Changes in basic soil properties and enzyme activities along an afforestation series on the dry Aral Sea Bed, Kazakhstan

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ABSTRACT
Afforestation of the desiccated Aral Sea Bed is needed for the rapid introduction of vegetation and rehabilitation of the soil environment. The present study aimed to detect the soil amelioration effect by afforestation of the Aral Sea Bed with respect of changes in topsoil properties and enzyme activities. In August and November 2018, soils were sampled from the barren areas and from areas afforested in 1991, 2005, 2009, 2010, and 2013. The exchangeable base cation concentrations (Ca²⁺, K⁺, Mg²⁺, and Na⁺), cation exchange capacity (CEC), plant-available P concentration, electrical conductivity, pH, and enzyme activities (acid phosphatase, β-glucosidase, and N-acetyl-glucosaminidase) were analyzed in the surface soil (0–10 cm). Base cation concentrations, CEC, and electrical conductivity decreased following afforestation possibly because of root absorption. The observed increase in soil pH could be affected by both root absorption and decomposition of plant residues. Enzyme activities, which are early indicators of soil recovery, might have been increased by afforestation through the release of nutrients from litter and root exudates. Our findings indicate that the establishment of vegetation through afforestation can provide supportive microenvironments for plants and microorganisms by decreasing soil salinity and activating soil microbial enzymes; these effects of afforestation are amplified over time.

Introduction
Depletion of the Aral Sea has been considered as one of the world's most severe environmental disasters in the last century (Micklin 2014). Its once rich and diverse shoreline ecosystems deteriorated with decreasing water level and increasing salinity (Micklin 2014). Erosion of the exposed sea bed resulted in salty sand and dust storms, and inflicted damage on vegetation, crops, and public health in adjacent regions. A large amount of salts from the desiccated seafloor affected the physicochemical properties of soils, which subsequently affected soil microbial activities and vegetation growth (Singh et al. 2012a).

Afforestation is considered as a relevant approach to reduce the damage from soil erosion and salty dust storms considering the pace and scale of the desertification and the longer time required for natural vegetation to populate the desiccated seafloor (Ravindran et al. 2007). Nonetheless, time for soil amelioration and the extent of the effects of afforestation can vary (Shirato et al. 2004; Singh et al. 2012b; Zhang et al. 2013). The soil dynamics following afforestation should be monitored to evaluate the improvement in environmental conditions (Shirato et al. 2004).

The establishment of vegetation generally contributes to the increase in soil water content and fertility, decreases pH and electric conductivity, and induces changes in soil enzyme activities (Singh et al. 2012a). These changes lead to the creation of supportive microenvironments for plants and microorganisms (Zhang et al. 2013). In particular, soil enzymes play an important role in organic matter decomposition and nutrient cycling (Cao et al. 2011). Decreased enzyme activity results in a reduction in the cycling of C, N, P, and a decrease in soil aggregation because microbial activity is the key to the formation and stabilization of soil aggregates (Rietz and Haynes 2003). Moreover, enzyme activities are very sensitive to environmental changes induced by afforestation of degraded lands (Singh et al. 2012a). Therefore, soil enzyme activities are regarded as useful indicators to recognize the changes in soil ecosystem functions such as microbial functioning, soil fertility, and nutrient availability to plants (Caldwell 2005; Sinsabaugh et al. 2009).

The current study aimed to investigate the effects of afforestation on soil properties and enzyme activities in the dry Aral Sea Bed. We compared soil physicochemical properties of degraded areas with those of afforested areas. Thereafter, the changes in soil...
properties in the afforested areas were analyzed to specify the differences associated with the period after afforestation (restoration span). We hypothesized that (1) vegetation establishment through afforestation ameliorates topsoil by changing soil properties and enhances enzyme activities and (2) the ameliorating effects on topsoil properties and enzyme activities differ depending on the restoration span.

Materials and methods

Study area

The research area is located near Kazalinsk, which is on the southern part of the north Aral Sea (Figure 1). A total of eight soil sampling sites were selected, i.e. DA, degraded area without any vegetative cover (neither natural nor through plantation), 2010N and 2013N where afforestation was carried out in 2010 and 2013, respectively (soils were sampled in November 2018); 1991A, 2005A, 2009A, 2010A, and 2013A where afforestation was carried out in 1991, 2005, 2009, 2010, and 2013, respectively (soils were sampled in August 2018). Black saxaul (*Haloxylon ammodendron* (C.A. Mey.) Bunge) was planted at all sites except DA.

The average air temperature in Kazalinsk was $-9.9^\circ$C (January) and $27.2^\circ$C (July) in 1970–2000 (Giese et al. 2007). The average annual rainfall was $123$ mm in 1985–2000 (Breckle and Wucherer 2012). The period between late August and early November is autumn in Kazakhstan, when the soil samples were collected, and it belongs to a period of low rainfall (Nezlin et al. 2005). Solonchak and takyr (takir) are the dominant soil types in the exposed Aral Sea Bed (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 (Pachkin et al. 2014). Soil texture of the sampling sites was classified as sand, composed of 90% of sand, 7% of silt, and 3% of clay.

Soil sampling and analyses

In August and November 2018, soil samples were collected using a digging knife from three points at depths of 0–10 cm and were mixed together to acquire one representative sample in each of the sites after removing surface residues. All samples were air-dried at room temperature and passed through a sieve to remove sea shells prior to the analysis of chemical characteristics. Sieve-pipette method was used to analyze the soil texture. Concentrations of exchangeable cations ($\text{Ca}^{2+}$, $\text{K}^+$, $\text{Mg}^{2+}$, and $\text{Na}^+$) and available P ($\text{P}_{2}\text{O}_5$) were measured using ICP-OES (730 series, Agilent Technologies Inc., CA, USA) after Mehlich 3 extraction. Cation exchange capacity (CEC) was calculated by adding the total amount of measured exchangeable $\text{Ca}^{2+}$, $\text{K}^+$, $\text{Mg}^{2+}$, and $\text{Na}^+$. Electric conductivity ($\text{EC}_{1:5}$) was measured using an EC meter (Orion Star A212, Thermo Scientific, MA, USA) after shaking the 1:5 soil-to-distilled water mixture for 30 min. 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 h.

Soils sampled in November were brought in a cool bag and then refrigerated. Activities of three enzymes (acid phosphatase (AP), β-glucosidase (BG), and N-acetyl-glucosaminidase (NAG)) were measured within a week from soil sampling and before air-drying to represent the biological activities of microbes in the soil (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 suspensions were prepared with 2 g soil and 100 mL of Tris buffer (pH 8.0). The other procedures followed the method of DeForest (2009) to incorporate the soil suspension, references, and substrates into the microplates. The microplates were covered and incubated at 25°C for 4 h. To terminate the reaction, 50 μL of 0.2 mol L \(^{-1}\) NaOH solution was added to each microplate well. Fluorescence was measured at 355 nm excitation and 460 nm emission levels with a Multi-Detection Microplate Reader (Sense, HIDE, Turku, Finland).

### Results

#### Changes in soil properties and enzyme activities following afforestation

Cation concentrations were 0.71–3.66 cmol\(_e\) kg \(^{-1}\) for Ca\(^{2+}\), 0.00–0.02 cmol kg \(^{-1}\) for K\(^+\), 0.07–0.21 cmol kg \(^{-1}\) for Mg\(^{2+}\), and 0.01–0.14 cmol kg \(^{-1}\) for Na\(^+\) across all sites. Ca\(^{2+}\) was significantly lower in 1991A as compared with DA, while there were no differences between the other afforested areas and the degraded area. Mg\(^{2+}\) and Na\(^+\) decreased in afforested areas, and they had the lowest value in 1991A. The CEC values of 2013N and 1991A were significantly lower than that of DA. There was no significant difference in P\(_2\)O\(_5\) concentrations among the sites. EC\(_{1:5}\) was significantly lower in all afforested sites than in the DA (23.40 ± 2.45 dS m \(^{-1}\)), and showed the lowest value in 1991A (1.94 ± 0.24 dS m \(^{-1}\)) (Table 2). Soil pH in afforested areas showed no significant difference with DA except for 1991A.

Linear regression analysis showed that Ca\(^{2+}\) (\(R^2 = 0.9174\)), Mg\(^{2+}\) (\(R^2 = 0.8352\)), Na\(^+\) (\(R^2 = 0.7467\)), CEC (\(R^2 = 0.9246\)), EC\(_{1:5}\) (\(R^2 = 0.9437\)), and soil pH (\(R^2 = 0.9072\)) had a strong correlation with restoration span, and there was no association between K\(^+\) (\(R^2 = 0.0006\)) and the restoration span. Except K\(^+\), all the other variables decreased with increasing restoration span, while soil pH showed a positive correlation with the restoration span.

The enzyme activities in 2010N (AP: 12.58 ± 1.44, BG: 26.27 ± 3.82, NAG: 2.25 ± 0.38 nmol h \(^{-1}\) g \(^{-1}\) soil) and 2013N (AP: 21.31 ± 4.72, BG: 27.88 ± 8.14, NAG: 1.31 ± 0.51 nmol h \(^{-1}\) g \(^{-1}\) soil) were significantly higher than those in DA for AP (1.46 ± 1.46 nmol h \(^{-1}\) g \(^{-1}\) soil), BG (0.08 ± 0.04 nmol h \(^{-1}\) g \(^{-1}\) soil), and NAG (0.00 ± 0.00 nmol h \(^{-1}\) g \(^{-1}\) soil) (Table 2).

#### Linkage of soil properties and biological activities following afforestation

Results of redundancy analysis showed that five out of the total soil properties (EC\(_{1:5}\)) concentrations of Ca\(^{2+}\) and Na\(^+\), CEC, and P\(_2\)O\(_5\)) presented in Table 2 explained approximately 92% of the variance in enzyme activity data (Figure 2). The first two axes of the redundancy analysis explained almost all of this variance (92%). EC\(_{1:5}\) was the most influential factor driving the changes in enzyme activities by stepwise selection, and this variable explained 76% of the total variance in the enzyme activities (Figure 2).

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

Table 1. Soil enzymes assayed for activity with abbreviation and substrate, the corresponding Sigma-Aldrich product number (Sigma no.), related elements, and enzyme functions.
Table 2. Soil properties of each study site.

| Soil properties | Study sites |
|-----------------|-------------|
|                | DA | 2010N | 2013N | 1991A | 2005A | 2009A | 2010A | 2013A | p       |
| Ca$^{2+}$ (cmol, kg$^{-1}$) | 2.98 | 2.97 | 2.34 | 0.71 | 2.88 | 2.85 | 3.04 | 3.66 | <.0001 |
| K$^+$ (cmol, kg$^{-1}$) | (0.37)$^{ab}$ | (0.06)$^{ab}$ | (0.50)$^{b}$ | (0.19)$^{f}$ | (0.04)$^{b}$ | (0.09)$^{b}$ | (0.03)$^{bc}$ | (0.06)$^{c}$ | <.0001 |
| Mg$^{2+}$ (cmol, kg$^{-1}$) | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | (0.00)$^{b}$ | <.0001 |
| Na$^+$ (cmol, kg$^{-1}$) | 0.14 | 0.08 | 0.05 | 0.01 | 0.04 | 0.03 | 0.04 | 0.06 | <.0001 |
| (cmol, kg$^{-1}$) | (0.02)$^{d}$ | (0.01)$^{d}$ | (0.01)$^{d}$ | (0.00)$^{d}$ | (0.00)$^{d}$ | (0.00)$^{d}$ | (0.00)$^{d}$ | (0.01)$^{d}$ | <.0001 |
| CEC | 3.34 | 3.27 | 2.52 | 0.80 | 3.03 | 3.00 | 3.20 | 3.88 | <.0001 |
| Available P (P$_{2}$O$_{5}$) (ppm) | 2.92 | 2.60 | 1.55 | 1.89 | 1.99 | 1.99 | 2.19 | 1.62 | 2.26 |
| EC$_{1:5}$ | 2.45$^{a}$ | (2.19)$^{bc}$ | (1.39)$^{bc}$ | (2.42)$^{c}$ | (0.92)$^{b}$ | (3.32)$^{b}$ | (1.14)$^{b}$ | (1.15)$^{b}$ | <.0001 |
| Soil pH | 8.93 | 8.97 | 8.85 | 9.38 | 8.80 | 8.69 | 8.63 | 8.62 | .0060 |
| AP | (0.15)$^{s}$ | (0.06)$^{s}$ | (0.11)$^{s}$ | (0.15)$^{s}$ | (0.08)$^{s}$ | (0.20)$^{s}$ | (0.02)$^{s}$ | (0.02)$^{s}$ | .0284 |
| BG | 1.46 | 12.58 | 21.31 | 1.46 | 1.46 | 1.46 | 1.46 | 1.46 | .0155 |
| NAG | 0.00 | 2.25 | 1.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | .0137 |

Means (standard errors) with different letters within a variable indicate significant difference among study sites at $p<.05$. EC$_{1:5}$: electric conductivity; CEC: cation exchange capacity; AP: acid phosphatase; BG: β-glucosidase; NAG: N-acetyl-glucosaminidase.

Discussion

Previous studies have investigated the soil amelioration effects of afforestation, e.g. changes in concentrations of base cations (Qadir et al. 2007; Matsui et al. 2019), electrical conductivity (Mishra et al. 2004), soil pH (Singh et al. 2012b), and soil microbial activity (Liu et al. 2019; Zhang et al. 2019) in various dryland soils.

In our study, conducted in the Aralum Desert, the concentration of each base cation was lower in the afforested sites, particularly in 1991A. Base cations are absorbed by plant roots through the cation exchange process by releasing the equivalent amount of protons (Duan et al. 2004), and it seems to be the main cause for the decrease in base cation concentrations. Meanwhile, K$^+$ and P$_{2}$O$_{5}$ showed different aspects of change compared with others, and it seems that their concentrations themselves were very low to show meaningful change compared with previous studies (Khamzina et al. 2006; Egamberdiyeva et al. 2007; Khamzina et al. 2008).

In addition, the root absorption of base cations and water might affect the distinct decrease in soil EC$_{1:5}$ of afforested sites compared with the degraded area. It was reported that afforestation of degraded saline land decreased soil EC$_{1:5}$ in the upper layer (Mishra et al. 2004), and this was accompanied by water absorption from the root. The increase in the root mass and water absorbed in afforestation sites leads to leaching of soluble salts in the rhizosphere (Abrol et al. 1988). In terms of soil amelioration, the reduction of EC$_{1:5}$ due to vegetation establishment could solve the problem of higher electrical conductivity that hinders nutrient uptake by increasing the osmotic pressure of the nutrient solution (Ding et al. 2018). When referring to the results of regression analysis, the restoration span and EC$_{1:5}$ showed a strong negative correlation, which indicates that there is a difference in soil amelioration effect depending on the restoration span. In particular, the EC$_{1:5}$ of 1991A was classified as non-saline. The other afforested areas belong to moderate or high salinity soils, while the EC$_{1:5}$ of DA indicated severe soil

Figure 2. Redundancy analysis of enzyme activities (AP, BG, and NAG) constrained by the soil properties including Ca$^{2+}$, K$^+$, Mg$^{2+}$, Na$^+$, CEC, and P$_{2}$O$_{5}$ concentrations, soil pH, and EC$_{1:5}$ ($p<.05$). Values in the table show the results of the stepwise selection. The asterisk indicates statistical significance at $p<.05$ based on the confidence interval which was estimated from the Monte Carlo simulation. EC$_{1:5}$: electric conductivity; CEC: cation exchange capacity; AP: acid phosphatase; BG: β-glucosidase; NAG: N-acetyl-glucosaminidase.
salinity soil according to the FAO classification (Abrol et al. 1988). It was reported that at least 20 years are needed for soil recovery because soil properties change slowly (Shirato et al. 2004). Our results generally corroborate this conclusion given the onset of salinity decline only in the 14th year since the afforestation.

Singh et al. (2012b) reported a reduction in soil pH by natural or artificial vegetation establishment in a study on amelioration of sodic soils. However, a different result was observed in the present study. Soil pH was not significantly different between the degraded area and afforested areas except in 1991A, where soil pH was even higher than that in the other sites. These results show that pH does not change drastically by afforestation, but over a long period of time. As mentioned above, the decrease in soil pH in the vegetation area might be due to the uptake of cations by roots, which requires the reverse flux of H⁺ (Nilsson et al. 1982). However, the absolute amount of cations in this region is too low for lowering the soil pH by root absorption of vegetation. No significant decrease in soil pH over time was observed in afforested areas and even in the natural vegetation areas on the dried Aral Sea bed (An et al. 2018). Meanwhile, soil pH was reported to increase because of the addition of organic matter and plant residue (Bakar et al. 2011). Organic anions accumulate in the plants as protons and are released from roots initially. After that, soil pH can increase when the accumulated organic anions in the plant residue are decomposed by microbes (Yan et al. 1996). Thus, the additional measurement of anion concentrations in the plant residue and microbial activities would help examine the effects of vegetation on soil pH.

Microbes in the soil exude some enzymes that play an important role in organic matter decomposition and nutrient cycling. Because they are affected by various soil properties (Davidson and Janssens 2006; Steinweg et al. 2013), enzyme activities of soil are very early and sensitive indicators for changes during restoration (Cao et al. 2011). In the current study, enzyme activities of AP, BG, and NAG distinctly increased in the afforested sites compared with the degraded area. It was reported that the establishment of vegetation can increase enzyme activities either naturally or in an artificial manner in arid lands (An et al. 2018). Increasing nutrient supplements from vegetation e.g. plant materials would promote the secretion and activity of soil enzymes (Sileshi et al. 2007). An et al. (2018) reported positive correlations of enzyme activities and total N and organic C concentrations in this region. Enzyme activities of AP, BG, and NAG could be interpreted as an index of microbial investment in the nutrient acquisition of each substrate (Keeler et al. 2009). Therefore, increasing enzyme activities could be regarded to reflect an increase in nutrient usage according to the increased nutrients from vegetation establishment through litter and root exudes.

Consequently, as stated in the hypothesis, soil improvement was observed with respect of the decrease of soil salinity and increase of enzyme activities, and the effects differed by restoration span in this study. Moreover, the change in enzyme activity compared to soil properties is more sensitive and occurs faster; therefore, it can be an early indicator of the effectiveness of afforestation.

Furthermore, our results showed that enzyme activities are mainly susceptible to EC1:5. Soils can be generally classified as saline when their electric conductivity is 4 dS m⁻¹ or more (Rietz and Haynes 2003). Smaller and less active microbes in saline soil release less enzyme, and increasing salt concentration tends to reduce the solubility and denature enzyme proteins through disruption of the tertiary protein structure, which is essential for enzymatic activity (Rietz and Haynes 2003). EC1:5 decreased with an increase in restoration span, and therefore, as time passed after afforestation, the negative effects of EC1:5 on soil enzyme activity seem to be attenuated, thereby contributing to soil improvement.

These are the small scale results of soil investigation; thus, a wider range of site selection and recurrent monitoring would be required to understand the spatial heterogeneity in soil properties which varies by vegetation distribution. The results of soil amelioration by afforestation could be gathered as a database and used to assess the outcome of afforestation and to propel further ecological restoration projects using vegetation in this area.

Conclusion

Distinct soil improvement was found in the afforested areas with respect to the decrease in soil salinity and the increase in soil enzyme activities in the desiccated bed of the Aral Sea. Concentrations of base cations, CEC, and EC1:5 decreased by root absorption of vegetation, and soil pH might be raised by the decomposition of plant residues. Soil enzyme activities seemed to increase by the introduction of vegetation and subsequent nutrient supplements in the afforested areas. Changes in basic soil properties and enzyme activities had a positive (pH and enzyme activities) or negative trend (base cations and EC1:5) along the restoration span of 27 years. The initial increase in enzyme activities, which can be considered as an early indicator of soil amelioration, might convert soil nutrients into plant-available forms. Along with the decrease in salinity, soil nutrient improvement indicated by the enzyme activity not only helps survival of pioneering afforestation species but might also facilitate natural vegetation succession in the vicinity.

Disclosure statement

The authors declare no competing interests.

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