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Improving runoff behavior resulting from direct inoculation of soil micro-organisms

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ABSTRACT

Surface hydrology can significantly influence biological soil crusts through altering runoff behavior. Hence, micro-organisms enrichment of degraded soil crust may be a novel and practical strategy to improve soil quality and surface runoff behavior. This study evaluated the impact of inoculating (i) bacteria, (ii) cyanobacteria and (iii) bacteria + cyanobacteria on soils, with the hypothesis this would improve runoff properties. Accordingly, we assessed the influence of microbial enrichment after inoculating the native cyanobacteria (*Nostoc*, *Oscillatoria* and *Lyngbya*) and bacteria (*Azotobacter* and *Bacillus*) on runoff onto a degraded soil under laboratory conditions. The cyanobacteria and bacteria were isolated from the studied soil, purified and proliferated in the laboratory, and then inoculated in individual or combined treatments onto soils placed into small experimental boxes. After 15, 30 or 60 days, the treatments were subjected to a simulated rainfall. We observed a significant ($p < 0.01$) decrease in both coefficient and peak of runoff in the bacteria, cyanobacteria, and bacteria + cyanobacteria inoculated boxes relative to the control boxes. There was also a significant ($p < 0.01$) delay in runoff start time (38–205%) and time to peak runoff (48–52%), and decrease in coefficient (74–96%) and peak of runoff (48–86%) in the bacteria, cyanobacteria, and bacteria + cyanobacterial-treated boxes compared to the control boxes. The most effective treatment was the inoculation of cyanobacteria after 60 days ($p < 0.01$). In this treatment, the runoff coefficient was reduced 96%, the peak reduced 83%, the start time delayed 168% and time to peak reduced by 34% compared to the control. While these results need verification in the field, they suggest that inoculation of native micro-organisms particularly cyanobacteria, can be practically used to restore local hydrological cycles in the soil.

1. Introduction

Altered hydrologic cycles as a result of land degradation is a common environmental problem with undesirable impacts, as this causes loss of soil and water, agricultural production, and an increase in flooding and threats to human and ecosystem health across scales (Bowker et al., 2008; Sadeghi et al., 2009; Kheirfam et al., 2017b). Several approaches have been suggested or implemented by former investigators (e.g., Lee et al., 2010; Hansen et al., 2012; Karami et al., 2012; Ritchey et al., 2012; Sadeghi et al., 2015a, 2016; Mamedov et al., 2016) seeking environmentally sound, economically effective, and practically feasible techniques to restore hydrologic function. They included sawdust and wood ash, municipal wastes, gypsum, lime (Sojka et al., 2007; Lee et al., 2010; Hansen et al., 2016; Sadeghi et al., 2016); animal and crop manures, organic composts, crop and food industry remnants (Ojeda et al., 2003; Sadeghi et al., 2015a; Gholami et al., 2016; Mamedov et al., 2016), and oil mulches and biodegradable polymers (Zohuriaan-Mehr and Kabiri, 2008; Awad et al., 2012; Lentz, 2015). Although the application of aforementioned amendments have been found to reduce runoff (Wilson et al., 2008; Liu et al., 2009; Leys et al., 2010; Sadeghi et al., 2015a, 2016), their applications in most field conditions are limited due to detrimental environmental effects, instability, cost, time-consuming application, and limited site accessibility (Sojka and Entry, 2000; Blanco and Lal, 2008; Klaunig, 2008; Woodrow et al., 2008; Epelde et al., 2013; Rossi et al., 2015). Hence, improvement of soil properties by using native biological amendments has long been a desired way to improve soil hydrological responses. Several past and on-going efforts have utilized the inoculation of

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biological soil crusts (BSCs) as a practical and effective strategy (Bowker et al., 2005; Chamizo et al., 2012b; Rossi et al., 2015; Kheirfam et al., 2017a). Inoculation-based techniques (IBT), such as transplantation of BSCs slurries of bacteria and cyanobacteria or introduction of *ex situ* cultivated crustal organisms in a given site. It has been shown that inoculation of micro-organism would be an effective stabilization technique, and thus likely beneficial for recovering hydrologic cycles as well (Colica et al., 2014; Rossi et al., 2015; Kheirfam et al., 2017a). Accordingly, we tested using cyanobacteria and bacteria inoculation as an eco-friendly way to improve hydrological responses of a highly erodible and degraded soil.

There are many ways soil cyanobacteria and bacteria can aid in improving hydrologic cycles. They secrete exopolysaccharides, thus aiding formation of soil aggregates and creating soil macro-structures by forming micro-networks that increase soil porosity and thus infiltration (Dorioz et al., 1993; Miralles et al., 2011; Chamizo et al., 2012a). These polysaccharides also increase water retention capacity (Chamizo et al., 2013), and soil stability (Strauss et al., 2012; Linsler et al., 2015). Biocrusts also increase soil surface roughness, thus slowing water flow and increasing infiltration (Reynolds et al., 2001) and consequently reducing runoff. But, the population of soil cyanobacteria and bacteria is low in degraded soils (Gans et al., 2005; Schloss and Handelsman, 2005; Kemprai, 2013). Hence, the richness of soil by organic matters and photosynthetic or free-living bacteria are the main factors for effectiveness of heterotrophic soil micro-organisms in improving soil properties (Kheirfam et al., 2017a). Microbial-rich BSCs have been shown to improve soil hydrologic cycles (e.g., Belnap et al., 2013; Rodríguez-Caballero et al., 2013; Zhao and Xu, 2013; Zhao et al., 2014; Wei et al., 2015). In this context, Belnap et al. (2013) reported that the runoff volume in microbial-rich BSCs decreased by 75% compared to microbial-poor BSCs. Zhao and Xu (2013) have also observed 11.8%-reduction in runoff generation in rich BSCs. Some investigators (e.g., Chen et al., 2006; Wang et al., 2009; Li et al., 2014; Colica et al., 2014) further reported that inoculation of cyanobacteria on desert sand hills improved soil properties.

The literature shows that many studies have been conducted to assess the effectiveness of micro-organisms in improving soil quality in general. However, we could not find studies that addressed whether cyanobacterial or bacterial inoculation could improve hydrologic cycles. Therefore, this study assessed the role of such inoculation on runoff characteristics of experimentally applied water.

2. Materials and methods

2.1. Soil sampling procedure

The experimental soil was collected from the Chalusrood Watershed (36°24′42″ to 36°30′59″ E, 50°30′42″ to 51°20′57″ N; Elevation: 1015 m above mean sea level) located in Mazandaran Province, Iran. The mean annual precipitation and temperature of the study area are 432 mm and 12 °C, respectively. The experimental soil consisted of limestone and limestone marl with a lime percentage of about 28%. Based on the USDA Soil Taxonomy classification method (USDA Soil Taxonomy, Soil Survey Staff, 2010), the soil was classified as fine loamy, mixed, Mesic, Typic Calcixerepts, Inceptisols. The soil texture was silty clay loam with bulk density of 1.16 g cm^{-3} and medium granular structure.

Twenty five soil samples were collected from the top soil at 0–2 cm depth (Carter and Gregorich, 2008) using a 10 cm-high and 5 cm-diameter PVC tube. The soil samples were then placed in plastics bags, stored at approximately 4 °C (Paul, 2014) and transported to the laboratory. The soil samples were then air-dried and sieved with a 2-mm sieve (Paul, 2014) and combined all together. Afterwards, 100 g of the sieved soils was sampled to isolate cyanobacteria and bacteria with three replications (Lecomte et al., 2011).

2.2. Provision of cyanobacteria and bacteria for inoculation

In order to select and introduce appropriate micro-organisms, the entire cyanobacteria and bacteria in the soil bank were first quantified and identified. We found these soils contained low amounts of cyanobacteria (2.6×10^3 cfu) and bacteria (7.6×10^4 cfu). We then identified them, based on available protocols (Bergey and Breed, 1957; Buchanan and Gibbons, 1974; Jett et al., 1997; Benson, 2002; Kaeberlein et al., 2002; Abrusci et al., 2005; Komárek, 2006; Whitton and Potts, 2012). The most dominant cyanobacteria, *Nostoc*, *Oscillatoria*, and *Lyngbya*, were purified and proliferated based on the procedures reported by Buchanan and Gibbons (1974), Komárek (2006) and Whitton and Potts (2012). The most appropriate and non-pathogenic bacteria, *Azotobacter* and *Bacillus*, were also purified using selective *Azotobacter* Agar, Modified II (Atlas, 2010) and DSMZ1 (Schrey et al., 2012) media and proliferated by liquid Luria Broth medium (Garbeva et al., 2011), afterwards. The cyanobacteria and bacteria were grown until a density of 10^{12} c l^{-1} was obtained (Janssen et al., 2002; Vieira and Nahas, 2005; Awad et al., 2011).

2.3. Preparation of experimental soils

The experiments were conducted at small boxes (laboratory plot, container or flume) scale with 0.5 × 0.5 × 0.5 m dimensions. The boxes were filled with a soil taken from the study area in layer basis, granulometrically arranged and carefully compacted by manual roller (Kukal and Sarkar, 2011; Khaledi Darvishan et al., 2014; Sadeghi et al., 2015b, 2016). The boxes were placed in water pools for 24 h and then left to drain for another 24 h to reach field capacity (~7%) of the study soil (Kukal and Sarkar, 2011). The soil moisture in boxes was continuously checked by a portable soil moisture meter before microbial inoculation as well as before rainfall simulation to ensure similar moisture conditions across all the boxes.

The experiment was conducted as a completely randomized block design with four treatments and three replicates each. The experimental treatments consisted of (I) individual inoculation of cyanobacteria, (II) individual inoculation of bacteria, (III) combined inoculation of cyanobacteria and bacteria (Wang et al., 2009; Ushio et al., 2013), and (IV) a control (without any inoculation). Furthermore, three time spans of 15, 30 and 60 days from the time of inoculation were used to assess the effect of time on inoculated micro-organism performance. The cyanobacteria and bacteria slurry with almost 10^{12} cells in 500 ml (Valencia et al., 2014) was first sonicated for 1 min at 19 W to homogenize the microbial distribution and then sprayed onto the surface of the experimental boxes by a 1-l handheld sprayer (Wang et al., 2009; Sears and Prithiviraj, 2012). The even spreading of the inoculation slurry on the soil was also visually assessed.

The study boxes were then placed outside the Rainfall and Erosion Simulation Laboratory of Tarbiat Modares University to be exposed to natural climatic circumstances during the study. The time spanned mimicked conditions found between in dry season (i.e., summer) and rainy season (i.e., autumn). It was also planned to find out the optimal opportunity for maximal growth and maturity of micro-organism population. The lowest and highest recorded daily temperature and solar radiation during the experiment time were 5.4 and 35.4 °C, and 300 and 2500 W m^{-2} day^{-1}, respectively. The boxes were covered during rain events.

The boxes were then placed on standard ramps with 25% slope similar to the slope of soil origin area (Kheirfam et al., 2017b). Boxes were then subjected to an artificial rain using a portable rainfall simulator with a height of about 4 m and BEX: 3/8 S24 W pressure nozzles with 50 ± 2 mm h^{-1} intensity and duration of 100 min, similar to rain events found in the study area (Sadeghi et al., 2016). Runoff amount was sampled every 2 min after commencement of the runoff. Afterward, runoff coefficient was calculated by dividing runoff volume to rainfall volume and expressed as percentage. The rainfall