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Biodynamics of Degraded Pastures at the Grazing Exclusion in Permafrost Region.

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ABSTRACT

The goal of this work was to monitor changes in these particular figures: above-ground phytomass – below-ground phytomass – soil organic substance – soil microbial activity during short-term (4-5 years) isolation of alas pasture in order to evaluate speed and self-healing features of cryolithozone ecosystems. The experiment was performed in the Central Yakutia (62°28´29.7" N 130°56´40.5" E 62°33´24,3" N, 130°54´01,4" E) in 2009-2013.. It was found out that flora, stock of above and below-ground phytomass, SOC, CFU, activity, resistance and resilience of microbial community of permafrost soil during short-term (4-5 years) of isolating degraded alas pasture reached background values. Grazing exclusion contributed to FS stabilization especially in meadow belt. Activity of autotrophic nitrification increased during pasture digression and did not decrease after 5 years of isolation, remaining 3 times higher than background values. Thus, grazing cessation on degraded pasture in permafrost zone led to similar phenomena observed in similar cases in other regions of the world. Sward productivity increase and release of nutrients from manure were common causes of these changes. Biodynamic features of degraded alas meadows in early years after isolation indicate capability of these ecosystems to regenerate. At the same time, high activity of autotrophic nitrification corroborates continuing imbalance in turnover in the soils of the isolated area.

Key words: Permafrost soil, pastoral digression, grazing exclusion, microbial community, Central Yakutia.

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INTRODUCTION

Studying rates and features of self recovery of disturbed ecosystems remains an important practical and theoretical ecological problem. State of pasture ecosystems after grazing cessation is a specific object of study. Most of pastures in the world are located in arid and semi-arid zones that are extremely vulnerable to anthropogenic load. Overgrazing resulting in environmental degeneration is one of the most serious contemporary problems. Components of pasture ecosystems in terms of exploration in isolation are set up in descending order: flora – above-ground phytomass – below-ground phytomass – physicochemical features of soil – microbial complex. Published long-term observations are predominantly applied to arid pastures located in climatic zones with average annual temperatures of 0 °C and higher (Stepes ..., 2002, Su et al., 2005, Pei et al., 2008, Slimani et al., 2010, Wang, 2010, Xiaoqi Zhou et al., 2012, Jing et al., 2014, Luan et al., 2014, Raiesi , Riahi, 2014, Wang et al., 2014 and others). At the same time contemporary science lacks comprehensive analysis that could take into consideration the basic components of ecosystem during isolation. In other words, the process of restoration of pasture ecosystems in cryoarid zones during isolation remains unexplored. This kind of information is definitely significant not only for recultivation of degraded lands, but also during evaluation of self-healing ecosystems located in severe climate zones. In this respect, the alas ecosystems of Central Yakutia exist in severe conditions such as permafrost, lack of precipitation and soil salinization.

The goal of this work was to monitor changes in these particular figures: above-ground phytomass – below-ground phytomass – soil organic substance – soil microbial activity during short-term isolation of alas pasture. As a result, it became possible to evaluate speed and self-healing features of cryolithozone ecosystems.

MATERIALS AND METHODS

Study site. The experiment was performed in the Central Yakutia (62°28´29.7" N 130°56´40.5" E 62°33´24,3" N 130°54´01,4" E). Climate in this region is cryoarid continental. The annual precipitation reaches 247 mm, including 162 mm during May–September and 85 mm in October–April with evaporation of 420–500 mm. The average annual temperature varies from –7 to –10°C, while summer temperatures frequently reach 30–35°C and the soil surface is warmed to 50°C. However, the close permafrost layer makes these soils the coldest in the Northern Hemisphere (Alas Ecosystems, 2005).

Alases are geomorphological structures only characteristic for cryolithozone. A specific ecosystem has established in the thermokarst hollow, which is represented by a set of biomes from marsh (lower hydrothermal belt) ranging from meadow (middle belt) and steppificated (upper belt) to a typical. According to the regional classification (Elovskaya, 1987, Desyatkin, 2008), the soils of the lower belt are permafrost sod–gley, soils of the middle belt are permafrost meadow, and soils of the upper belt are permafrost steppificated. Russian and international classification of these soils has not been developed yet. All of the examined soils are salty (sod–chloride types) with water pH reaching 9.5. Granulometric composition is light loamy.

We have examined two typical mature hollow thermokarst alases with different degrees of human impact localized to the Tyungyulyun terrace (the fifth above_floodplain terrace of the Lena River) in the northern part of the Lena–Amga interfluvial area. Grass stand of Toburuon background alas is a hayfield where animal grazing is limited. Grass stand of Uelan alas is degraded as a result of overgrazing (III degree digression).With a recommended pasturing level of 0.5 livestock units/ha, pasturing in this alas reaches six livestock units. This is a typical situation for most Central Yakutian alases located in the vicinity of settlements. In order to study self-regeneration of alas ecosystem components after grazing exclusion in 2009 degraded Uolan alas were fenced off areas to prevent access of animals.

Vegetation sampling

Geobotanical investigations were conducted during 2009 - 2013. Botanical hay harvest was taken from the square 1m^2 in the period of mass flowering herbs. There were 3-5 replicates for each sample. Projecting cover was evaluated in following scale: + - species cenopopulation had insignificant share in process of phytocenosis; 1 point – the projective cover of plants up to 5%, 2 – is within 6-15%, 3 – is within 16-25%, 4 – is within 26-50 %, 5 – more than 51 (Mirkin et al., 1989). Latin names of plants were provided by Cherepanov (1995) report.

Soil sampling and analysis

The data provided in this report is based on the analysis of soil samples selected in June 2013 and 2014 from 0-10 cm layer. There were 6 replicates for each sample. Below-ground phytomass was determined by washing 10x10x10 cm of soil monoliths away using 0.25 mm strainer. Soil organic carbon (SOC) was determined by bichromate oxidation (Nikitin, 1999), labile organic carbon (LOC) – in direct 0.1n NaOH extract, total organic nitrogen (TON) – by Kjeldahl method. Invertas activity was determined using liquid Fehling's reagents (Methods, 1980).

Microbial analysis

Nitrification rate was found out by soil composting upon optimum hydrothermal conditions. The total numbers of cultivable bacteria were determined as colony forming units (CFUs) on agar plates. The medium composition was following, g/l: fish extract peptone medium 0.3, peptone 0.6, glucose 0.1. To account the number of CFU we used highly diluted organic medium. Small diameter of colonies and relatively low rate of its' growth on agar surface allowed more detailed differentiation of observation variants. The functional diversity of the soil microbial complex was assessed using a CLPP (Garland, Mills, 1991, Gorlenko, Kozhevin, 2005) with some modifications (Danilova, 2014). This test estimates the activity of soil microbial complex according to the utilization intensity of various carbon sources, where the intensity is assessed by the color reaction with tetrazolium salts. 24 substrates (Dulcic, Inositol, lures, sorbitol, glycerol, maltose, lactose, sucrose, raffinose, glucose, arabinose, rhamnose, xylose, galactose, fructose, starch, cellulose, urea, K citrate, NH_4-citrate, K malate, K-Na tartrate, NH_4 oxalic, TWIN 80) were involved in the test. Soil suspensions were prepared by suspending 10 g of fresh soil in 100 ml of sterile phosphate buffer solution (0.05M Na_2HPO_4/NaH_2PO_4, pH 6.8). Aliquots 0, 1 ml from 10^{-1} dilution were pipetted in tubes of Eppendorf containing 0.5 ml of a mixture of the substrate, Czapek's medium without sugar, peptone. The final concentration of substrate in the reaction mixture was equal to 0.15, peptone – 0.10%.The tubes were incubated in the dark at 28^0C, after incubation of 40 h mixture were pipetted to each well in the microplate and the color of mixture is considered visually.

Richness of function spectrum was calculated as a sum of points of all 24 substrates utilization (Zak et al., 1994). Evenness of function spectrum was calculated using this formula:

$$\mathbf{E = 1/\sum (p_i)^2}$$

where p_i is a proportion of each substrate utilization in sum of all substrates (Degens et al., 2001).

Resistance of function spectrum of soil microbial community was estimated according to scope of E-index variation (**V_E**= σ/M *100%, where σ – standart deviation, M – mean) during stress (soil composting without additional source of carbon in optimal hydrothermal conditions). Accounting was conducted after 0, 15 and 30 days since the beginning of the experiment. The decrease **V_E** indicates an increase functional stability of microbial communities.

Data analysis

Experimental data was through traditional dispersion analysis (ANOVA). Similarities between variants of tests were estimated through results multivariative analysis that were based on principal component analysis, PCA (Statsoft STATISTICA software package) (Borovikov, 2003).

RESULTS

The object of this study was a part of catena. There is steppe meadow on the upper part of the poorly moisturized slope (steppificated belt). On overgrazing pasture *Carex duriuscula* and *Artemisia jacutica* are prevailing species while others are rare. Above-ground biomass amounts 0.3-0.6 t per ha. Average height of grass stand equals 5-10 cm. *Polygonum aviculare и Suaeda corniculata* are prevailing in central part of the slope (middle meadow belt). Above-ground phytomass varied yearly. In some years it reached 3 t/ha when *Suaeda corniculata* galophyte weed overgrew. In two or three years after isolation graminoid share increased to 50-57% of overall phytomass when initial amount was less than 1%. At the same time *Hordeum* *brevisubulatum* dominated the higher belt while *Puccinellia tenuiflora* became widespread in meadow belt (Table 1).

Table 1: Projecting cover of grass stand at the grazing exclusion (degraded alas Uelan)

Parameter	2009	2010		2014	
	Before exclusion	Pasture	Exclusion	Pasture	Exclusion
Steppificated belt					
Projecting cover (%)	70	60	70	60	80
Height of grass stand, cm	5-10	10	50	10-15	60
Hordeum brevisubulatum (Trin.) Link	+	+	3	2	3
Elytrigia repens (L.) Nevski	1	1	1	+	
Poa transbaicalica Roshev.	+		1	+	3
Carex duriuscula C.F. Mey.	4	4	3	5	1
Artemisia jacutica Drob.	2	2	2	1	
Crepis tectorum L.	+	+	1	+	2
Plantago media L.	+	+	+	1	+
Taraxacum ceratophorum (Ledeb.) DC.	1	1		+	+
Potentilla norvegica L.	+		1	+	+
Descurainia sophia (L.) Webb. ex Plantl	+				
Saussurea amara (L.) DC.	+				
Polygonum aviculare L.	+	+	+	+	
Knorringia sibirica (Laxm.) Tzvel.	+		1	+	2
Lepidium densiflorum Schrad.	1		+	+	
Artemisia commutata Bess.		+			
Suaeda corniculata (C.A. Mey.) Bge		+	+		
Chenopodium album L.		+			
Atriplex patula L.			+		
Thalictrum simplex L.					+
Erigeron acer L.					+
Meadow belt					
Projecting cover (%)	80	60	80	60	70
Height of grass stand, cm	3-5	5-10	60	10-15	50
Puccinellia tenuiflora (Griseb.) Scriber et Merr	1	1	3	1	3
Suaeda corniculata (C.A. Mey.) Bge	4	5	2	4	2
Knorringia sibirica (Laxm.) Tzvel.	1	2	3	2	5
Glaux maritima L.	1	2		3	
Potentilla anserina L.	2	1			
Chenopodium album L.	+				
Polygonum aviculare L.	4				
Taraxacum ceratophorum (Ledeb.) DC.	1				
Atriplex patula L.	1				
Corispermum sibiricum Iljin	1				

Judging by stock of above-ground phytomass, productivity of examined undisturbed grass ecosystems was similar to productivity of Tuvinian and Transbaikalian steppes. Rates of productivity significantly decreased due to pasture digression. However, a year after isolation the rate reached background values on steppificated belt. In 4 years the both steppe and meadow belt rates exceeded background values by 40% and 50% (Fig.1A). After 4-5 years of isolation the amount of below-ground phytomass increased only on meadow belt perhaps due to increased share of graminoids in this variant (Fig.1B). The level of SOC upon grazing exclusion almost reached the undisturbed alas level. However, due to highly spatial variability of the index there not always was a possibility to statistically prove this inference (Fig.1C). The amount of CFU in steppe belt of degraded alas exceeded background alas indexes. There were no differences on meadow belt (Fig.1 D).

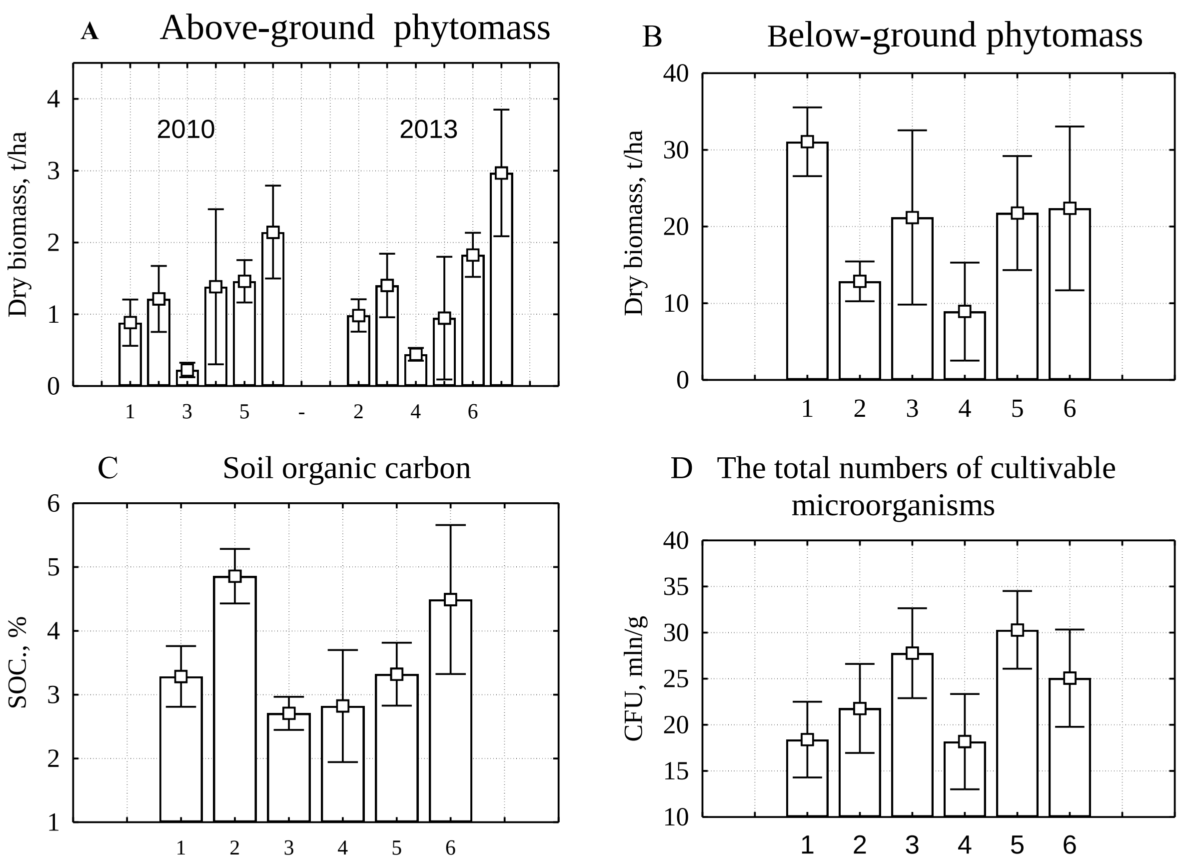

Fig.1 Biodynamic of degraded pasture at the grazing exclusion (0-10 cm)

1,2 - background alas steppificated and meadow belts respectively
3,4- degraded alas steppificated and meadow belts respectively
5,6 – pasture exclusion steppificated and meadow belts respectively
Vertical bars denote confidence interval P 095

Judging by stable C/N, LOC/SOC ratios, the changes in soil organic matter were quantitative and did not impact its quality (Table 2). Invertase activity of soil as a resulting index of rootage interaction, microbial complex and solid phase of the soil (Burns et al., 2013) after exclusion increased by 40% on meadow belt and by 30% on steppe belt isolation and reached background alas indexes. Activity of autotrophic nitrification increased during pasture digression and did not decrease after 5 years of isolation, remaining 3 times higher than background values.

Table 2: Some properties of alas soils after 5 years of isolation (0-10 cm)

Parameter	Background alas		Degraded alas: Pasture		Degraded alas: Exclusion		CI
	A	B	A	B	A	B	
C/N	12	10	14	9	12	10	2
LOC/SOC.,%	14	4	16	9	16	9	4
Nitrification rate, mg $N\text{-}NO_3$/kg 14 days	19	16	75	55	70	62	18
Invertase, mg glucose / g 18 h	33	35	30	24	39	34	9
A- steppificated belt, B - meadow belt, CI – confidence interval, P 095, n=6							

Functional diversity (FD) of soil microbial community during isolation had changed significantly in comparison to original one (Fig.2). At the same time, steppe belt indexes reached background values. Meadow belt index during isolation differed from background value as well as from degraded analog and resembled steppe belt of degraded alas. Judging by total activity (richness 84 points) and evenness (E=24), steppe belt soils on background alas after isolation had more active and diverse microbial community than the meadow ones (7, 8 respectively). Isolation furthered activation of meadow belt microbial community, indexes reached 48 and 12 respectively, variant reached steppe belt of degraded alas.

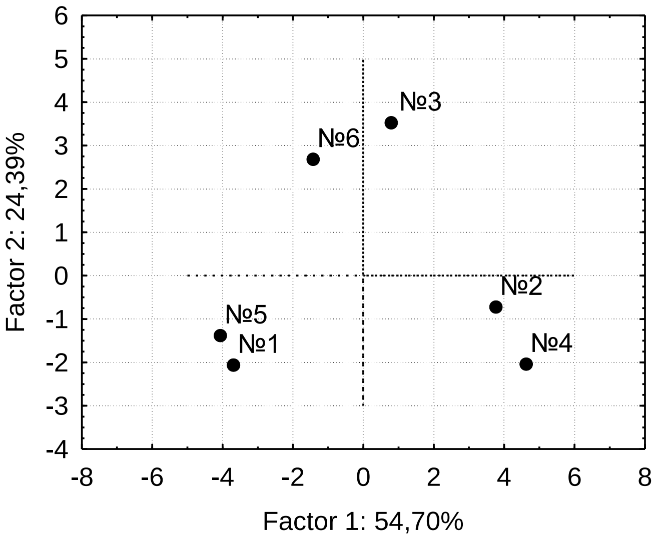

Fig.2 Functional diversity of soil microbial community in 4-5 years after isolation

1,2 – background alas, steppificated and meadow belts respectively, 3,4 – degraded alas, steppificated and meadow belts respectively, 5,6 – grazing exclusion, steppificated and meadow belts respectively

In order to determine the ecological significance of mentioned changes, comparative resistance of function spectrum (functional resistance) of microbial community was studied (Table 3).

Table 3: Relative increase of soil microbial community's resistance ability after 5 years of grazing exclusion

Evenness (E)	Background alas		Degraded alas			
			Pasture		Exclusion	
	A	B	A	B	A	B
Afer 0 days	16	19	13	13	15	18
After15 days	23	22	19	13	22	16
After 30 days	14	18	12	7	20	16
Mean	17	19	15	11	19	17
V_E.,%	30	10	30	**30**	20	**8**
A – steppificated belt, B – meadow belt, n = 9						

Judging by the scale of fluctuations of evenness (**V_E**) under stress, the functional resistance of soil microbial community of steppe belt of background alas and pasture were almost the same. On meadow belt overgrazing contributed to a sharp decrease in the resistance of the soil microbial community. Grazing cessation contributed to functional diversity stabilization in both objects, especially in meadow belt.

DISCUSSION

As is known, during grazing exclusion, vegetation is restored quickly enough and the stock of above-ground phytomass usually reaches the background values in 2 - 6 years, but the botanical composition of grass stand recovers at least 10-25 years. (Gorshkova, Saharovskiy, 1983, Stepi .., 2002, Rusanov A., 2011, Wang et al, 2010).

According to our data, phytomass stock on third-stage degraded alas pasture exceeded backround values after 4 years of isolation. Thus, despite adverse conditions, productivity of alas meadows recovered quickly enough during isolation.

Recovery patterns of below-ground part ecosystem after grazing exclusion has been studied relatively less than the aboveground one. Geographically these data relates to soils of Khakassian, Tuvinian, Mongolian arid pastures (Stepes ., 2002), to loess plains of China (Jing et al., 2014 and others), Inner Mongolia (Su et al., 2005, Pei et al., 2008, Li et al., 2012), Iran (Raiesi , Riahi , 2014), North Africa (Slimani et al., 2010) etc. It was determined that in genuine steppe conditions below-ground phytomass accumulates 5 years slower than above-ground phytomass and reaches maximum amount after 15 years since the start of the experiment (Jing et al., 2014).

According to our data, stock of below-ground phytomass on the steppe belt alas pastures reached 30t/ha in soil layer 0-10 cm. This rate is higher than that of pastures mentioned in literature above. Presumably, our object showed even higher rate. The fact is that in mortmass the fraction smaller than 0.25 mm became significant, also the share of carbonized plant residues that were impossible to separate from sand during decantation was large, so these two parts were impossible to take into account. These particular features of below-ground phytomass of alas steppe belts are obviously related to specific conservation of plant residues in cryoarid conditions under salinization. Smaller stock of below-ground phytomass in meadow belt is obviously related to decomposition in favorable moisture conditions.

According to literature data, total reserve of below-ground phytomass changes insignificantly during short-time isolation (2-3 years) (Stepes.., 2002). Such pattern was detected on steppe belt of alas pasture, whereas meadow belt showed significant increase of this index which is due to increased amount of graminoids in grass stand.

Increased stock of above-ground and below-ground phytomass after isolation was accompanied by increased amount of SOC (Su et al., 2005, Pei et al., 2008, Slimani et al., 2010, Li et al., 2012, Raiesi , Riahi , 2014, Jing et al., 2014). This process is the most intense during first years of isolation, in arid conditions in poor soils after 6-12 years the index reached background values (Su et. al., 2005, Li et al., 2012).

Marked changes in the contents of SOC not have any impact on the quality of its composition, which follows from the fact that the ratio C/N and LOC/SOC remained stable, typical steppe and meadow soil.

According to several authors, increased content of SOC is related to soil enzymatic activity during isolation (Su et al 2005, Shi et al 2013, Raiesi, Riahi 2014). In our case, we did not observe a stable correlation between these two indicators. Probably, the invertase activity level of investigated soils was related to the number of living roots in greater extent. As known, the synthesis of extracellular enzymes by the cells of the microorganisms in response to receipt in the soil with fresh organic matter increases abruptly only in the presence of roots of higher plants (Averill, Finzi, 2013).

It is known that soil microbial complex is an important part of the below-ground ecosystem and is recognized as a major factor in the cycle of matter. Microbial biomass carbon usually is used to characterize soil microbial system in conditions of long-term isolation. In case of short-term isolation this index is not sensitive enough. In our work the number of CFU and functional diversity of cultivated part of the microbial community were used to indicate changes in soil microbial system. This approach is highly informative because this particular part of community is usually the first to respond to the supply of fresh organic matter. The number of CFU in our experiment probably correlated with the content of available nitrogen-containing organic compounds in soil. Apparently, manure was main source of these substances (Danilova et al, 2013). This was especially evident in steppe belt, where degraded alas indexes were higher than undergraded alas indexes. In meadow belt, manure influence was obviously mediated by other limiting factors, e.g. changes in number and composition of salts in soil.

The CLPP method is recognized as a complementary approach to the assessment of microbial diversity in soil. CLPP method used in our investigation allowed us to evaluate stability of soil microbial community during isolation. Stability of any community consists of two components – resistance and resilience. Resistance and resilience that are referred to as an ability to withstand perturbations and to recover from perturbations, respectively (Seybold et al., 1999; Griffiths et al., 2000, Zhang et al., 2013)

It was found out that the resilience of soil microbial complex of steppe belt is considerably high. After 4-5 years of isolation the FD of microbial community has almost reached background alas indexes. Indexes of meadow belt microbial community were equally low on background alas as well as on degraded alas. During isolation these indexes increased significantly and reached steppe soil indexes on degraded alas. Probably the main reason for this is convergence of plant residues as a result of increased ratio of graminoids in the vegetative cover of the meadow zone.

Special characteristics of succession of microbial communities during soil composting without additional carbon sources was used as main criteria for assessing the resistance of ecosystems. Analysis of the evenness (E) variation metric showed relative increase of community's resistance ability even after short-term isolation. Thus, increased functional diversity and resilience of cryoarid soil microbial communities in the short-term isolation of degraded pastures was observed.

In our previous publication (Danilova et al., 2013) it was shown that the excessive increase in the activity mineralization processes in the soil is the main reason of instability of degraded alas ecosystems. As follows from the presented data, in the short-term isolation the activity of autotrophic nitrification did not decrease. Therefore, the upturn of FD saprophytic community and other studied objects is only the beginning of the recovery process, while the ecosystem of degraded pasture remains sufficiently disturbed.

CONCLUSION

Thus, grazing cessation on degraded pasture in permafrost zone led to similar phenomena observed in similar cases in other regions of the world. Sward productivity increase and release of nutrients from manure were common causes of these changes. Biodynamic features of degraded alas meadows in early years after isolation indicate capability of these ecosystems to regenerate. At the same time, high activity of autotrophic nitrification corroborates continuing imbalance in turnover in the soils of the isolated area.

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