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Original Research

Positive Effects of Perennial Wheat on Soil Fertility, Carbon Stocks and Microbial Biomass in South-Eastern Kazakhstan

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Abstract

Perennial wheat yields for several years after sowing, and develops a deep and dense root system, thereby improving soil structure and preserving soil fertility. It is also drought resistant, which increases its future potential under the ongoing climate change. Moreover, perennial wheat offers and effective strategy for carbon sequestration through increasing soil carbon stocks. However, the effect of perennial wheat on soil properties remains uncertain in the conditions of south-eastern Kazakhstan due to the lack of data. To fill this gap, we conducted a study in which five varieties of perennial wheat were grown in the Almaty region in soils of different fertility for two years. As a result, in the plot where topsoil was removed to a depth of 25 cm, total carbon increased by nearly three times, from 0.54% to 1.42%, and the number of microorganisms increased from 715.1x103±0.2x103 to 48.0x103±0.6x103 colony forming units per ml of soil. The photosynthetic performance of perennial wheat varieties in the experimental plot was not negatively affected by the removal of top soil suggesting that perennial wheat can successfully restore soil fertility and replenish soil with chemical elements involved in photosynthesis. Our results confirm that in the conditions of the south-eastern Kazakhstan perennial wheat makes a significant contribution to the soil carbon stock accumulation and development of soil biota; in our study, these effects were particularly strong in the case of degraded soil.

Keywords: carbon sequestration, microorganism, number of microorganisms, perennial wheat, photosynthetic capacity

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Introduction

Perennial wheat is one of the most important grain crops and meets about two-thirds of the protein and energy needs of the world's population [1]. Perennial wheat is a staple food that feeds approximately 30% of the world's population [2]. Global food security is threatened by rapid population growth and abrupt climate change [3]. Water scarcity in developing countries, mainly in semi-arid and arid regions, is a major problem affecting crop growth and yields, and leading to food security concerns. An integrated foliar nutrient management system can improve growth, yields, and other components of crop management in arid and semi-arid regions. Studies addressing the effect of complex foliar application of nutrients on field crops are limited [4, 5].

Globally, about 30-40% of all cultivated lands has low crop yields due to the phosphorous (P) shortages. In soil, P is usually present in the soluble form, insoluble mineral form and as organic P. Approximately half of the total volume of soil P is organic [6, 7]. Therefore, top soils are the most important indicators showing the level of heavy metal pollution in the air [8, 9].

Global temperatures are rising rapidly due to increasing concentrations of greenhouse gases. It is a matter of concern that the atmospheric CO_2 concentrations are increasing despite the fact that carbon dioxide has been identified as a major greenhouse gas. Agricultural lime is a carbon source that eventually enters the atmosphere as CO_2 [10]. Microbial respiration is the main channel for the release of organic carbon from soil, which varies depending on soil management practices. That is why stabilization of greenhouse gas emissions from agricultural land, especially those of CO_2, is an essential component of climate change mitigation [11, 12]. Heavy metal concentrations and other environmental indices should be constantly monitored to comprehensively understand the behavior and associated risks of heavy metals in aquatic environments [13-15]. Among various microorganisms living in the rhizosphere, there are those stimulating plant growth and defensive mechanisms. Plants are the most important living groups in the world and form the basis of the food pyramid. Therefore, life on Earth is largely dependent on plants, directly or indirectly [16, 17]. In natural pollution, nature can clean itself in a short time through recycling mechanism [18, 19]. The association with microorganisms gives plants various benefits, including increased resistance to various biotic and abiotic stresses, increased production and accumulation of secondary metabolites, improved nitrogen fixation, osmotic regulation during drought stress, and increased photosynthetic capacity [20]. Millions of microorganisms are present in soil, and more than 85% of them are beneficial to plants and give vitality to soil systems [21]. Destruction in vegetation tends to bring about changes in biodiversity, in climate, and in ecosystem services [22].

Phosphate-soluble bacteria (PSB) reduce the negative impact of soil calcification on the soil phosphorus content. Unavailable phosphorus compounds may become available to plants due to phosphate-absorbing bacteria. The active strains involved in this transformation are *Pseudomonas, Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium*, and *Sinorhizobium* [23]. Soil is the main organic carbon pool for the terrestrial ecosystems. The reduction in soil organic carbon (SOC) decreases productivity, damages biological diversity and ecosystem resilience [24, 25]. Although micronutrients such as Mn, Zn, Cr, Cu, Fe, and Ni are required for living organisms including plants, they can also produce harmful effects in high concentrations [26-28]. Agricultural soil preservation and soil fertility improvement are considered adaptive restorative practices. Hybridization between wheat and wild wheatgrass has been found to be very effective in improving soil fertility; in particular, nitrogen and phosphorus increase carbon sequestration [29, 30].

Perennial agricultural crops have an advantage over annual crops in terms of reduced soil erosion, increased biomass and diversity of soil microorganisms, and increased soil carbon sequestration [31]. This is particularly relevant for Kazakhstan where, according to the United Nations Development Program, 66% of land surface is in danger of degradation and desertification (https://www.undp.org/kazakhstan/stories/landscape-planning-guardianship-safeguarding-ecosystem) [32]. One such crop is perennial wheat developed by the Land Institute, USA (https://landinstitute.org) through hybridization of winter durum wheat with intermediate wheatgrass. Because perennial wheat is a relatively new crop for Kazakhstan, to our knowledge there is no data on the impact of perennial wheat on soil fertility, including the number and diversity of soil microorganisms, and the soil carbon stocks [33].

Microbial biomass is an important living component of soil; it also plays a major role in the formation of soil aggregates [34-37]. In the conditions of sub-humid Mediterranean climate, a comparison of the rhizosphere environment of perennial wheat at the first and fourth year of growth revealed an increase in microbial biomass and enzymatic activity, and an increase in the diversity of microbial community composition [38, 39]. We hypothesized that similar changes will be observed in the conditions of the south-eastern Kazakhstan. Soil carbon sequestration is a conversion of atmospheric CO_2 into soil organic matter to achieve its long-term preservation [40, 41]. Plants absorb carbon dioxide in the process of photosynthesis. When plants die, the carbon in the leaves, stems, and roots turns into the soil organic matter. Therefore, green house gas emissions that drive global climate change can be reduced through biological carbon sequestration [42, 43]. In our study we tested the hypothesis that over time soil carbon would increase under perennial wheat lines.

Photosynthesis is a process in which light energy is captured by a crop and converted into biomass; thus crop yield is largely determined by the photosynthetic efficiency of plants in question [44]. The main factor determining the formation of photosynthetic capacity of field crops is the optimal provision of plants with available forms of nutrients during the vegetation season. Therefore, to adapt plants to the soil mineral conditions, it is necessary to take into account various processes and factors [45]. We tested the photosynthetic capacity of perennial wheat growing in two types of soil: undisturbed and degraded by the removal of the topsoil to a depth of 25 cm. Given a presumed ability of perennial wheat to restore soil fertility and replenish soil with chemical elements involved in photosynthesis.

The overall objective of our study prospects for growing perennial wheat in southeastern Kazakhstan by investigating the effects of perennial wheat on general soil properties, including microbial composition and carbon stocks, and by measuring leaf photosynthetic efficiency in different soils.

Material and Methods

Experimental Materials

The study was carried out in the Almaty region in 2021-2023. The experiments were carried out in three replications. We studied the soil chemical composition, evaluated the number and diversity of soil microorganisms, and measured the photosynthetic capacity of perennial wheat leaves.

Study Area and Experimental Settings

The soils of the experimental site are foothill dark or light chestnut soils formed on loess-like loams, with a clearly pronounced fertile horizon. According to the mechanical composition of soils, it belongs to coarse dusty medium loams, the content of physical clay is 29-32%, and that of the coarse dust, 40-50%. The availability of easily hydrolyzable nitrogen is medium, of mobile phosphorus is low, and of exchangeable potassium is medium. In the upper horizon, the humus content is up to 2.4%.

Topsoil removal has a strong negative effect on physical and chemical soil properties, and reduces soil microbial diversity and abundance [46-48]. To study the effect of growing perennial wheat in different soils, we carried out an experiment with the following treatments (Table 1): C1 and C2 – control without and with perennial wheat; T1 and T2 – topsoil removal to a depth of 0.25, without and with perennial wheat.

Soil Sampling for Agrochemical Analysis

Spot samples (12 in total) were taken according to GOST 17.4.4.02-2017 [49] by a soil drill from a depth of 0-20 cm, each sample weighing 200 g (Fig. 1).

To determine the chemical content of soils the following parameters were measured: total humus, total organic carbon, easily hydrolysable nitrogen, mobile phosphorus, mobile potassium, CO_2, and soil pH. The list of analyses performed and methods used is provided in Supplement (Table S1).

Microbiological Analyses

Soil samples were taken from a depth of 0-20 cm and placed in sterile paper bags before sowing and in the phase of the wheat full ripeness. In total, eight soil samples were taken for microbiological analysis.

To determine the number of microorganisms we used the Koch's method [50]. Following this method, 10 g of soil sample was placed in a 250 ml flask containing 100 ml of sterile tap water and shaken for 30 min on an orbital shaker at 120 rpm (BiosanOS-20, Latvia). The resulting soil suspension was inoculated by the method of limiting dilutions on meat-peptone agar (MPA). Petri dishes with MPA were incubated in a thermostat at a temperature of +27ºC. Bacteria were counted on the third day; the number of ecological-trophic groups of microorganisms was measured in colony-forming units (CFU) per 1 gram of absolutely dry soil.

Very rarely, pure cultures of microorganisms survive in vivo. It takes 2-3 days to isolate pure cultures of most bacteria. To isolate pure cultures, we obtained accumulative cultures, isolated pure cultures, and checked the purity of the isolated cultures.

The identification of soil bacteria in sites with perennial wheat was carried out using morphological, physiological and biochemical properties of bacterial cultures according to Mishustin et al. [51].

Table 1. Agricultural treatments used in the study.

Site	Number	Area, ha	Varieties of perennial wheat	Treatment
Control without perennial wheat	C1	0.25	-	Topsoil was not removed
Control with perennial wheat	C2	0.25	No.701, No.702, No.703, No.704, No.801	Topsoil was not removed
Soil treatment without perennial wheat	T1	0.25	-	Topsoil removed to a depth of 0.25 cm
Soil treatment with perennial wheat	T2	0.25	No.701, No.702, No.703, No.704, No.801	Topsoil removed to a depth of 0.25 cm

Fig. 1. Soil sampling for agrochemical and microbiological analyses.

Various representatives of bacilli, actinomycetes, filamentous fungi and other microorganisms can emit protease. The activity of proteases can be determined through cellular exterior using gelatine, casein, etc. proteins as a substrate. In our study, the activity of proteolytic enzymes was determined using gelatine as a substrate. To prepare the medium, 10-15 g of gelatine is added to 100 ml of meat-peptone broth (MPB) and allowed to swell for 20-30 minutes. Then, in a water bath, the medium is boiled until gelatine is completely dissolved. The resulting medium is poured into 8-10 ml test tubes. The tubes are disinfected for 15-20 minutes at 0.5 atm. The microorganisms are then inoculated with a microbiological needle. The germination time is 7-10 days. Liquefaction of gelatine was visually noted. We recorded the degree of gelatine liquefaction and shape (crater-shaped, swede-shaped, funnel-shaped, saccular, layered). The proteatic enzymes catalyze the cleavage of proteins into poly- and oligopeptides.

Measuring Photosynthetic Efficiency of Perennial Wheat Leaves

To determine chlorophyll fluorescence, 30 leaves were taken in total: 15 leaves in the control, and 15 leaves in the experimental site. Fluorescence-related parameters were evaluated by determining fluorescence levels. RLC were recorded using Junior-PAM (Heinz Walz, GmbH, Effeltrich, Germany) under 450 nm actinic illumination [52]. Each time, the top leaf was selected (n = 3). To measure chlorophyll fluorescence, the middle third of the leaf was used, since this area has the most homogeneous structure [53]. All measurements were taken on sunny days from 10 am to 12 am. The leaves were shaded with a clip before recording the RLC. For each measurement, the fluorometer produced eight light saturation pulses at 10,000 µmol (photon) m-2 s-1 every 20 s, while the actinic light increased after each pulse from 0 to 65, 90, 125, 190, 285, 420, and 625 µmol (photon) m-2 s-1. The equipment recorded the fluorescence shortly before the saturation pulse (Ft), and the minimum (F0’) and maximum (Fm’) values of the fluorescence output after each pulse in the open state of the PSII reaction center after far red illumination. The following parameters were calculated using WinControl-3.29 (Heinz Walz, GmbH, Effeltrich, Germany) software at PPFD 65 and 625 µmol m-2 s-1: (1) effective photochemical quantum yield of PSII, Y(II)65 and Y(II)625; (2) quantum output of non-photochemical energy conversion in PSII due to suppression of the light capture function, Y(NPQ)65 and Y(NPQ)625; (3) quantum output of non-photochemical energy conversion in PSII other than caused by downregulation of the light capture function, Y(NO)65 and Y(NO)625; and (4) PSII electron transfer rate, ETR.

Results and Discussion

The Influence of Perennial Wheat on Agrochemical Properties of Soil

According to the results of agrochemical analysis (Table 2), topsoil removal (T1) significantly reduced the carbon, nitrogen, phosphorus, potassium, and total humus content. In the treatment with topsoil removal (T2), perennial wheat had a strong positive effect on the accumulation of soil humus, carbon and nitrogen. In this treatment (T2), the relative total carbon content was almost three times higher than in the treatment without perennial wheat (T1), 1.42% and 0.54%, respectively. The relative total carbon content was also higher in the treatment with perennial wheat (T2) than in the control with perennial wheat (C2), 1.42% and 0.82%, respectively. This result can be explained by the fact that perennial wheat has to allocate resources to seasonal survival; it develops and maintains a strong root system thus contributing to carbon accumulation in soil [54].

Table 2. Results of soil agrochemical analysis.

Site	Treatment	Monitored parameters						
		Total humus %	Total carbon %	Mobile			pH	CO_2
				Nitrogen mg/kg	Phosphorus mg/kg	Potassium mg/kg		
C1	Control without perennial wheat, 0-20 cm, primary data	1.40±0.02	0.8±0.4	48 ±1	144±3	1000±	8.40	2.89±0.08
C2	Control with perennial wheat, 0-20 cm, final data	1.43±0.02	0.82±0.04	48 ±1	162±3	750±5	7.52	2.92±0.08
T1	Treatment, without perennial wheat, 0-20 cm, primary data	0.94±0.01	0.54±0.03	28.0±0.5	176±4	840±5	8.59	2.96±0.09
T2	Treatment, with perennial wheat, 0-20 cm, final data	2.4±0.4	1.4±0.1	33.6±0.8	88±2	530±3	8.15	2.51±0.07

The Diversity and Abundance of Soil Microbial Communities

The results of microbiological analysis show that in soils with perennial wheat there were more aerobic microorganisms than in soils without perennial wheat (Table 3). The total number of microorganisms in the soil with perennial wheat was 715.1 × 10^3 CFU/ml and 160.4 × 10^3 CFU/ml, while the number of microorganisms in soil without perennial wheat was much lower, 48.0x10^3 CFU/ml and 73.1x10^3 CFU/ml, respectively. A similar result was obtained when studying the spectrum of biological activity of microorganisms to clarify their biotic connections in the "soil-microbial community-plants" system; it was found that the number of soil microorganisms increases due to the increased amount of residue of perennial plants [55].

In our project, we isolated the following five pure cultures of microorganisms: MN-1, MN-2, MN-3, MN-4, and MN-5. The macromorphological characteristics of these cultures are presented in Table 4.

In the course of the project, the following physiological properties of the five aboriginal microorganisms were studied: mobility, spore formation, Gram staining (traditional), the ability to form capsules, proteolytic activity, and the ability to grow at high temperatures (46^{0}C). The results are presented in Table 5. As can be seen from Table 5, two cultures are proteolytically active, four are capsule-forming, three are Gram positive, five are mobile, and three are spore-forming.

Photosynthetic Capacity of Perennial Wheat

As follows from the data presented in Fig. 2, growing conditions in the site with treatment had a positive effect on the photosynthetic performance of perennial wheat. In the site with treatment, there was is a slight

Table 3. Total number of aerobic microorganisms in the soil at a depth of 0.2 m.

№	Treatment	Total number of microorganisms, CFU/ml
C1	Control without perennial wheat, 0-20 cm, primary data	73.1x10^3±0.1x10^3
C2	Control with perennial wheat	160.4x10^3±0.2x10^3
T1	Treatment without perennial wheat, topsoil was removed to a depth of 25 cm, primary data.	48.0x10^3± 0.6x10^3
T2	Treatment with perennial wheat, topsoil was removed to a depth of 25 cm.	715.1x10^3±0.2x10^3

Table 4. Macromorphological characteristics of soil microbial communities in soil with perennial wheat.

№	Medium	Pure culture	Colony description
1	MPA	MN-1	Round, white, smooth surface, smooth edges, shiny
2		MN-2	Round, grey, smooth surface, smooth edges, shiny
3		MN-3	Round, yellowish in color, with smooth surface and smooth edges, shiny
4		MN-4	Irregular shape, white, surface rough, edges fringed, dry
5		MN-5	Irregular shape, white, surface rough, edges fringed, dry

Table 5. Physical and biochemical properties of microbial communities in soil with perennial wheat.

№	Pure culture	Mobility	Sporulation	Capsule	Gram staining	Proteolytic activity	Growth at 46ºC
1	MN-1	+	-	-	+	+	++
2	MN-2	+	+	+	+	+	++
3	MN-3	+	-	+	-	-	+
4	MN-4	+	+	+	+	++	++
5	MN-5	+	+	+	+	+++	++

Note: “ + “ property is expressed; “ - “ property is not expressed.
Growth capacity: “+ + +” strong, “++” medium, “+” weak.

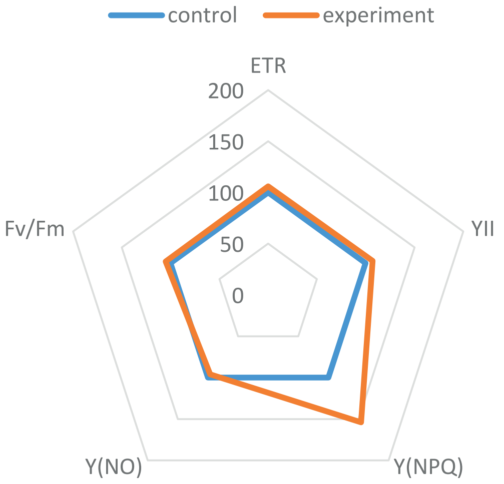

Fig. 2. Different activity of the photosynthetic apparatus of perennial wheat depending on soil conditions.

increase in the values of the maximum quantum yield of photosystem II (PSII) (Fv/Fm ratio), the effective photochemical quantum yield (YII) and the speed of cyclic electron transport through photosystem II (ETR): 105, 107 and 106% of the control, respectively. An increase in the values of these parameters is an indicator of the absence of photoinhibition and normalization of the performance of the reaction centers of photosystem II [56, 57]. The level of quantum yield of unregulated dissipation energy in photosystem II(Y(NO)) decreased slightly(down to 96% of the control) and the level of quantum yield of regulated energy dissipated in photosystem II (Y(NPQ)) increased significantly, up to 154% of the control. A decrease in the quantum output of unregulated heat dissipation and fluorescent radiation Y(NO) means that the fluxes of excess energy are under control, and an increase in Y(NPQ) indicates the normalization of the redistribution processes of excess light energy entering photosystem II [58, 59].

Perennial crop increased the soil carbon and nitrogen content compared to the annual crop. The physical characteristics of soil are of paramount importance for the root development, water and air movement, as well as for its subsequent impact on the soil chemical and biological processes. Management practices and their legacy can affect the physical quality of soil, and the cultivation of perennial crops has been proposed as a solution to maintain or improve the physical quality of soil in agro-ecosystems by providing year-round soil cover and enhanced root growth. In turn, increasing soil carbon with zero tillage has been shown to improve soil resistance to compaction. The main mechanism lies in the low density, high specific surface area and significant water absorption of soil organic matter [60]. It should be noted that carbon storage in the uppermost soil layers and comparison with cultivated systems should be based on samples taken outside the deepest depth of treatment [61]. The difference between the boundary of good physical quality of soil characterizes the lack of physical quality of soil, since the physical quality of soil is an important basis for the general condition of the soil [62, 63].

If a decrease in the physical quality of soil is associated with a decrease in the productivity and profitability of crops, improving or maintaining the physical quality of soil can improve and stabilize yields and profitability by increasing the availability of soil moisture [64], improving structure by increasing aggregation, and increasing biological activity [65]. However, the durability of an increased carbon content in the soil when growing a perennial crop increases, as evidenced by the increase in macroporosity caused by the perennial phase for two years after sowing alfalfa on soils sown with wheat and rapeseed. Thus, further studies are needed to assess the overall effect of including perennial crops in long-term diversified crop rotations to determine whether the improvements in soil physical quality obtained from perennial crops will continue in the next stages of crop rotation. As a result of our study, it was found that even if the topsoil is removed, the composition of the soil, the diversity of microorganisms and physiological characteristics improve compared to the soil without perennial wheat; in this regard, it is obvious that perennial wheat has a positive effect on the physical quality of the soil.

Conclusions

When studying perennial wheat in the south-east of Kazakhstan, the experiment was carried out on soils of different fertility. In the control, the soil was undisturbed, while in the treatment, topsoil was removed to a depth of 25 cm. To investigate the effect of perennial wheat on soil composition, agrochemical properties of samples of primary soils and the soils with perennial wheat growing for two years were studied. We also determined various characteristics of soil microorganisms in samples before and after the cultivation of perennial wheat. The photosynthetic capacity of the leaves was also compared between the control and treatment sites. The results of the study demonstrated that perennial wheat had a positive effect on agrochemical properties of soil as well as on the number and diversity of soil microbial communities. We conclude that in the conditions of the south-eastern Kazakhstan, perennial wheat offers a solution to the problems of soil erosion and degradation associated with traditional annual cropping systems.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Material

Table S1. Agrochemical analyses performed and methods used.

No.	Type of analysis	Method
1	Total humus, %	ST RK 3477-2019, Tyurina (ST RK 3477-2019 Soils. Determination of humus by the method of I.V. Tyurin)
2	Easily hydrolyzable nitrogen, mg/kg	Tyurin-Kononova (Determination of mobile nitrogen by the method of Tyurin and Kononova)
3	Mobile phosphorus (P_2O_5), mg/kg	GOST-26205-91 (GOST 26205-91 Determination of mobile forms of phosphorus and potassium according to the Machigin method modified by TsINAO)
4	Mobile potassium (K_2O), mg/kg	GOST-26205-91 (GOST 26205-91 Determination of mobile forms of phosphorus and potassium according to the Machigin method modified by TsINAO)
5	pH (aqueous)	GOST-26423-85 (GOST 26423-85 Soils. Methods for determining the specific electrical conductivity, pH and dense aqueous extract residue)
6	Aqueous Extract	GOST 26423-85-26428-85 (GOST 26423-85 Soils. Methods for determining the specific electrical conductivity, pH and dense residue of aqueous extract, GOST 26428-85 Soils. Methods for the determination of calcium and magnesium in water extract)