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Quality improvement of an erosion-prone soil through microbial enrichment

Hossein Kheirfam, PhD Student[a], Seyed Hamidreza Sadeghi, Professor[a,*], Mehdi Homaee, Professor[b], Behrouz Zarei Darki, Assistant Professor[c]

[a] Department of Watershed Management Engineering, Faculty of Natural Resources, Tarbiat Modares University, Noor 4641776489, Mazandaran Province, Iran
[b] Department of Soil Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[c] Department of Marine Biology, Faculty of Marine Science, Tarbiat Modares University, Noor 4641776489, Mazandaran Province, Iran

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ABSTRACT

Biological soil crusts on bare land have undeniable roles in reducing soil degradation through improving soil quality. Enriching soil crusts biological properties by increasing soil microorganisms' population can be an effective strategy to improve soil chemical properties. Accordingly, native nitrogen-carbon fixing cyanobacteria and bacteria were individually and combined inoculated on 0.25 m^2-plots filled by an erosion-prone, bare, and low quality soil collected from Marzanabad Region, Northern Iran. The important soil chemical properties, viz., total organic nitrogen, total organic carbon, soil organic matter, and total organic carbon-to-total organic nitrogen ratio were measured at time spans of 7–8 days for a period of 60 days. The results indicated that the total organic nitrogen, total organic carbon and soil organic matter increased significantly ($p < 0.01$) in all treatments. The total organic nitrogen and, total organic carbon and soil organic matter in the cyanobacteria, bacteria, and combined inoculated treatments increased up to 240, 188, and 172%; and, 40, 39, and 33%, respectively, compared to the control conditions. Additionally, the total organic nitrogen and total organic carbon in the study treatments were 46, 32, and 37%; and 40, 33, and 33% higher than those reported for the control plots. The rate of nitrogen fixing in all inoculated treatments had an increasing trend up to 38 days after which it declined. Nonetheless, carbon and organic matter storage increased throughout the experimental period. Meanwhile, the ratio of carbon-to-nitrogen were not significantly ($p > 0.17$) changed during the study period. Based on the study results, the positive role of microorganisms, especially cyanobacteria, was demonstrated in improving the chemical properties of the study soil.

1. Introduction

Degradation of soil quality and quantity is a threat worldwide, endangering food security and sustainable development. Physical, chemical, and biological properties are the principal indicators for soil health and potential evolution (Paz-Ferreiro and Fu, 2016; Merante et al., 2017). Soil physical and/or chemical properties, such as aggregate stability, bulk density, water retention capacity, organic matter (OM), carbon (C), and nitrogen (N) are influenced by biological properties, such as soil microbial activity and density (Veum et al., 2014). Soil microbes, particularly cyanobacteria and bacteria, make biological soil crusts (BSCs), functioning as ecosystem engineers (Chamizo et al., 2012). The richness of BSCs can preserve the quality and quantity of soil against degradation drivers (Bowker et al., 2006). Significant positive relationships between the richness of microorganisms and some soil physical and chemical properties have already been reported (Chamizo et al., 2012). Powell et al. (2015) confirmed that soil particles adhere through microorganisms' polysaccharides (PSs) secretion. Furthermore, soil structure improvement and hydraulic conductivity reduction through PSs' secretion of cyanobacteria have been reported by Colica et al. (2014). Rossi and De Philippis (2015) also reported that biofilms produced by cyanobacteria in Hobq Desert in China improved the physical and chemical properties of surface particle adhesion and C sequestration of the soil.

Previous research demonstrated that PSs secreted by BSC bacteria and cyanobacteria and their adhesive cell walls not only build soil microstructures, but also create soil macrostructures by

* Corresponding author.
E-mail addresses: H.Kheirfam@yahoo.com (H. Kheirfam), Sadeghi@modares.ac.ir, shrsadeghi@yahoo.com (S.H. Sadeghi), Mhomaee@modares.ac.ir (M. Homaee), Zarei@mail.ru (B. Zarei Darki).

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forming micro networks (Dorioz et al., 1993). The created soil macrostructures amend the soil physical properties, such as addressing surface roughness (Reynolds et al., 2001), raising porosity and expanding water retention capacity and improving both soil stability and soil aggregation (Chamizo et al., 2012). In addition, the role of richness in BSCs has been confirmed through changing soil biological and chemical properties, such as nutrient cycling, soil fertility and, C and N fixation rate (Acea et al., 2003; Nisha et al., 2007; Wang et al., 2009; Rossi et al., 2015; Rashid et al., 2016). Accordingly, a wide range of strategies have been used and/or assessed to amend soil physical, chemical, and biological properties, and, ultimately, BSCs' enrichment. These strategies include sawdust and wood ash, municipal waste, gypsum, lime (Sadeghi et al., 2016), animal and crop manures, organic composts, crop and food industries residues (Sadeghi et al., 2015; Chen et al., 2017) and selected oil mulches and biodegradable polymers (Awad et al., 2012).

The role of BSCs rehabilitation through biomineralization (Valencia et al., 2014), transferring rich BSCs into degraded soil crusts (Zhao et al., 2014), and inoculation-based techniques (IBT) (Wang et al., 2009; Rossi et al., 2015) have been shown to improve physical, chemical, and biological properties of the soils. So that, the results of cyanobacteria inoculation in desert regions in China by Wang et al. (2009) indicated that the cyanobacteria population increased up to 48% in the second year after inoculation. Furthermore, Sears and Prithiviraj (2012) indicated that the large-scale inoculation of cyanobacteria was effective in increasing soil nutrients, N fixation, and C sequestration.

Scrutinizing the available literatures showed no comprehensive study has been conducted to assess the effects of microbial enrichment through artificial inoculation on important properties of the quality of an erosion-prone soil in Iran where very rare studies have been documented in soil quality improvement. It is also inferred from the literature that the chemical properties, particularly the amounts of soil N, C, and OM, are the most important indicators for soil quality assessment over a short period of time. On the other hand, inoculation of soil with cyanobacteria and bacteria could be a new and an effective technique to increase soil N, C, and OM. Accordingly, the present study aimed to assess the effect of inoculation of native cyanobacteria and bacteria based upon increasing total organic nitrogen (TON), total organic carbon (TOC), soil organic matter (SOM), and carbon-to-nitrogen ratio (C/N) of an erosion-prone soil. To control external environmental factors, the study was planned to be conducted under laboratory conditions and with small plot scale.

2. Materials and methods

2.1. Site description and sampling

The study was conducted at a highly erosion susceptible sub watershed of the Chalus Watershed, located in western Mazandaran Province, northern Iran. Most of the parts (approximately 60%) of this watershed have experienced intensive soil erosion behavior, which is beyond the tolerable soil loss level. This threat has led negative on-site and off-site effects. The regional climate was semi-arid cold according to the Emberger classification method (Emberger, 1955). The soil of the study area was categorized as Xeric and Mesic type overlaid marl and limestone formations. The soil samples were taken from the upper (0–20 cm of the soil surface) in September of 2014. The sampled soil was air dried and sieved (<8 mm) prior to experimental use. The detailed properties of the study soil have been summarized in Table 1.

2.2. Isolation, purification, and proliferation of cyanobacteria and bacteria

In order to conduct the study, the upper 20 cm-surface soil was sampled and subjected to necessary preparation as described by Chamizo et al. (2012). The isolation and identification processes of existing cyanobacteria and bacteria in the soil bank were conducted using conventional microbiological techniques (Whitton and Potts, 2012). Accordingly, the cyanobacteria were identified by using Chu-10 medium (Andersen, 2005) complied with methodologies suggested by Whitton and Potts (2012), using high-resolution optical microscopes. While, bacteria were identified by using Tryptic Soy Agar (TSA) general medium (Lecomte et al., 2011) and serial dilution technique and consequently Gram stained and identified via the methodology proposed by Bergey and Breed (1957) by means of microscopic examination and distinguishing morphological characteristics (Kaeberlein et al., 2002). The *Nostoc, Oscillatoria*, and *Lyngbya* cyanobacteria, as well as the *Azotobacter* and *Bacillus* bacteria, were ultimately selected based on their capacity in N-C fixing, secreting extracellular polymeric substances, and having a self-feeding system. Accordingly, the selected cyanobacteria were purified through a multi-stage purification technique and then transferred for proliferation into 2-l Erlenmeyer flask containing Chu-10 medium. In addition, *Azotobacter* Agar, Modified II (Atlas, 2010) and DSMZ1 (Schrey et al., 2012) selective media were used to purify the *Azotobacter* and *Bacillus* bacteria, respectively. The liquid Luria Broth medium was also used to proliferate the bacteria (Garbeva et al., 2011). Eventually, the proliferated cyanobacteria and bacteria were counted to achieve the appropriate rate of 10^{12} c l^{-1} (Vieira and Nahas, 2005). They were counted by using the Neubauer chamber cell counting under high-resolution optical microscopes and optical density (OD) in spectrophotometers (Janssen et al., 2002).

2.3. Preparation of experimental treatments

The laboratory simulation of soil profile was carried out in order to control environmental drivers and to create equal conditions for experimental treatments and credible evaluation. Towards this attempt, a series of 0.25 m^2-small plots with a depth of 0.5 m were manufactured. The study soil was then transferred to the Rainfall and Erosion Simulation Laboratory of Tarbiat Modares University at Faculty of Natural Resources in Noor City, Northern Iran, and appropriately well-set as per available procedures (Sadeghi et al., 2015). Finally, the cyanobacteria and bacteria with almost 10^{12} cells in 500 ml (Valencia et al., 2014) were inoculated on surface of the plots by spraying technique (Wang et al., 2009; Sears and Prithiviraj, 2012). The inoculation treatments viz. (i) individual

Table 1
Description of physical and chemical properties of the study soil.

Property	Description	Property	Description
Texture	Silty clay loam	pH	7.55
Structure	Medium granular with fragile stability	EC (ds. m^{-1})	0.21
Soil depth (cm)	37.5	Sand (%)	14
Bulk density (g cm^{-3})	1.16	Silt (%)	46
Lime (%)	28	Clay (%)	40