



Changes in biological soil quality indicators under saline soil condition after amelioration with alfalfa (*Medicago sativa* L.) cultivation in meadow Solonchak

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Abstract

Land use and amelioration practices are considered as main drivers in change of biological soil quality indicators in meadow Solonchaks. To gain insight into the impact of amelioration with alfalfa (*Medicago sativa* L.) cultivation on the underlying soil microbiological and biochemical properties, the objective of this study was to determine the effect of alfalfa (*Medicago sativa* L.) cultivation on biological soil quality indicators such as microorganisms counts, microbial biomass, basal soil respiration and enzyme activities (dehydrogenase, catalase, β -glucosidase, protease, urease, alkaline phosphatase and arylsulphatase) in meadow Solonchak. Post-amelioration with alfalfa cultivation influenced the soil microbiological and biochemical properties and increased soil organic matter content and improved biological soil quality indicators. The results of this study may contribute to future researches for soil microbial communities in different type of amelioration practices in soil quality and sustainable productivity meadow Solonchaks.

Keywords: Amelioration, microorganisms, saline soil, soil quality.

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Introduction

Soil salinity is one of the most serious abiotic factors restricting productivity of field, plant diversity and plant growth in arid and semi-arid regions, where soil salt content is high and precipitation is insufficient (Kazemi and Eskandari, 2011) Worldwide, about 30 million ha are severely affected by salinity and an additional almost 80 million ha are estimated to be affected to some extent (Umali, 1993). The area of saline soils in Kazakhstan, including Solonetz, alkaline soils, and complexes of Solonetz with other soils, is 111.55 million ha, or 41% of the national territory (FAO, 2015). Saline soils are present everywhere in the country except in mountainous areas. They are common in the steppe zone, where they cover about 30% of the area. In dry steppe, semi-desert and desert zones these soils occupy up to 50% of the area. Salt-affected soils are represented mainly by Solonetz and alkaline soils. Solonchaks cover only 1–3% of the area of salt-affected soils in the steppe zone, and 7–13% of the area of salt-affected soils in the semi-desert and desert zones (FAO, 2015).

The amelioration of problem soils is a very important goal throughout the world, especially with saline or saline-sodic soils (Mady, 2011). Sodic and saline-sodic soils possess poor physical properties and fertility problems that adversely affect the growth and yield of most crops (Sumner 1993; Grattan and Grieve, 1999). Saline-sodic soils reclamation is one of the main problems for humans in the future. The reclamation of

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saline soils uses many different methods such as physical amelioration (deep ploughing, subsoiling, sanding, profile inversion), chemical amelioration (amending of soil with various reagents: gypsum, calcium chloride, limestone, sulphuric acid, sulphur, iron sulphate) and biological amelioration (Oad et al., 2002). The biological amelioration methods using living (sowing new forms of leguminous plants) or dead organic matter (crops, stems, straw, green manure, barnyard manure, compost, sewage sludge) (Matsumoto et al., 1994) have two principal beneficial effects on the saline and alkaline soils reclamation: the improvement of the soil structure and permeability, thus enhancing salt leaching, reducing surface evaporation, and inhibiting salt accumulation in the surface layers; and the release of carbon dioxide during respiration and decomposition. For saline or sodic soils, the addition of organic matter can accelerate the leaching of Na, decrease the exchangeable Na percentage and electrical conductivity, and increase water infiltration, water-holding capacity, and aggregate stability (Lax et al., 1994; Qadir et al., 2001). Thus, the increase in the yield and quality of agricultural cultures, particularly in arid areas and on saline soils, can be achieved through the high-culture farming by scientifically sound ecologically safe use of new types of bio-fertilizer and biologics, sowing new forms of leguminous plants (especially leguminous vegetable), bio-amelioration with halophytes that have a positive effect on the biological soil quality indicators such as microbiological and enzymatic activities, soil respiration and the processes of humus-forming in soil, and, finally, soil fertility and productive capacity (Mady, 2011).

Soil quality has been defined as “the capacity of a soil to function within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality and promote plant and animal health” (Karlen et al., 1997) and can be assessed using a wide variety of biological, physical and chemical indicators (Doran and Parkin, 1994). Biological indicators typically include microbial biomass carbon (Jordan et al., 1995; Karlen et al., 1997) and microbial enzyme activities (Bandick and Dick, 1999; Eivazi et al., 2003). Microbial enzyme activities reflect metabolic factors and may serve as early indicators of soil quality improvement or degradation in agroecosystems (Dick, 1994). The purposes of the present work were to compare biological soil quality indicators such as microbial biomass carbon, enzyme activities and basal soil respiration between saline soil conditions and ameliorated soil with alfalfa (*Medicago sativa* L.) cultivation in meadow Solonchak of South Kazakhstan.

Material and Methods

Study site description

Experimental studies were carried out at the Teskensu (Kaz.Teskensu) village in the Enbekshikazakh district (43°32' N, 77°51' E) of the Almaty region of Kazakhstan (Figure 1). The study area is situated between 601 m elevation from sea level.



Figure 1. Location map of the study area

The climate type of study area is “Continental Climate” which can be described as low humidity, plenty of sunlight, a short but rather cold winter. The average annual air temperature is 12.3°C, total rainfall is 275.7 mm. The average long-term sum of precipitation for a period with temperature above 10°C is 198-245 mm.

The field experiment

Between 1985 and 1989, the field experiment established by S. Kaldybayev to ameliorate with alfalfa (*Medicago sativa* L.) cultivation soils in some parts of meadow Solonchaks (Beketova et al., 2017; Yerteyeva et al., 2018). From 1989 until 2017, alternating leguminous plants (especially leguminous vegetable and alfalfa) were grown integrated production without other amelioration methods in bio-ameliorated meadow Solonchaks. Other parts in meadow Solonchak is virgin land.

Soil sampling

Soil samples were taken on 27 May 2017 as a bulked sample from ameliorated soils and virgin lands from 0 to 20 cm soil depth (plough layer). The soil samples had 43.56% clay, 40.29% silt and 16.15% sand. Soil samples were dried under atmospheric condition and passed through a 2 mm sieve to prepare for laboratory analysis. In soil samples, CaCO₃ content by Scheibler calsimeter, pH in 1:1 (w/v) soil:dH₂O suspension by pH-meter, electrical conductivity (EC) in the same soil suspension by EC-meter, exchangeable cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) were measured using a 1 N NH₄OAc (pH 7) extraction, water soluble exchangeable cations pH in 1:1 (w/v) soil:dH₂O. All soil samples were sieved through a 150 µm mesh before determining the total organic carbon content (C_{org}) by the wet oxidation method (Walkley–Black) with K₂Cr₂O₇ (Rowell, 1996).

Measurement of biological soil quality indicators

Microbiological indicators

Microbiological counts were expressed as a number of colony forming units (CFUs) per g of dry soil. The total number of bacteria was determined by the dilution method on agarized soil extract. The total number of fungi was determined on the Martin’s medium (Martin, 2003). Ten grams of each soil sample were added to 95 mL of 0.1% (w/v) solution of sodium pyrophosphate. After homogenization for 30 min, this solution was decimally diluted (10⁻¹ to 10⁻⁷) and aliquots of the resulting solutions plated on appropriate culture media. After incubation at 25 or 30°C, for up to 10 days, the colony forming units (CFU) were counted. Data were expressed as colony forming units (CFU) g⁻¹ dry soil.

Microbial biomass carbon (C_{mic}) was determined by the substrate-induced respiration method of by Anderson and Domsch (1978). A moist sample equivalent to 10 g oven-dry soil was amended with a powder mixture containing 40 mg glucose. The CO₂ production rate was measured hourly using the method described by Anderson (1982). The pattern of respiratory response was recorded for 4 h. Microbial biomass carbon (C_{mic}) was calculated from the maximum initial respiratory response in terms of mg C g⁻¹ soil as 40.04 mg CO₂ g⁻¹ + 3,75. Data were expressed as mg C g⁻¹ dry soil.

Basal soil respiration (BSR) at field capacity (CO₂ production at 22°C without addition of glucose) was measured, as reported by Anderson (1982); by alkali (Ba(OH)₂·8H₂O + BaCl₂) absorption of the CO₂ produced during the 24h incubation period, followed by titration of the residual OH⁻ with standardized hydrochloric acid, after adding three drops of phenolphthalein as an indicator. Data were expressed as µg CO₂-C g⁻¹ dry soil.

Enzyme activities

Dehydrogenase activity (DHA) was determined according to Pepper et al. (1995). To 6 g of sample 30 mg glucose, 1 ml of 3% TTC (2,3,5-triphenyltetrazoliumchlorid) solution and 2.5 ml pure water were added and the samples were incubated for 24h at 37°C. The formation of TPF (1,3,5 triphenylformazan) was determined spectrophotometrically at 485 nm and results were expressed as µg TPF g⁻¹ dry sample. Catalase activity (CA) was measured by the method of Beck (1971). Ten ml of phosphate buffer (pH, 7) and 5 ml of a 3% H₂O₂ substrate solution were added to 5 g of sample. The volume (ml) of O₂ released within 3 minutes at 20°C was determined. Three replicates of each sample were tested and controls were tested in the same way, but with the addition of 2 ml of 6.5% (w/v) NaN₃. Results were expressed as ml O₂ g⁻¹ dry soil. β-glucosidase activity (GA) was measured according to Eivazi and Tabatabai (1988). 0.25 ml toluene, 4 ml TRIS (hydroxymethyl) aminomethane buffer (pH, 12) and 1 ml of 0.05 M *p*-nitrophenyl β-D-glucopyranoside solution were added to the 1 g sample and the samples were incubated for 1 h at 37°C. The formation of *p*-nitrophenol was determined spectrophotometrically 410 nm and results were expressed as µg *p*-nitrophenol g⁻¹ dry soil. Protease activity was measured according to Ladd and Butler (1972). Using casein as a substrate, soil samples were incubated for 2 h at 50°C at pH 8.1. Amino acids released during the

incubation period were extracted, and the remaining substrate was precipitated after the addition of trichloroacetic acid. Aromatic amino acids react with Folin-Ciocalteu phenol reagent in an alkaline solution to form a blue complex, which was determined spectrophotometrically and results were expressed as $\mu\text{g Tyrosn g}^{-1}$ dry soil.

Urease activity (UA) was measured by the method of Hoffmann and Teicher (1961). 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7) and 1 ml of 10% urea substrate solution were added to the 1 g sample and the samples were incubated for 3 h at 37°C. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as $\mu\text{g N g}^{-1}$ dry soil. Alkaline phosphatase activity (APA) was determined according to Tabatabai and Bremner (1969). 0,25 ml toluene, 4 ml phosphate buffer (pH,8.0) and 1 ml of 0,115 M *p*-nitrophenyl phosphate (disodium salt hexahydrate) solution were added to the 1 g sample and the samples were incubated for 1 h at 37°C. The formation of *p*-nitrophenol was determined spectrophotometrically at 410 nm and results were expressed as $\mu\text{g p-nitrophenol g}^{-1}$ dry soil. Arylsulphatase activity (ASA) was measured according to Tabatabai and Bremner (1970). 0.25 ml toluene, 4 ml acetate buffer (pH,5.5) and 1 ml of 0.115 M *p*-nitrophenyl sulphate (potassium salt) solution were added to the 1 g sample and the samples were incubated for 1h at 37°C. The formation of *p*-nitrophenol was determined spectrophotometrically 410 nm and results were expressed as $\mu\text{g p-nitrophenol g}^{-1}$ dry soil. All determinations of biological soil quality indicators were performed in triplicate, and all values reported are averages of the three determinations expressed on an oven-dried soil basis (105°C).

Results and Discussion

Some chemical properties of ameliorated and virgin land soils are given in Table 1. As shown in Table 1; soil pH, EC, CaCO_3 , water soluble cations and exchangeable cations were higher in virgin land than in the ameliorated land.

Table 1. Some soil chemical properties of ameliorated and virgin land of meadow Solonchack.

Soil chemical properties	Ameliorated land	Virgin land
pH	8.35	9.02
EC, dSm^{-1}	0.76	3.32
Organic matter, %	2.60	0.56
CaCO_3 , %	12.19	18.22
Water soluble cations, dSm^{-1}		
Na ⁺	1.09	5.89
K ⁺	0.39	0.42
Ca ²⁺	1.02	18.59
Mg ²⁺	0.76	6.59
Exchangeable cations, dSm^{-1}		
Na ⁺	1.05	3.79
K ⁺	0.70	0.72
Ca ²⁺	15.24	58.11
Mg ²⁺	4.19	5.17

Biological soil quality indicators

In this study two categories of biological soil quality indicators were used: microbiological indicators and enzyme activities. Biological soil quality indicators of ameliorated and virgin lands are presented in Table 2.

Table 2. Biological soil quality indicators in ameliorated and virgin land of meadow Solonchack.

Biological soil quality indicators	Ameliorated land	Virgin land
Microbiological indicators		
Bacteria count, CFU g^{-1} dry soil	5×10^8	2×10^6
Fungi count, CFU g^{-1} dry soil	3×10^3	5×10^2
C_{mic} , mg C g^{-1} dry soil	326,36	162,72
BSR, $\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil	75,41	36,37
Enzyme activities		
DHA, $\mu\text{g TPF g}^{-1}$ dry soil	58,15	25,11
CA, ml O ₂ g^{-1} dry soil	5,69	5,11
GA, $\mu\text{g p-nitrophenol g}^{-1}$ dry soil	12,69	3,58
UA, $\mu\text{g N g}^{-1}$ dry soil	8,68	4,29
PA, $\mu\text{g Tyrosn g}^{-1}$ dry soil	89,54	36,72
APA, $\mu\text{g p-nitrophenol g}^{-1}$ dry soil	27,36	5,96
ASA, $\mu\text{g p-nitrophenol g}^{-1}$ dry soil	19,56	8,75

Microbiological indicators

Soil microorganisms play an important role as regulators of major biogeochemical cycles and can significantly affect the ecosystem functioning (Tiedje et al., 1999), being involved in organic matter dynamics, nutrient cycling and decomposition processes (Nannipieri et al., 2003). Because of these reasons, microbial populations are important in soil fertility and quality. The anthropogenic activities affect the diversity of natural habitats modifying the number of species occurring in the environment at the landscape scale. In this study, it was determined that melioration practices strongly influenced bacteria and fungi populations in soils, and the virgin land soil had the lower bacteria and fungi population than ameliorated land soil (Table 2). Therefore, microbial population and their activities in response to amelioration practices is a fundamental indicator for sustainability of ecosystem processes.

We accept that C_{mic} gives an idea of the potential microbial activity of a soil (Nannipieri et al., 1990). The incorporation of organic matter by amelioration with alfalfa (*Medicago sativa* L.) cultivation in soil raised the C_{mic} level significantly, which reflects the increased number of microorganisms (Table 2). The general increase in C_{mic} noted can be attributed to the incorporation of easily biodegradable organic materials (Perucci, 1992). In addition, favorable conditions in ameliorated land and the higher content of organic matter (Table 1), which acts as energy source for the microorganisms, thus contributing to an increase in their activity and biomass. Anderson (1982) defined BSR as a useful parameter in measuring a soil's biological activity. High contents of organic matter and microbial populations significantly raised the BSR in ameliorated land (Table 2). Hence, favorable conditions result in increase in the size of the microbial biomass and the efficiency of C substrates degradation, conducting to an increase in respiration rate per unit of microbial biomass.

Enzyme activities

Enzyme activity is essential in both mineralization and transformation of organic C and plant nutrients. Hence, enzyme measurements have been used to determine effects of agricultural and/or amelioration practices on soil microorganisms as Biological soil quality indicators. As presence of dehydrogenases, which are intracellular to the microbial biomass, is common throughout microbial species and they are rapidly degraded following the cell death, the measurement of microbial DHA in soils has been used extensively (Bolton et al., 1985; Rossel and Tarradellas, 1991; Obbard, 2001). Therefore, usage of DHA as an index of microbial activity has been suggested (Benfield et al., 1977; Nannipieri et al., 1990; Tabatabai, 1994; Masciandaro et al., 2000). The CA is based on the rates of oxygen release from the added hydrogen peroxide, and may be related to the metabolic activity of aerobic organisms (Kızılkaya et al., 2004). The virgin land soil had the lower DHA and CA enzyme activity than ameliorated land soil (Table 2).

The GA, PA, UA, APA and ASA are good markers of biological fertility since they are involved in microbial cycling of C, N, P and S. The GA catalyzes the hydrolysis of β -D-glucopyranoside and is one of the three or more enzymes involved in the saccharification of cellulose (Bandick and Dick, 1999; Turner et al., 2002). The PA hydrolyze proteins to polypeptides, oligopeptides, and amino acids. As most N compounds in mineral soils are organically bound, these transformations are necessary to release N for plant uptake (Okur et al., 2009). The UA is involved in the hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants. It acts on carbon-nitrogen (C-N) bonds other than the peptide linkage (Bremner and Mulvaney, 1978). The APA hydrolyzes compounds of organic phosphorus and transforms them into basically phosphate ions of inorganic phosphorus, which are assimilate by plants (Amador et al., 1997). The ASA is the enzyme involved in the hydrolysis of arylsulphate esters by fission of the oxygen-sulphur (O-S) bond. This enzyme is believed to be involved in the mineralization of ester sulphate in soils (Tabatabai, 1994). In this study, activity of all the studied extracellular enzymes was significantly higher in the ameliorated land soil than in the virgin land soil samples (Table 2). Higher extracellular enzyme activity in ameliorated land soil is related higher content of organic matter content (Table 1).

Several studies (Kızılkaya and Bayraklı, 2005; Aşkın and Kızılkaya, 2006; Kızılkaya and Hepşen, 2007) showed that the soils including high organic matter content have higher enzyme activities such as; intracellular (DHA and CA) and extracellular-hydrolytic enzymes (GA, PA, UA, APA and ASA) than the soils including low organic matter content. During the biological amelioration period with alfalfa, post harvesting material might have decomposed, resulting in higher enzymes in ameliorated land soil. In addition, increased content of organic carbon and nutrients may increase enzymes in ameliorated land soil. Some research were reported that increasing organic matter and available nutrients were increased to enzyme activity, (Leirós, et al., 2000; Kızılkaya, 2005). It is possible that the increasing organic material had stimulated microbial production of enzymes (GA, PA, UA, APA and ASA) in soil, or supported more enzymes accessible to substrate.

Conclusion

Overall, data obtained in this work revealed an important effect of amelioration practices on biological soil quality indicators. Soil microbial communities and their enzymatic activities exhibited compositional shifts that tracked with changes in land amelioration. This study, combining the microbiological and biochemical data furnishes a good methodological approach to describe the influence of bio-amelioration with alfalfa (*Medicago sativa* L.) cultivation on biological soil quality indicators. In fact, the results demonstrate that in the same pedological conditions, amelioration activities that influence the microbiological properties and their activities in soils, showed a more stable and higher microbiological and biochemical soil composition as well as intra and extracellular enzyme activities compare with virgin land soil. Further researches are required to determine whether the observed shifts in microbial community composition produce parallel changes in the functional attributes of these communities across soil types under different type of amelioration practices. The use of culture-independent approaches, like metagenome sequencing, will make it possible to identify the specific drivers of land amelioration and land use dynamics exhibited by soil microbial communities and to give a complete picture of the microbial communities in a typical meadow Solonchaks.

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