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Abstract: Soil salinization is a major problem in irrigated agriculture. A field study was conducted in the Sariosiyo district in the Surkhandarya region of southeast Uzbekistan to evaluate soil nitrogenase activity and nitrogen-fixing bacteria populations in saline serozem soils under wheat, maize, and alfalfa, as well as from adjacent fallow land. Composite soil samples were randomly collected from depths of 0-10, 10-20, and 20-30 cm in autumn, winter, spring, and summer, which were then 2-mm sieved and subjected to selected microbial population analysis and enzymatic studies. The results showed that alfalfa contributed both to high nitrogenase activity and to a large nitrogen-fixing bacteria population. The fallow soil had a lower nitrogenase activity and bacterial population. The nitrogenase activity was higher in the soil from a depth of 10-20 cm in spring. Results suggest that cropping, especially suitable crop rotation, is essential to support greater microbial biomass population and nitrogenase activity for improving the biological fertility of saline and nitrogen-poor calcareous arid soils.

Key Words: Irrigated agriculture, salinity, season, soil depth, nitrogenase activity, microbial population

Tuzlu ve Kurak Topraklarda Mikrobiyal Populasyon ve Nitrojenaz Aktivitesi Üzerine Tarımın Etkisi

Özet: Toprak tuzluluğu sulu tarımda en önemli problemdir. Özbekistan'ın güney doğusunda Surkhandarya bölgesinde Sariosiyo'da buğday, mısır, alfalfa tarımı yapılan serozem topraklarda nitrojenaz aktivitesi, nitrojeni fikse eden bakteri populasyonunu araştıran bir arazi çalışması yapılmıştır. Kompozit toprak örnekleri 0-10, 10-20, ve 20-30 cm derinlikte sonbahar, kış, ilkbahar ve yaz aylarında alınmış ve seçilen mikrobiyal populasyonlar incelenmiş ve enzimatik çalışmalar yapılmıştır. Sonuç olarak alfalfa tarımı yüksek nitrojenaz aktivitesine ve nitrojeni fikse eden bakterilerin artmasına neden olmuştur. Nadasa bırakılan toprağın ise daha az nitrojenaz aktivitesine ve bakteri populasyonuna sahip olduğu gözlenmiştir. Nitrojenaz aktivitesine baharda 10-20 cm'lerde daha fazla raslanmıştır. Sonuçlar nitojence fakir kalkerli kurak topraklarda biyolojik verimliliği artırmak için daha fazla mikrobial biomass populasyonu ve nitrojenaz aktivitesi açısından tarım yapılması özellikle ürün değişiminin çok önemli olduğunu göstermiştir.

Anahtar Sözcükler: Sulu tarım, tuzluluk, nitrojenaz aktivitesi, mikrobyial populasyon

Introduction

Salinity leads to degradation of soil fertility under continental climate conditions in Central Asia, which is of major concern (1,2). In regions with low rainfall and high evaporative demand, the causes of soil salinity are mostly related to the traditional cultivation of naturally saline lands, inflow of saline groundwater from higher plateaus, and increased soluble salt concentration of water recycled for irrigation (1). Acceleration of irrigated soil salinization is recognized as the major factor limiting crop productivity in Uzbekistan (2).

Nitrogen (N) is an element essential for the support of all forms of life. It is found in amino acids, and proteins, and many other organic compounds are derived from the N fixation process (3,4). Biological nitrogen fixation is carried out only by prokaryotes, which may be symbiotic or free living in nature (5,6). It is well documented that biological nitrogen fixation mediated by nitrogenase enzymes is a process important to the biological activity of soil (6). Nitrogenase activity in soil depends on ecological conditions in association with the specific nitrogen fixation capabilities of certain microorganisms and plant genotypes under various climatic conditions (7-

10); however, the degree of nitrogenase activity is plant specific (11). The nitrogen fixing activity of free-living, non-photosynthetic aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen concentration, and a supply of organic C substrates. Nitrogen-fixing organisms are generally active in plant root zone soil. Plants that are capable of releasing exudates exhibited higher nitrogen fixation activity in soil (12,13).

Over the years, a number of studies have characterized microbial populations and nitrogenase activity in soils (14,15); however, there are only a few studies of nitrogenase activity in arid saline soils of Central Asia, and especially rare are reports on the saline serozem soils of Uzbekistan, which are characterized by extremely low levels of nitrogen and organic matter. The objectives of the present study were to enumerate populations of nitrogen-fixing bacteria and to determine the nitrogenase activity in saline serozem soils, with respect to season, crop cultivation, and soil depth

Materials and Methods

Sample Collection Region

Composite soil samples were collected from Surkhandarya province in southeastern Uzbekistan. The soil is calcareous serozem, having a calcic horizon within 50 cm of the surface. The climate of the region is semi-arid, with mean annual air temperature of 18 °C and mean annual rainfall of 200 mm.

Soil samples from depths of 0-10, 10-20, and 20-30 cm were randomly collected with an auger (3.5 cm in diameter) in close proximity of the roots of maize, wheat, and alfalfa in traditionally cultivated fields (CT), as well as from an adjacent area that was fallow and had never been cultivated (NT). Conventional mineral fertilizer (N, P, and K) use ranged from 150 to 200 kg ha^{-1} yr^{-1} for maize and wheat. There was no fertilizer applied to alfalfa. Soil samples were collected at 3-month intervals, in October (autumn), January (winter), April (spring), and July (summer). The soil cores were pooled, sieved (< 2 mm), and stored at 4 °C. The field-moist sieved soil samples were used for microbial and enzymatic studies. A portion of the soil was air-dried at room temperature prior to selected chemical and physical analyses.

Soil Chemical and Physical Analysis

Air-dried soil samples were analyzed for pH, total C (C_T), N (N_T), total P, exchangeable K and Mg concentration, and particle size properties. Selected chemical and physical properties of the soil are presented in Table 1. C_T was measured by elementary analysis, while N_T was determined by the Kjeldahl method. The molybdenum blue method was used to determine soil total P. Exchangeable K was determined by using flame photometry (16). An atomic absorption spectrophotometer was used to measure $CaCl_2$ and extractable Mg (17). Soil pH was measured with an electrometer. Soil particle size distribution was determined by the natrium phosphate method.

Soil Microbiological Analyses

For the enumeration of nitrogen-fixing bacteria, soil samples were gently sieved through a 2-mm mesh, visible pieces of crop residue and roots were removed, and 10 g of field-moist soil was mixed with 90 ml of sterile water for 15 minutes. Subsequently, the suspensions were serially diluted on Eshbi agar containing 0.2 g of K_2HPO_4, followed by 0.2 g of $MgSO_4$, 0.2 g of NaCl, 0.1 g of K_2SO_4, 5 g of $CaCl_2$, and then 20 g of saccharose per liter. The number of CFUs (colony forming units) was determined. Nitrogenase activity was measured using a standard acetylene reduction assay in which 12% (v/v) acetylene was added to triplicate serum bottles. The amount of ethylene formed at 28 °C in 12 h was determined by gas chromatography, as described by

Table 1. Selected properties of serozem soil (0-30 cm depth).

CT	NT.	P	K	Mg	C:N	pH	Soil particle distribution (%)		
		mg kg^{-1}					Sand	Silt	Clay
1000	60	300	900	600	16.7	8.0	2.2	54.5	43.3

C_T = C total; N_T = N total

Haahtela et al. (18). The acetylene reduction assay was performed 1-3 times per day, each time in separate triplicate bottles.

Statistical Analyses

Microbial and enzymatic data were analyzed according to a $4 \times 4 \times 3$ factorial design. Analysis of variance was performed with SAS (Statistical Analysis System) to separate the main and interactive effects of the independent factors. Linear contrast was performed to compare the effects of cropping vs. fallow on the seasonal distribution of nitrogen-fixing bacteria and their enzymatic activity in soil.

Results

Averaged across crops and soil depth, a significantly greater density (28%-61%) of nitrogen-fixing bacteria was observed in spring than in the other seasons (Table 2). Among the crops, alfalfa was able to support the highest number (50%-80%) of nitrogen-fixing bacteria in all seasons, while fallow soil had the lowest. Linear contrast showed that cropping significantly increased (>70%) the density of nitrogen-fixing bacteria, as compared to fallow soil (Table 3). The distribution of microbial populations varied according to soil depth. The total number of bacteria was higher at soil depths of 10-20 cm and 20-30 cm than at 0-10 cm, regardless of plant type.

Analysis showed that alfalfa significantly increased (55%-90%) nitrogenase activity and that the effect was more pronounced in spring and summer than in autumn and winter (Table 4). Averaged across soil depth, nitrogenase activity was higher during the winter, spring, and autumn, for wheat, alfalfa, and maize, respectively. Linear contrast showed that crops had significantly greater (> 80%) nitrogenase activity, as compared to fallow soil (Table 5). Nitrogenase activity was higher in the 10-20 cm horizon and for some plants in the 20-30 cm horizon. At this soil depth, the microbial population was also higher.

Discussion

The significantly greater density of bacteria and nitrogenase activity observed in soil under alfalfa during

Table 2. Seasonal distribution of nitrogen-fixing bacteria (M CFU/g soil) under different crops in serozem soil (30 cm depth).

Tillage	Crops	Soil depth (cm)	Winter	Spring	Summer	Autumn	Mean
C_T	Wheat	0-10	3.9 ± 0.6	14.9 ± 0.9	16.5 ± 0.6	13.4 ± 0.8	
		10-20	7.1 ± 0.8	15.2 ± 0.7	18.6 ± 0.5	12.6 ± 0.9	
		20-30	8.2 ± 1.2	18.3 ± 0.6	17.2 ± 0.8	17.5 ± 0.7	
			6.4b	16.1c	17.4b	14.5b	13.6a
C_T	Alfalfa	0-10	14.7 ± 0.8	33.4 ± 0.5	24.5 ± 1.0	31.9 ± 0.7	
		10-20	16.9 ± 0.8	41.2 ± 0.7	24.3 ± 0.8	33.2 ± 1.2	
		20-30	18.2 ± 0.6	36.1 ± 1.1	23.9 ± 0.8	32.8 ± 0.6	
			16.6a	36.9a	24.2a	32.6a	27.6 a
C_T	Maize	0-10	5.9 ± 0.5	21.9 ± 0.9	10.4 ± 0.9	10.6 ± 0.7	
		10-20	6.2 ± 0.8	24.1 ±1.2	10.6± 0.7	8.8 ± 0.7	
		20-30	9.4 ± 1.2	27.2 ± 0.7	11.9 ± 1.2	9.9 ± 1.1	
			7.2b	24.4b	11c	9.8c	13.1c
N_T	Fallow	0-10	2.3 ± 1.4	6.9 ± 1.1	0.9 ± 1.2	5.9 ± 1.1	
		10-20	4.2 ± 0.9	10.2 ± 0.9	1.2 ± 0.8	6.2 ± 0.9	
		20-30	5.1 ± 0.7	13.1 ± 0.8	1.4 ± 0.7	6.1 ± 0.7	
			3.9c	10.1d	1.2c	6.1d	5.3c
Seasonal effect			8.5C	21.9A	13.5B	15.7B	

C_T = Conventional tillage, N_T = No tillage, M = Million, and CFU = Colony forming units

Table 3. Linear contrast between cropped and fallow soils for seasonal distribution of nitrogen-fixing bacteria (M CFU/g soil).

Tillage	Crops	Winter	Spring	Summer	Autumn	Mean
C_T	Crops	10.1a	25.8a	17.5a	19a	18.1a
N_T	Fallow	3.9b	10.1b	1.2b	6.1c	5.3b

C_T = Conventional tillage, N_T = No tillage, M = Million, and CFU = Colony forming units

Table 4. Nitrogenase activity under different crops in serozem soil (0-30 cm).

Tillage	Crops	Soil depth (cm)	Nitrogenase Activity (μM C_2H_4 kg h^{-1})				
			Spring	Summer	Autumn	Winter	Mean
C_T	Wheat	0-10	23.1 ± 1.2	9.8 ± 1.1	14.0 ± 0.6	89.2 ± 0.9	
		10-20	35.6 ± 0.8	11.5 ± 0.8	23.1 ± 1.1	112.3 ± 0.6	
		20-30	8.9 ± 0.9	9.2 ± 0.7	18.3 ± 0.8	148.0 ± 1.3	
			22.5c	10.2c	18.5c	116.5a	41.9b
C_T	Alfalfa	0-10	183.7 ± 1.2	91.8 ± 1.1	115.0 ± 1.1	21.5 ± 0.8	
		10-20	107.0 ± 1	103.0 ± 0.7	150.0 ± 0.7	56.1 ± 0.7	
		20-30	91.8 ± 1.4	75.9 ± 0.7	90.0 ± 1.2	53.6 ± 1.1	
			127.5a	90.2a	118.3a	43.7b	95a
C_T	Maize	0-10	11.5 ± 0.7	25.0 ± 1.4	35.0 ± 0.9	9.8 ± 1.4	
		10-20	69.0 ± 1.1	90.0 ± 1.1	87.0 ± 0.7	74.0 ± 1.2	
		20-30	17.8 ± 0.7	40.0 ± 0.8	35.0 ± 1.1	8.9 ± 0.9	
			32.8b	51.7b	52.3b	30.9c	41.9b
N_T	Fallow	0-10	10.9 ± 1.1	6.5 ± 0.9	5.3 ± 1	10.1 ± 0.8	
		10-20	14.0 ± 0.9	8.3 ± 1.3	8.9 ± 1.4	12.0 ± 1.2	
		20-30	16.3 ± 1.4	4.2 ± 1.4	7.5 ± 1.2	17.3 ± 1.4	
			13.7d	6.3c	7.2d	13.1d	10.1c
Seasonal effect			49.1A	39.6A	49.1A	51.1A	

C_T = Conventional tillage, N_T = No tillage, M = Moles, and C_2H_4 = Acetylene

Table 5. Linear contrast between cropped and fallow soils for seasonal distribution of nitrogen-fixing bacteria (M CFU/g soil).

Tillage	Crops	Season				Mean
		Winter	Spring	Summer	Autumn	
C_T	Crops	60.9a	50.7a	63a	63.7a	59.6a
N_T	Fallow	13.7b	6.3b	7.2b	13.1b	10.1b

C_T = Conventional tillage, N_T = No tillage, M = Moles, and C_2H_4 = Acetylene

spring is most probably related to greater release of exudates and availability of C substrates, due to alfalfa's extensive rooting system. It is reported that C exudation by roots into the surrounding soil and the availability of mineral nutrients in soil are of considerable importance to increasing microbial populations (19). Root exudates, as a source of energy, are important for the ecological functions, especially nitrogen fixation, of heterotrophic soil microflora. Plants that produce exudates exhibit higher nitrogen fixation (12). Alfalfa had a versatile capacity to produce greater root exudates and enrich the soil with nitrogen through nitrogen-fixing activities (20).

Seasonal changes in the nitrogen-fixing bacteria showed that with an increase in atmospheric temperature their numbers increased. Zou et al. (21) reported similar changes in microbial populations during different seasons; during winter the number of microorganisms was lower than in spring and summer.

Similarly, lower nitrogenase activity in fallow soil than in cropped soil might be related to fallow soil having a smaller bacterial pool. A high concentration of organic material is essential for supporting an active bacterial pool and hence, high microbiological activity in soil. Govedarica (22) and Rovira (23) suggested that soil containing greater organic matter will have higher nitrogenase activity.

Conclusion

Experimental results suggest that variation in agricultural crops significantly influenced microbial populations and enzymatic properties in the tested soil. To improve the fertility of saline soil, particularly biological fertility, it is necessary to use suitable crops, especially crop rotation, which can increase microbial numbers and nitrogenase activity in the soil. This is especially important for low organic and nitrogen content soils of the arid and semi-arid saline regions of the world.

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