analysis of soil

No	Before biostimulant treatment							pH
	Total humus	Gross			Mobile			
		Nitrogen, %	Phosphorus, %	Potassium, %	Nitrogen mg/kg	Phosphorus mg/kg	Potassium mg/kg	
1	0.55	0.056	0.100	2.000	33.6	41	390	9.45
2	0.32	0.028	0.100	2.062	16.8	18	240	9.50
3	0.71	0.070	0.080	2.062	14.0	34	150	9.37
4	0.20	0.028	0.056	1.937	8.4	14	150	9.67
No	After biostimulant treatment							pH
	Total humus	Gross			Mobile			
		Nitrogen, %	Phosphorus, %	Potassium, %	Nitrogen mg/kg	Phosphorus mg/kg	Potassium mg/kg	
1	0.611	0.062	0.111	2.220	37.296	45.51	432.9	8.31
2	0.355	0.031	0.111	2.289	18.648	19.98	266.4	8.36
3	0.788	0.078	0.089	2.289	15.540	37.74	166.5	8.24
4	0.222	0.031	0.062	2.150	9.324	15.54	166.5	8.51

Table 3. Results of the research of alkali metal uptake process before and after treatment with biostimulant (per 100 g of soil)

No	Results of the research							
	Ca, mg		Mg, mg		Na, mg		K, mg	
	before	after	before	after	before	after	before	after
1	3.96	4.63	1.98	2.32	0.23	0.27	0.61	0.71
2	3.47	4.06	1.49	1.74	0.23	0.27	0.32	0.37
3	4.46	5.22	1.49	1.74	0.23	0.27	0.18	0.21
4	2.48	2.90	0.99	1.16	0.23	0.27	0.18	0.21

al. 2022; Legay et al. 2016; Heyburn et al. 2017). The assimilated form of nitrogen (N), phosphorus (P) and other organic nutrients follow a stable course through the microbial network. The observations made have shown that soil properties and cover crop season will directly proportionally affect the dynamics of soil microorganisms. These observations prove a theoretical basis for future research on the inextricable relationship between cover plants and soil microbiota in arid pastures. In the observation, natural indicators such as oligotrophic microorganisms were used.

Oligotrophic microorganisms (autochthonous or native group) do not need rich substrates for life activity, so they participate in the last phase of mineralization of organic residues. They play the main role in creation of fertile soil because of their numerous colony-forming capacity. It should be taken into account that under the influence of concentrated mineral fertilizers $N_n P_n K_n$ or ammophos, a uniform increase in the colonies of oligotrophs from December month to August (from 6.2 ± 3.5 to

37.8 ± 2.5 million CFU, $P < 0.05$) was studied. It was also found that when manure composts were used instead of mineral fertilizers, they exhibited slow action, reducing the number of oligotroph colonies in late July (to 7.3 ± 1.4 and 3.7 ± 1.2 million CFU, during low moisture, $P < 0.05$), and if the beginning of summer is hot, they go into anabiosis. The decrease in the number of oligotrophic microorganisms indicates that the available carbon-containing organic nutrients are available in sufficient quantities, according to the sources (Tedersoo et al. 2014; Beaumelle et al. 2020) not all bacteria and fungi are useful for the plant, the task is to identify the right microorganisms and create a favorable environment for them (Figure 1).

Influence of biostimulants on microbial communities

A short-term increase in plant productivity due to the use of mineral fertilizers leads to a decrease in stability and subsequently to the degradation

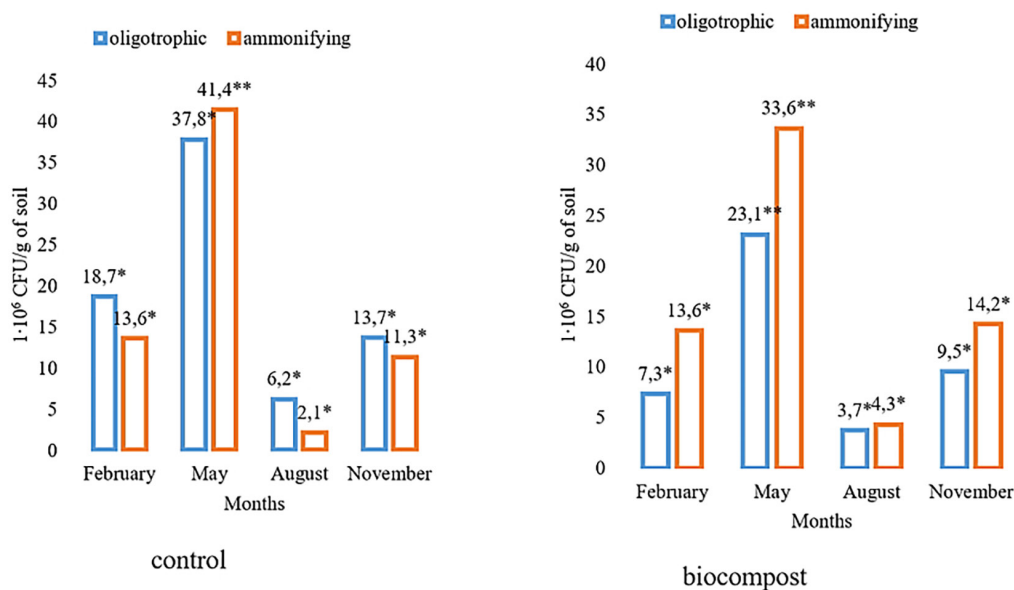


Figure 1. Dynamics of oligotrophic and ammonifying microorganisms' abundance in light Calcisol. Compared with the control variant we see a decrease in oligotrophic bacteria in the variant with biocompost. Decrease of oligotrophic bacteria to 3.7 ± 1.2 million CFU is an indicator of increased soil fertility (*= $P < 0.05$, **= $P < 0.01$)

of individual components of the ecosystem. For example, the use of nitrogen-phosphorus, nitrogen-potassium and phosphorus-potassium fertilizers had little effect on the biocenosis. The use of these fertilizers did not increase the number of microorganisms, including ammonifiers, actinomycetes and micromycetes, as well as cellulolytic microorganisms. Taking into account these indicators, a series of experiments was conducted using the MERS biostimulator and the “biofertilizer” prepared by the authors.

From the ecological point of view, the group of oligonitrophilic microorganisms that can develop at low levels of bound nitrogen in the medium and use atmospheric nitrogen is of the greatest interest in sulfur soils. They are weak fixers of atmospheric nitrogen and enrich the soil with protein nitrogen after dying off. As a rule, a very high number of oligonitrophils in the soil indicates a decrease in nitrogen content and creates the conditions unfavorable for other microorganisms. In the experiments with biostimulant and biofertilizer, the number of oligonitrophil microorganisms depended largely on the fertilizer composition and plant development phase (Fangueiro et al. 2015; Edesi et al. 2020; Fangueiro et al. 2009a, 2016b; Pereira et al. 2010; Tiquia et al. 2000; R. Sun et al. 2015). Thus, the high content of oligonitrophils in the soil was observed at the beginning of the experiment (before sowing and fertilizing 57.4 ± 2.5 million CFU/g of soil, $P < 0.05$).

Background readily available, not fully used by ephemera plants, mineral compounds maintained the number of oligonitrophils at a very high level (73.5 ± 2.1 million CFU, $P < 0.05$) during the growing season, reducing it to 4.5 ± 1.3 million CFU/g soil by the phase of ripening, while biofertilization contributed to a decrease in the microorganisms of this group. Biofertilizer uniformly reduced the number of oligonitrophils throughout the growing season (57.4 ± 2.5 in February, 37.4 ± 1.7 in May, 1.5 ± 1.2 million CFU in August, and 9.3 ± 2.2 million CFU in November, $P < 0.05$). The biostimulant showed its effect only after repeated application – in May with further uniform decrease of oligonitrophils amount by the end of vegetation (beginning with 57.4 ± 2.5 ; in May with repeated application 65.1 ± 1.1 ; in August 1.8 ± 0.5 ; and in November increased with 21.6 ± 0.7 million CFU, $P < 0.05$) (Figure 2).

One of the main causes of nitrogen losses from fertilizer application in light gray loam in the experimental area is biological denitrification. Denitrifying microorganisms in anaerobic respiration are capable of transferring electrons from organic matter to nitrates, resulting in oxidized forms of nitrogen being reduced to gaseous oxides or molecular nitrogen. The ability to conduct the denitrification process is widespread among soil bacteria and has been experimentally shown in genera *Bacillus*, *Pseudomonas*, *Micrococcus*, *Achromobacter*. It was found that in the initial

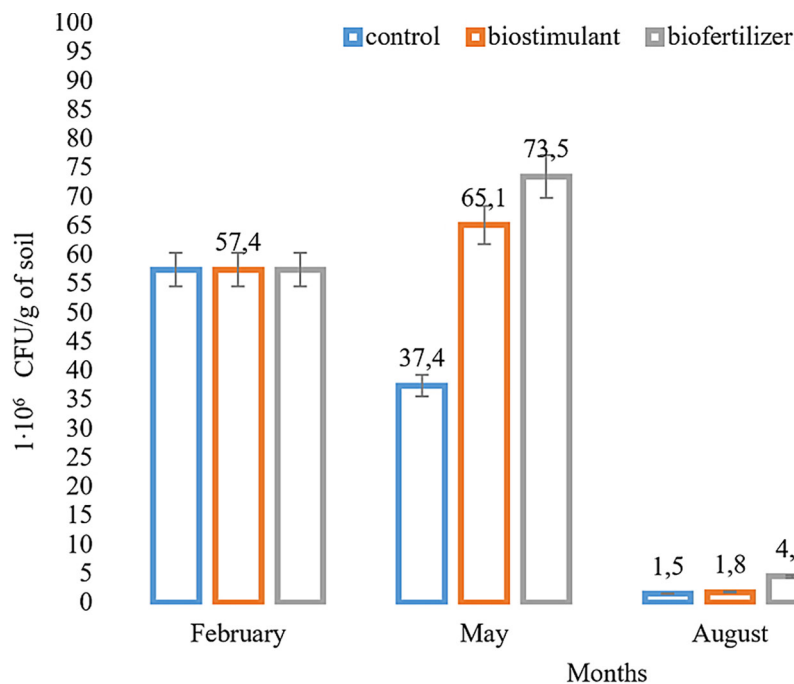


Figure 2. Dynamics of changes in oligonitrophilic microorganisms abundance under the influence of biostimulant and biofertilizer. On the control variant oligonitrophilic microorganisms significantly decreased during the year from $57.4 \cdot 10^6$ CFU to $1.5 \cdot 10^6$ CFU ($P > 0.05$), but on the biostimulant variants from $1.8 \cdot 10^6$ CFU (August) to $65.1 \cdot 10^6$ CFU (May) and biofertilizer from $4.5 \cdot 10^6$ CFU (August) to $73.5 \cdot 10^6$ CFU (May) they significantly increased. The increase in colony of oligonitrophilic microorganisms indicates the inhibition of nitrogen compounds in the soil

soil in early spring the number of denitrifiers was quite low – 0.2 million cells/1 g of soil (Figure 3).

After biofertilizer application, the number of denitrifying microorganisms decreased significantly from $37.2 \pm 0.3 \cdot 10^6$ to $13.5 \pm 0.3 \cdot 10^6$ CFU in the study period, and when biostimulator was used their number decreased to $25.1 \pm 0.4 \cdot 10^6$ CFU, indicating a vigorous recovery of soil nitrate nitrogen (Figure 3). Application of biofertilizer and biostimulator led to a significant increase in the number of denitrifiers (it is noticeable in May during the rapid growth of ephemeral plants), with a decrease in their number as the formation of plant seeds. It can be assumed that the mineral fertilizers applied to the soil are more affected by denitrifying microorganisms, and there may be significant losses of fertilizer and soil nitrogen in the form of reduced nitrogen compounds (NO_2 , N_2O , NO , N_2). On the contrary, the bacterial fertilizers containing an organic component and having slow-acting properties have a protective effect on mineral easily accessible forms of nitrogen (Netthisinghe et al. 2023, Das et al. 2017). Of great theoretical and practical interest is the study of autotrophic nitrifying microorganisms, because these microorganisms, despite their

moderate abundance in calcisol soils, can significantly reduce the nitrogen use ratio of fertilizers (Wakelin et al. 2009). The increase in the number of autotrophic nitrifiers is a reliable indicator of the presence of available forms of nitrogen and, consequently, the full nutrition of plants. Autotrophic nitrification is the sequential oxidation of NH_3 and NO_2 by two specialized groups of microorganisms that gain energy from these reactions and use CO_2 as a carbon source for growth.

Effectiveness of biostimulants on degraded pastures

The microbial ecosystem effectively maintains soil homeostasis. The reactions of microorganisms to changes in environmental factors are manifested at both the ecosystem and population levels. At the ecosystem level, they are expressed in changes in the quantitative and qualitative composition of the community. As habitat conditions change, some species decrease in number (or disappear), while others emerge, i.e., the composition of dominants and co-dominants changes. The reactions of microorganisms at the population level are expressed in changes in the kinetics

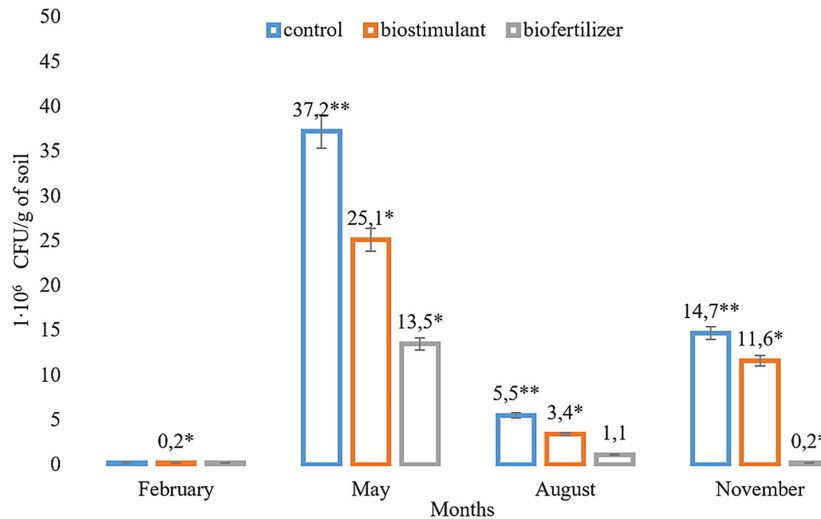


Figure 3. Dynamics of numbers of denitrifying microorganisms under the action of biostimulant and biofertilizer (*= $P < 0.05$, **= $P < 0.01$)

of their growth and development depending on certain ecological conditions. The sensitivity and high indication ability of microorganisms allow them to be selected as a tool for monitoring anthropogenic changes in the biosphere (Turner et al. 2013). In this regard, it seems appropriate to identify the differences in the structure and functioning of microbial communities under the influence of new phosphorus-containing fertilizers.

Thus, the most effective representation of the structure in the form of a histogram with accumulation, reflecting the contribution of each group of microorganisms to the total abundance, as well as a normalized histogram were considered (Figure 4). In particular, a study of the composition of microbial communities in the original soil without treatment showed the dominance of 2 groups of microorganisms – oligotrophs (oligocarbophiles) and oligonitrophils (41.3 and $34.8 \cdot 10^6$ CFU, $P < 0.05$), with co-dominants – amylolytic microorganisms ($21.3 \cdot 10^6$ CFU, $P < 0.05$).

In spring months under the influence of biostimulator and biofertilizer, the number and proportion of oligotrophs significantly decreased (to $19.8 \cdot 10^6$ CFU), while the number of oligonitrophic microorganisms was stabilized and their number in the community was: in the version with biostimulator – $33.5 \cdot 10^6$ CFU (Figure 4 b), and biofertilizer – $41.3 \cdot 10^6$ CFU (Figure 4 c). The position of amylolytic and microorganisms increased in the variant with biofertilizer, the number of phosphate mobilizers increased (to 18.1 million CFU), and the quantity decreases denitrifiers (up to $0,3$ – $1,2 \cdot 10^6$ CFU) and actinomycetes appeared

(3.3 – $10.1 \cdot 10^6$ CFU) (Figure 4 c). In May, the total number of microorganisms in all variants changes sharply, with an increase in the number of all defined groups of oligonitrophils, amylolytic, ammonificators, actinomycetes, phosphormobilizers and denitrifiers. A significant increase in the number and proportion of amylolytic species in the variants with biofertilizer ($40.7 \cdot 10^6$ CFU) with a significant decrease in the participation of oligonitrophils and oligotrophs was observed (Figure 4) (Wierzchowski et al. 2021).

Rather indicative was the experiment of wormwood-ephemera plants in the control plot with a small total number of microorganisms the only dominant group were oligotrophs $47.2 \cdot 10^6$ CFU (Figure 4 a), which may indicate a deficit of nutrient compounds in this period (Reynolds et al. 2003). It should be particularly noted that biofertilization contributes to community restructuring, and despite the significant dominance of oligotrophs, ammonificators, oligonitrophils, actinomycetes, microorganisms assimilating mineral nitrogen, and phosphate mobilizers are found in the microbial complex. All these manipulations on soil microorganisms showed reliable results in increasing the number and green mass of arid plants (Bellabarba et al. 2019). A total of 8 plant species were identified in the study area, and their average number for the three years of the study is shown in Figure 5.

After the treatment of experimental plots according to the proposed technology, the density of cover plants increased significantly, for example, grass mixture of *Halimodendron halodendron*,

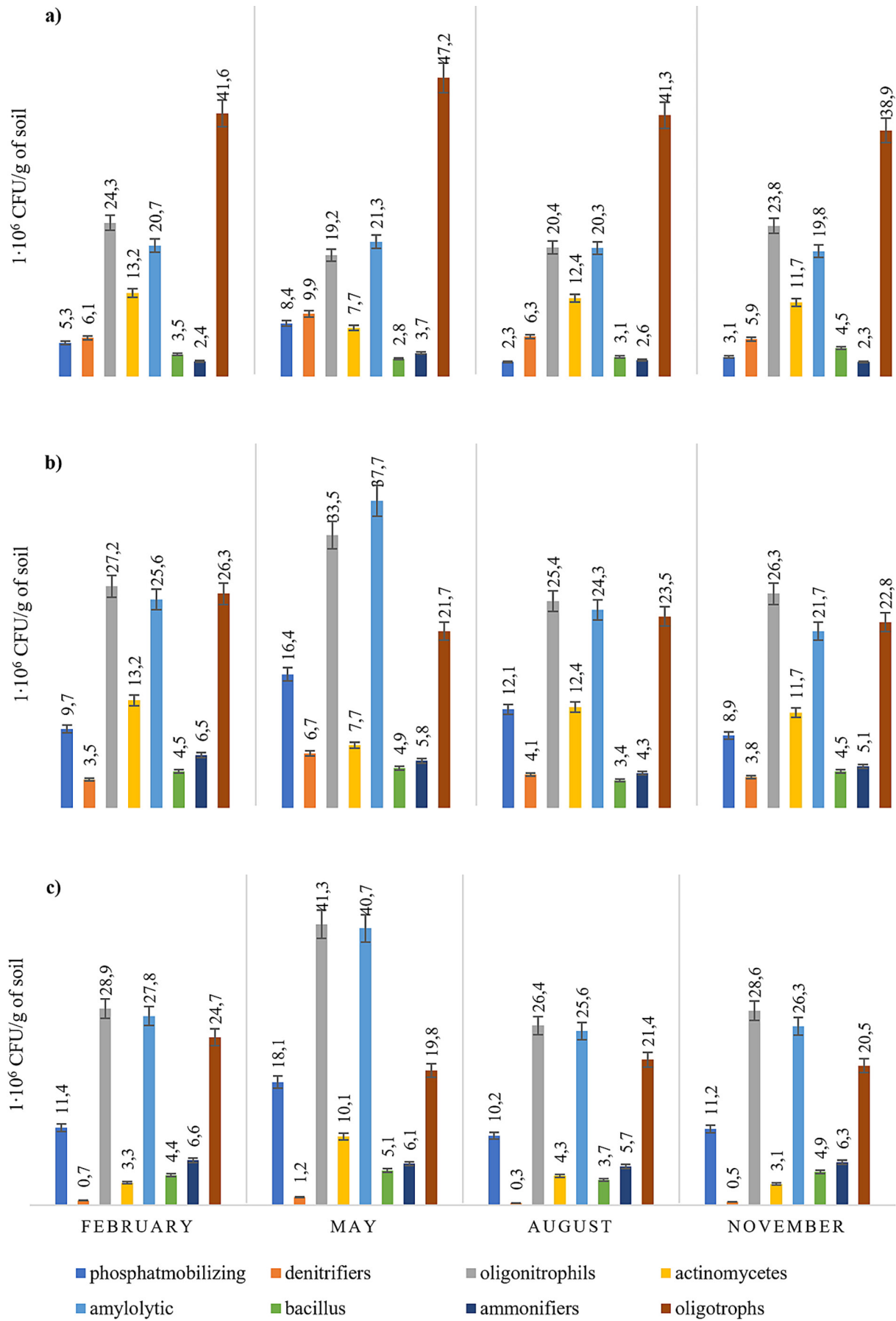


Figure 4. The number of dominant microorganisms in the studied soils. (a) control; (b) biostimulator; (c) biofertilizer, (P<0.05)

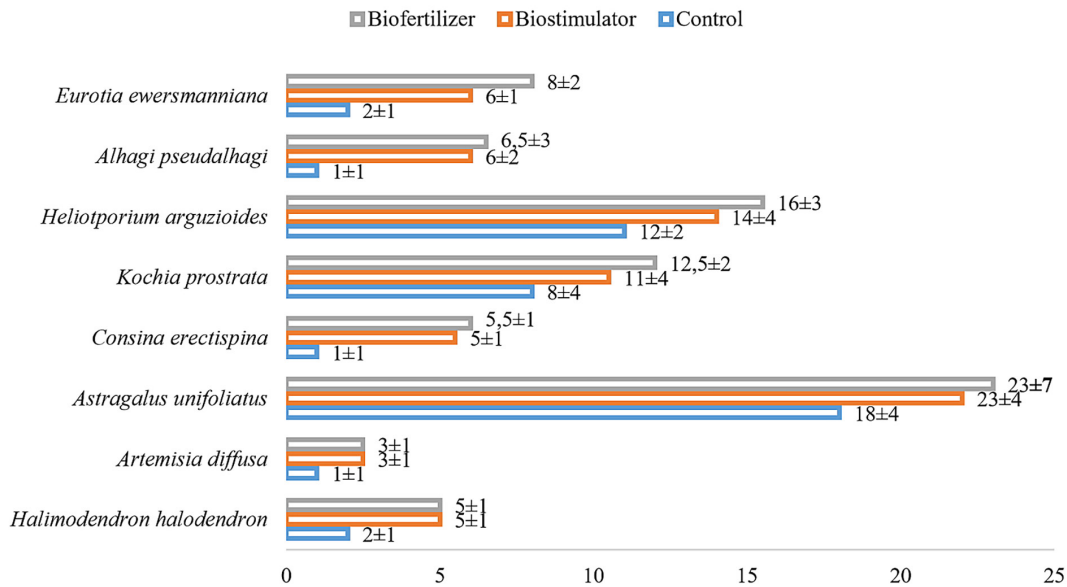


Figure 5. Average percentages of herbage after tillage for 2020–2022

Artemisia diffusa, *Astragalus unifoliatus*, *Consina erectispina*, *Kochia prostrata*, *Heliotporium arguzioides*, *Alhagi pseudalhagi*, *Eurotia ewersmanniana* – 81–93 pcs/60 m². In plants of *Artemisia ephemera* association, the stems became juicy, flowering began earlier. This phenomenon shows that plants have an abundance of phosphorus fertilizer (Figure 4a, b, c). Nevertheless, after applying the proposed technology, it is recommended to refrain from grazing sheep and goats for one year. Because during the geobotanical survey, it was found that sheep were pulling out young plants with roots. After 2 years, the plant is not afraid of anything (frost, drought, steppe fires and overgrazing), the roots go very deep up to 15–25 meters.

CONCLUSIONS

It is received that soil microbial communities in the variants with application of biostimulator and biofertilizer have the greatest stability to anthropogenic load and ability to self-regeneration. This can be judged from the values of CO₂ consumption and respiratory activity and the metabolic coefficient. Biofertilizer containing up to 60% of organic compounds mitigates the activating effect of macronutrients and, reduces the absolute number of microorganisms – “participants” – harmonizes the soil community. In the variants with biofertilizer a high number of phosphate-mobilizing microbial communities

is noted, which slightly decreases in the case of biostimulator. Using them in the near-settlement pastures of villages Kozhatogai, Shogirli, Baytogai, Shoshkabalak, Darbaza showed that the developed methods of soil treatment are innovations that provide flexibility and allow adjusting the humus cover, which in turn increases the soil cover. Taking into account the perspectives of the obtained data, the technology of biostimulator and biofertilizer application was developed. It led to significant results in terms of increasing green mass of arid plants and sustainable development of degraded near-settlement pastures. The authors are well aware that due to the complexity of interactions in the microbiocenosis, the study cannot fully account for all influencing background factors. However, the authors made an attempt to make a significant contribution to the development of rational methods for restoring degraded pastures using microbial-based biostimulant. The authors hope that this direction will be in demand at the national and international levels to create sustainable agricultural systems. In the near future, a joint research roadmap will be drawn up on the application of soil microbiota as a promising “biological tool” for biogeocenosis restoration.

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