DOI: 10.5152/forestist.2018.001
Forestist 2018, 68(1): 1-6

İSTANBUL
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Original Article

Vegetation establishment improves topsoil properties and enzyme activities in the dry Aral Sea Bed, Kazakhstan

Kazakistan'da kuruyan Aral Deniz Yatağında bitki örtüsü gelişiminin üst toprak özellikleri ve enzim aktivitelerini iyileştirmesi

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ABSTRACT

Afforestation has been conducted for preventing desertification in the desiccated Aral Sea Bed. The present study aimed to investigate the changes in topsoil properties and enzyme activities owing to vegetation establishment. In August 2017, soils were sampled from degraded area devoid of vegetation (DA), areas afforested in 2002 (P1) and 2013 (P2), and naturally vegetated area (NA) in the northern part of the exposed Aral Sea Bed. Soil water content, pH, electrical conductivity, total N and organic C concentrations, exchangeable cation concentrations (K^+, Mg^{2+}, Ca^{2+}, and Na^+), available P (P_2O_5) concentration, cation exchange capacity, and enzyme activities (acid phosphate, N-acetyl-glucosaminidase, and β-glucosidase) were analyzed in the topsoil up to a depth of 10 cm. Soil water content, total N and organic C concentrations, K^+ and Mg^{2+} concentrations, and enzyme activities were higher in P1 and NA than in DA. Moreover, no significant difference was found between P1 and NA in soil water content, total N and organic C concentrations, and some of the exchangeable cation concentrations. Our findings indicate that vegetation establishment increased the soil organic matter which is strongly associated with soil water content, organic C concentration, and overall soil fertility. The effects of plantation on soil amelioration are similar to those of natural vegetation in the long-term (15 years). Moreover, soil enzyme activities increased with rise in soil water content and total N and organic C concentrations in both vegetated areas (P1 and NA).

Keywords: Aral Sea, desertification, enzyme activity, restoration, soil amelioration

ÖZ

Kurumuş olan Aral Denizi Yatağında çölleşmeyi önlemek amacıyla ağaçlandırma yapılmıştır. Bu çalışma vejetasyon (bitkilendirme) çalışmasına bağlı olarak yüzey toprağı ve enzim aktivitelerindeki değişiklikleri araştırmak amacıyla yapıldı. Ağustos 2017'de, vejetasyondan yoksun çorak alandan (DA), 2002 (P1) ve 2013 (P2) yıllarında ağaçlandırılmış alanlardan ve Aral Deniz Yatağının kuzey bölümündeki doğal bitki oluşumuna sahip alandan (NA) toprak numuneleri alındı. Toprağın su içeriği, pH, elektrik iletkenliği, total N ve organik C seviyeleri, değiştirilebilir katyon seviyeleri (K^+, Mg^{2+}, Ca^{2+},ve Na^+), mevcut P (P_2O_5) seviyesi, katyon değişim kapasiteleri ve enzim aktiviteleri (fosfat asit, N-asetilglukozaminidaz ve β-glukosidaz) yüzey toprağından 10 cm derinliğe kadar analiz edildi. Toprak su içeriği, total N ve organik C seviyeleri, K^+ ve Mg^{2+} seviyeleri ve enzim aktivitelerinin P1 ve NA örneklerinde DA'ya göre daha yüksek olduğu görüldü. Ayrıca P1 ve NA arasında topraktaki su içeriği, total N ve organik C seviyeleri ve bazı değiştirilebilir katyon seviyeleri açısından anlamlı bir fark bulunmadı. Bulgularımıza göre vejetasyon çalışması, toprak su içeriği, organik C seviyesi ve genel toprak verimliliği ile oldukça ilişkili olan toprak organik maddesini artırdı. Toprağın iyileştirilmesinde bitkilendirmenin etkileri uzun dönemde (15 yıl) doğal vejetasyonun etkileriyle benzerdir. Ayrıca, toprağın enzim aktiviteleri toprağın su içeriği ve total N ve organik C seviyelerindeki artışla P1 ve NA numunelerinde yükselmiştir.

Anahtar Kelimeler: Aral Denizi, çölleşme, enzim aktivitesi, restorasyon, toprağın iyileştirilmesi

Cite this paper as:
An, J., Kim, S., Chang, H., Khamzina, A., Son, Y., 2018. Vegetation establishment improves topsoil properties and enzyme activities in the dry Aral Sea Bed, Kazakhstan. *Forestist* 68(1): 1-6

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Received Date:
12.12.2017
Accepted Date:
31.12.2017

INTRODUCTION

Desiccation of Aral Sea is considered one of the world's most serious environmental disasters in the recent past (Micklin, 2014). Aral Sea was the world's fourth-largest inland water body in 1960, with an area of nearly 67,000 km^2 (Waltham and Sholji, 2001). However, diversion of the tributary rivers for large-scale irrigation for supporting the extensive cotton industry caused its depletion starting from the 1960s. Aral Sea split into two water bodies as northern Small Aral Sea and southern Large

Aral Sea by the late 1980s, and the area decreased to 11,000 km^2 in 2015 (Jin et al., 2017). The decreasing water level and the increasing salinity caused soil degradation, vegetation decline, and deterioration of rich and diverse shoreline ecosystems (Micklin, 2014). Furthermore, a large amount of salts affected the physical and chemical properties of soils which in turn affected plant growth and enzyme activities (Singh et al., 2012a).

Considering the pace and scale of the desertification in this region, vegetation establishment is regarded as a more appropriate approach for reducing the associated damage from soil salinization and salty dust storms than e.g. chemical methods (Salt et al., 1998; Van et al., 2005; Park et al., 2013). Although naturally vegetated regions are present, establishment of vegetation by natural processes takes a long time. Rehabilitation of the dried seabed through afforestation has been attempted in several restoration projects (Micklin, 2014) aiming to reduce soil erosion, thereby increasing nutrient concentration and soil organic matter and improving soil structure (Shirato et al., 2004; Yüksek and Yüksek, 2011). Nonetheless, time for soil amelioration and extent of the effects through afforestation varied (Shirato et al., 2004; Singh et al., 2012b; Zhang et al., 2013); therefore, the degree of soil recovery following afforestation should be monitored and evaluated (Shirato et al., 2004).

Detecting soil properties and enzyme activities can be relevant for understanding biological processes in soil recovery. The interactions between physical and chemical properties of soils and exchangeable cations affect plant growth by controlling nutrient availability. In addition, they contribute to the increase in soil fertility and creation of supportive microenvironments for plants and microorganisms (Zhang et al., 2013). The increase in concentrations of nutrients such as P, N, and organic C are good indicators of soil quality and productivity because they affect the physicochemical and biological properties of soil (Cao et al., 2011). Furthermore, soil enzymes play an important role in organic matter decomposition and nutrient cycling (Cao et al., 2011). Enzyme activities are highly sensitive to the changes derived from plantations of different species and management practices on degraded lands (Singh et al., 2012a). Therefore, soil enzyme activities are considered to be useful indicators of soil ecosystem function, including microbial functioning, fertility, biological diversity, productivity, resource requirements, and nutrient availability to plants (Caldwell, 2005; Sinsabaugh et al., 2009). Therefore, the simultaneous measurement of soil properties and enzyme activities are required for evaluating the effect of plants on soil biochemical processes. Moreover, the vegetation establishment contributes to the increase in soil water content and fertility, decrease in pH and electric conductivity, and induced changes in microbial activities (Singh et al., 2012a).

The present study aimed to investigate the effects of the vegetation establishment on soil properties and enzyme activities in Aral Sea Bed. To this end, we compared soil biochemical properties in a degraded area with afforested and naturally vegetated area. Thereafter, the soil in the afforested area was compared with that in the naturally vegetated area to specify the differences associated with the type of vegetation establishment. We hypothesized that (1) vegetation establishment ameliorates soils by reducing soil pH and salinity and enriching its fertility, (2) enzyme activities would increase after the vegetation establishment on degraded soil, and (3) the ameliorating effects on topsoil properties and enzyme activities differ between afforested and naturally vegetated areas.

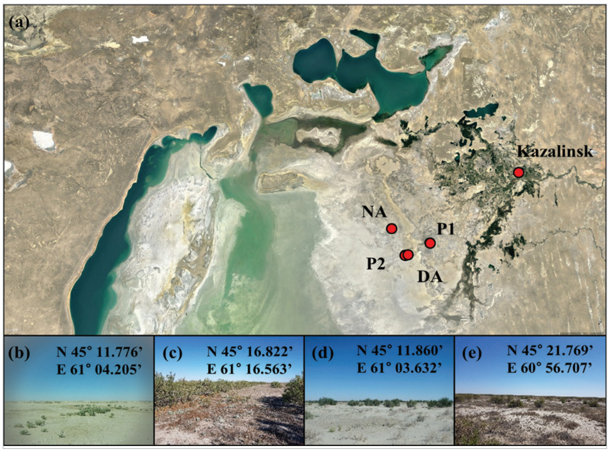

Figure 1. a-d. Location of the study area (a) and the photographs and GPS coordinates of study sites (b: degraded area (DA), c: area afforested in 2002 (P1), d: area afforested in 2013 (P2), and e: natural vegetation area (NA))

MATERIALS AND METHODS

Study Area

The research area is located on the southwest of Kazalinsk (Figure 1) near northern Aral Sea. Four soil sampling sites were selected, i.e. degraded area (DA), without plant cover; plantation areas P1 and P2, where afforestation was carried out in 2002 and 2013, respectively; and a natural vegetation area (NA). Aral Sea shoreline has receded starting from 1970s in all the sites. The annual mean temperature of Kazalinsk is 7.5°C (Issanova and Abuduwaili, 2017), and the annual rainfall is less than 90 mm (Waltham and Sholji, 2001). Soil of the exposed bed of Aral Sea is dominated by solonchak and takyr (Breckle and Geldyeva, 2012) which are types of gray-brown desert soil characterized by high quantity of carbonates, low organic carbon content, and the presence of a superficial porous crust (Pachikin et al., 2014). The sampled soils were dominated by coarse sandy texture.

Soil Sampling and Analyses

After removing surface residues, soil samples were collected using a digging knife from three points at depths of 0-10 cm, and mixed in each of the four sites in August 2017. Soil water content was measured gravimetrically, after oven-drying fresh soil samples at 105°C. The samples were air-dried at room temperature and passed through a sieve to remove sea shells prior to the analysis of soil chemical characteristics. Soil pH was measured using a refillable pH electrode (ROSS Ultra pH/ ATC Triode, Thermo Scientific, MA, USA) in a 1:5 soil-to-distilled water mixture with shaking for 1 hour. Electric conductivity ($EC_{1:5}$) was measured by EC meter (Orion Star A212, Thermo Scientific, MA, USA), after shaking the 1:5 soil-to-distilled water mixture for 30 minutes. Total N concentration was determined by dry combustion at 1,000°C with an elemental analyzer (vario Macro, Elementar Analysensysteme GmbH, Langenselbold, Germany), and the soil organic C concentration was measured by the Walkley and Black wet digestion and titration method (Walkley and Black, 1934). Soil concentration of exchangeable cations (K^+, Mg^{2+}, Ca^{2+}, and Na^+) and available P (P_2O_5) were measured using ICP-OES (730 series, Agilent Technologies Inc., CA, USA) after Mehlich 3 extraction. Cation exchange capacity (CEC) was calculated by adding up the total amount of exchangeable K^+, Mg^{2+}, Ca^{2+}, and Na^+ measured.

Three enzyme activities in soil (acid phosphate (AP), N-acetyl-glucosaminidase (NAG), and β-glucosidase (BG)) were used to represent the degradation of main soil biochemical compounds (Table 1) (Sinsabaugh et al., 2009). These enzymes were measured by fluorometric method (DeForest, 2009) in black polystyrene 96-well microplates (300 µL, SPL Life Sciences Co. Ltd, Pocheon-si, Korea), using substrate analogs linked to the fluorescent molecules of 4-methylumbelliferon (4-MUB, Sigma-Aldrich Co. Ltd, Yongin-si, Korea). For enzyme assays, soil suspension was prepared with 2 g soil and 100 mL of the Tris buffer (pH 8.0). The other procedures followed DeForest (2009), who used a strict order to incorporate the soil suspension, references, and substrates into the microplates. The microplates were coved and incubated at 25°C in the incubator for 4 hours. To terminate the reaction, 50 µL of 0.2 mol L^{-1} NaOH solution was added to each well. Fluorescence was measured at 355 nm excitation and 460 nm emission levels with a Multi-Detection Microplate Reader (Sense, HIDEX, Turku, Finland).

Statistical Analysis

One-way ANOVA was used for assessing the differences in soil water content, pH, $EC_{1:5}$, total N and soil organic C concentrations, concentrations of exchangeable cations and available P, CEC, and enzymes activities among the sites. Duncan's test was applied to indicate significantly different means ($p<0.05$). All statistical analyses were performed using SAS 9.4 (SAS Institute, NC, USA).

RESULTS AND DISCUSSION

P1 and NA had higher soil water content (% of weight) (DA: 2.46, P1: 6.93, and NA: 5.61; p=0.0021), total N and soil organic C concentrations (%) (DA: 0.01, P1: 0.05, and NA: 0.03; p=0.0010 and DA: 0.14, P1: 0.48, and NA: 0.38; p=0.0041, respectively), and K^+ and Mg^{2+} concentrations ($cmol_c$ kg^{-1}) (DA: 0.71, P1: 1.49, and NA: 1.65; p=0.0005 and DA: 7.51, P1: 9.61, and NA: 11.03; p=0.0097, respectively) than those of DA (Table 2). CEC ($cmol_c$ kg^{-1}) was

Table 1. Soil enzymes assayed for activity with abbreviation and substrate, corresponding Sigma-Aldrich product number (Sigma no.), related elements, and enzyme functions

Enzyme (Abbreviation)	Substrate	Sigma no.	Related element	Enzyme function
Acid phosphate (AP)	4-MUB-phosphate	M8883	P	Hydrolysis of phosphate from phosphosaccharides and phospholipids
N-acetyl-glucosaminidase (NAG)	4-MUB-N-acetyl-β-glucosaminide	M2133	N	Hydrolysis of chitin N-acetyl-β-glucosaminide
β-glucosidase (BG)	4-MUB-β-D-glucopyranoside	M3633	C	Hydrolysis of terminal β-D-glucosyl residues

Table 2. Soil properties of study sites

	Study site				
Soil properties	DA	P1	P2	NA	p
Soil water content (% of weight)	2.46 (0.69)^b	6.93 (1.70)^a	0.26 (0.07)^b	5.61 (0.68)^a	0.0021
pH	9.09 (0.10)	8.81 (0.04)	8.71 (0.04)	8.82 (0.13)	0.0835
EC1:5 (dS m^{-1})	13.62 (3.10)^{ab}	17.14 (3.57)^a	5.87 (1.41)^b	21.61 (4.06)^a	0.043
Total N (%)	0.01 (0.00)^b	0.05 (0.01)^a	0.01 (0.00)^b	0.03 (0.00)^a	0.0010
Organic C (%)	0.14 (0.01)^b	0.48 (0.11)^a	0.09 (0.00)^b	0.38 (0.04)^a	0.0041
K^+ (cmolc kg^{-1})	0.71 (0.10)^b	1.49 (0.16)^a	0.30 (0.03)^b	1.65 (0.24)^a	0.0005
Mg^{2+} (cmolc kg^{-1})	7.51 (0.53)^{bc}	9.61 (1.23)^{ab}	4.94 (0.26)^c	11.03 (1.38)^a	0.0097
Ca^{2+} (cmolc kg^{-1})	82.50 (11.26)	87.23 (1.21)	71.37 (4.43)	103.36 (2.60)	0.089
Na^+ (cmolc kg^{-1})	9.19 (2.15)	10.71 (2.63)	3.27 (0.33)	13.28 (6.16)	0.1654
Available P (P_2O_5) (ppm)	2.22 (0.74)^b	46.80 (17.52)^a	2.64 (0.18)^b	10.81 (1.58)^b	0.0359
CEC (cmolc kg^{-1})	99.92 (8.74)^{bc}	109.04 (5.18)^{ab}	79.87 (4.90)^c	129.32 (5.18)^a	0.0087

$EC_{1:5}$: electric conductivity, CEC: cation exchange capacity
Means (standard errors) with different letters within a variable indicate significant difference among study sites at $p<0.05$.

Table 3. Acid phosphatase (AP), N-acetyl-glucosaminidase (NAG), and β-glucosidase (BG) enzyme activities (nmol/h/g soil) in study sites

	Study site				
Enzyme	DA	P1	P2	NA	p
AP (nmol h^{-1} g^{-1} soil)	0.69 (0.25)^c	36.68 (6.32)^b	0.90 (0.29)^c	50.30 (2.83)^a	<0.0001
NAG (nmol h^{-1} g^{-1} soil)	0.00 (0.00)^b	12.13 (4.20)^a	0.08 (0.01)^b	8.07 (2.15)^a	0.015
BG (nmol h^{-1} g^{-1} soil)	0.00 (0.00)^b	49.84 (7.77)^a	0.78 (0.27)^b	63.18 (6.84)^a	<0.0001

Means (standard errors) with different letters within a variable indicate significant difference among study sites at p<0.05.

significantly higher in NA than in DA (DA: 99.92 and NA: 129.32; p=0.0087), and P_2O_5 concentration (ppm) was significantly higher in P1 than in the other sites (DA: 2.22, P1: 46.80, P2: 2.64, and NA: 10.81; P=0.0359). Soil pH did not differ significantly among the sites, all of which had alkaline soils (pH 8.71-9.09). No significant difference in $EC_{1:5}$ was found in P1, P2, and NA, as compared with DA. Analysis of the effect of vegetation establishment for different durations on enzyme activities (nmol h^{-1} g^{-1} soil) showed that AP (DA: 0.69, P1: 36.68, P2: 0.90, and NA: 50.30; P<0.0001), NAG (DA: 0.00, P1: 12.13, P2: 0.08, and NA: 8.07; p=0.015), and BG (DA: 0.00, P1: 49.84, P2: 0.78, and NA: 63.18; p<0.0001) were significantly higher in the vegetated areas (P1 and NA) than in the degraded area (DA), whereas the difference between DA and P2 was not significant (Table 3).

Consistent with the first hypothesis, our results combined point at an increase in soil nutrient because of long-term vegetation establishment, either naturally (since 1970s) or by afforestation (since 2002). Positive interaction of vegetation with soil water content has been observed previously (D'Odorico et al., 2007; Singh et al., 2012b). Soil water content could increase in vegetated areas owing to reduction in air and soil temperature and lower evaporation from the soil surface shaded by plants (Kizito et al., 2006). In contrast, soils in degraded area tend to dry out rapidly owing to the higher exposure to solar irradiance, which causes greater soil water evaporation (D'Odorico et al., 2007). In addition to the shading effect, vegetation may improve soil infiltration capacity owing to the action of roots (D'Odorico et al., 2007). However, soil water depletion has been reported in the case of large-scale afforestation in drylands (Jackson et al., 2005).

In addition, enrichment of fertility is a well-known indicator of soil amelioration (Singh et al., 2012b). The superior total N and organic C concentrations in P1 than in DA might be due to foliar litter inputs, fine root turnover (Singh et al., 2012b), and formation of humus (Jobbágy and Jackson, 2004). Exchangeable cation concentration in the study sites tended to differ according to the type of cation. A previous study on coastal sand dunes reported a higher concentration of K^+ in the topsoil of afforested areas, whereas that of Na^+ did not change because of the differential importance of cations for plants (Jobbágy and Jackson, 2004). Exchangeable K^+ is known to accumulate in surface soils because of upward transport by plants and plant litter decomposition (Jobbágy and Jackson, 2001).

Reduction of pH by natural or artificial vegetation establishment, as reported in studies on amelioration of sodic soils (Singh et al., 2012b) was not relevant in the present study on primary vegetation succession and afforestation in the Aral Sea Bed. In addition, the uptake of nutrient cations by roots requires the reverse flux of H^+, which could decrease the pH of soil in vegetated areas (Nilsson et al., 1982). However, the absolute amount of nutrients is very low in this region (Table 2). Therefore, longer time might be required for nutrient absorption and lowering of pH by vegetation. Several previous studies, which reported that the success of plantations in ameliorating saline-alkali land depends on the duration time of the plantation and the corresponding amount and quality of litter deposited (Singh et al., 1994; Singh et al., 2012b; Zhang et al., 2013). Particularly, a significant change in pH with plantation duration has been reported (Zhang et al., 2013). Shirato et al. (2004) has specified that approximately 20 years is needed for soil recovery because soil properties change slowly. Previous afforestation studies in Central Asia revealed either decrease (Hbirkou et al., 2011) or increase (Khamzina et al., 2016) in soil salinity following vegetation establishment. In the Aral Sea Bed, both, long-term vegetated and exposed areas exhibited high salinity, suggesting that abiotic factors such as groundwater oscillations might play a role in the soil salinity dynamics (Schachtsiek et al., 2014).

No statistically significant difference was found between P1 and NA for most of the soil properties studied, which indicates similarity in the state of amelioration in spite of the longer duration of plant cover in NA than in P1. Increasing soil organic C concentration during the vegetation restoration process is associated with the increase in soil CEC (Shirato et al., 2004). The variation in exchangeable cation concentrations and CEC would change along with soil chemical properties, such as pH, C and N concentrations, and biological properties, which affect the availability of nutrients to plants (Zhang et al., 2013).

Similar to the second hypothesis, increases in soil enzyme activities, which reflect that microbial activity in the soil, might be promoted by the plantation. Enzyme activities of soil are very early and sensitive indicators for changes during restoration (Cao et al., 2011), and they are affected by various soil properties, such as soil water content and nutrient supplements (Davidson and Janssens, 2006; Steinweg et al., 2013). Similarly, distinct correlations of enzyme activities with soil water content and total N and organic C concentrations in the present study (Figure 2). In-

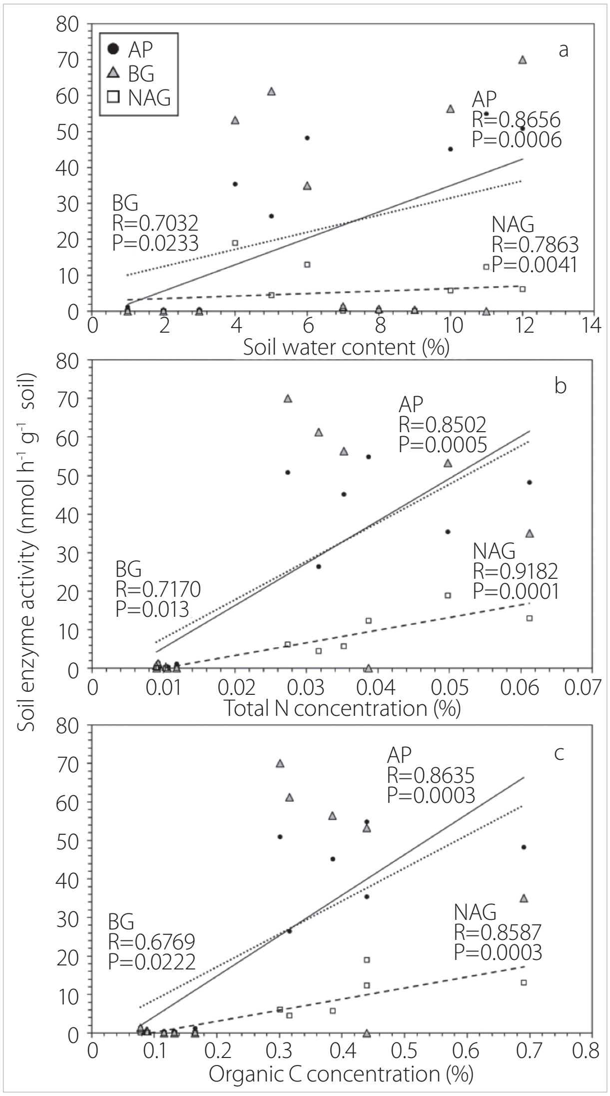

Figure 2. a-c. Correlations of enzyme activity (nmol h^{-1} g^{-1} soil) (Acid phosphatase (AP), N-acetyl-glucosaminidase (NAG), and β-glucosidase (BG)) and soil water content (a) and total N (b) and organic C concentrations (%) (c)

creases in soil organic C concentration and water content might be favorable for soil microbes, thereby promoting the secretion and activity of enzymes (Sileshi et al., 2007). However, enzyme production could be stimulated with increase in the diffusion of substrate or increase in bioavailability of nutrients in the soil (Davidson and Janssens, 2006; Steinweg et al., 2013). Even if nutrients available in soil were sufficient for microbial activity, available nutrient diffusion limitations in environments with low soil moisture content can reduce the production of microbial enzymes (Steinweg et al., 2013). Thus, enhanced microbial activity in vegetated areas can contribute to plant productivity through the regulation of mineral nutrient availability (Van Der Heijden et al., 2008).

Nevertheless, both positive and negative effects of plantation with soil water and salinity have been reported (Qi et al., 2015), whereas the results of the present study are based on the one-off, small-scale soil survey. Therefore, recurrent monitoring at larger spatial scales would be required for capturing the spatial heterogeneity in soil properties, which are dependent on vegetation distribution (Li et al., 2011), and the manifestation of the detailed effects of soil amelioration by vegetation establishment after plantation.

CONCLUSION

Distinct effects of vegetation establishment on soil chemical characteristics and microbial enzyme activities were found on the desiccated bed of Aral Sea. The changes were pronounced in P1, which was afforested in 2002, and in NA, which was naturally vegetated. However, no change was found in P2, the recently planted area (2013) as compared with the degraded area. Vegetation establishment increased soil organic matter, soil organic C concentration, and soil water content. These changes might have been responsible for the increase in CEC and nutrient retention capacity, resulting in increases in K^+ and Mg^{2+} concentrations. Improvements in soil properties, such as increases in soil water content, nutrient concentrations, and enzyme activities were comparable between P1 and NA. Evidently, plantation exerts similar amelioration effects as those exerted by natural vegetation, subject to the availability of sufficient time for vegetation development. Furthermore, afforestation appears to be quicker method that is as effective as natural vegetation establishment. Therefore, rehabilitation through plantation might be recommended for establishing soil cover and reinforcing the process of the natural succession.

Acknowledgement: This study was carried out with the support of Korea Forest Service (2012021C10-1718-AA01).

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