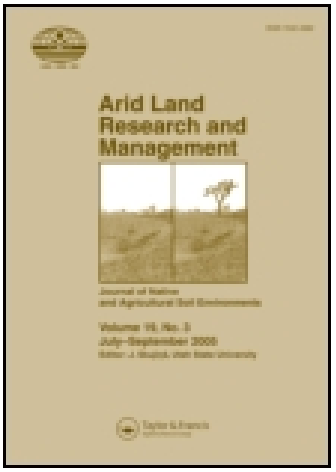

Arid Land Research and Management
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uasr20

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Published online: 03 Sep 2014.

To cite this article: Chan-Ho Park, Xinrong Li, Rong Liang Jia & Jae-Seoun Hur (2015) Effects of Superabsorbent Polymer on Cyanobacterial Biological Soil Crust Formation in Laboratory, Arid Land Research and Management, 29:1, 55-71, DOI: 10.1080/15324982.2014.928835

To link to this article: http://dx.doi.org/10.1080/15324982.2014.928835

Arid Land Research and Management, 29:55–71, 2015

ISSN: 1532-4982 print/1532-4990 online
DOI: 10.1080/15324982.2014.928835

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Effects of Superabsorbent Polymer on Cyanobacterial Biological Soil Crust Formation in Laboratory

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Numerous attempts have been made to control desertification and restore soil by inducing biological soil crusts (BSCs) formation through inoculation with cyanobacteria or application of soil fixing chemicals. In this study, combined application of biological (cyanobacteria) and chemical (superabsorbent polymer: SAP) materials to sand particles was conducted under laboratory conditions. Nostoc sp. was applied to sand at 5 to 25 mg fresh weight (FW)/cm^2 and incubated at 25°C under 100 μmol photons m^{-2} s^{-1} for 12 hr per day for 3 months. MWD (Mean Weight Diameter) analysis showed that the minimal cell dose for artificial BSCs induction was 10 mg FW/cm^2, and the resulting BSC was stable enough to resist various destructive factors. Aggregate stability of the combined biological and chemical treatment (ACSAP) was higher than that of samples treated with Nostoc *cells alone (AC) in the fast and slow wetting tests, but less stable than that of AC treatment in the wet stirring test. The SAP showed no harmful effect on* Nostoc *cell growth because of increased biomass during ACSAP treatment. However, the levels of extracellular polysaccharides (ESP) were lower upon ACSAP treatment than AC treatment. The wet stirring test demonstrated that ESP content was more important than cyanobacterial biomass for maintaining stability of the induced BSC. Overall, this study provides useful information regarding the interaction of* Nostoc *cells and superabsorbent polymer during artificial BSC formation. The results indicate that future studies investigating application of combined treatment of* Nostoc *cells and SAP in the field are warranted.*

Keywords cyanobacteria, extracellular polysaccharides, induced biological soil crusts (IBSCs), *Nostoc* sp., superabsorbent polymer

Introduction

Biological soil crusts (BSCs) are highly specialized communities composed of cyanobacteria, green algae, lichens, mosses, bacteria, and micro-fungi (West, 1990). BSCs are classified as cyanobacterial crusts, lichen crusts, or moss crusts

Received 14 October 2013; accepted 24 May 2014.
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based on their succession stage and dominant components (Belnap et al., 2001). The organisms comprising BSCs can adapt themselves to extreme environmental conditions such as high temperature, pH, and salinity, low precipitation, strong irradiation, and desiccation. They can also fix mobile sand dunes, as well as alter top soil moisture (Galun et al., 1982) and resistance to wind and water erosion, resulting in an upturn of the surrounding environment and influencing seed germination (Harper and Marble, 1988; Su et al., 2007). Additionally, BSCs improve soil fertility through mineral chelation, dust entrapment, and metabolism, which is beneficial to invertebrates and reptiles, as well as vascular plants (Belnap et al., 2001; Bielders et al., 2002). Accordingly, BSCs have been considered as a solution for restoration of degraded desert soil. However, the recovery time required for cyanobacterial BSCs after disturbance is predicted to be 45 to 110 years (Belnap and Eldridge, 2003). Many researchers have conducted experiments investigating the rapid induction of BSCs artificially through the use of moss and cyanobacteria. Chen et al. (2006) constructed man-made desert algal crusts in Inner Mongolia, China by inoculating *Microcoleus vaginatus* Gomont ex Gomont onto unconsolidated sand dunes. Wang et al. (2009) also tested the feasibility of mixed cyanobacterial inoculation with straw checkerboards and automatic sprinkling micro-irrigation techniques in desert areas. Xiao et al. (2011) examined artificial propagation of crusts in the Loess Plateau of China by inoculation with moss-dominated biological soil crusts and found that the settled BSC positively affected infiltration, overland flow, runoff, and water redistribution. Furthermore, Malam Issa et al. (2007) demonstrated that cyanobacteria inoculation improved the soil physical quality. Yu and He (2011) observed that polysaccharides were secreted by cyanobacteria and played a critical role in stabilizing the soil surface. Wu et al. (2013) showed that the soil fertility and microenvironment of top soil improved as cyanobacterial crusts were developed.

Although the aforementioned studies provide some useful information regarding artificial BSCs generated using cyanobacteria, it is still unclear how efficiently cultured biomass of cyanobacteria can be used for artificial BSCs formation. Because massive amounts of cyanobacterial cells should be cultured in the laboratory or by other equipped facilities to induce artificial BSCs in a large area, we should determine the amounts of cyanobacterial biomass that are sufficient to induce formation of BSCs strong enough to prevent destructive stresses in the field. Therefore, we investigated the minimal inoculation dose of cyanobacterial biomass in this study.

Many chemical agents, such as organic polymers, have been investigated for sand stabilization because they play an important role in increasing sand aggregate stability and protecting sand particles against wind erosion. To prevent wind and water erosion in arid or semi-arid areas, sand fixing chemical agents such as polymerized by the monomer of vinyl acetate (PVIN), polyvinyl alcohol (PVA), poly aspartic acid (PASP), and polyacrylamide (PAM) have been used. These chemical agents are normally less expensive than physical (mechanical) and vegetative materials (Liu et al., 2012), and are effective at fixing sand particles in the short term, but not reliable and sustainable in the long term due to microbial degradation (Corti et al., 2002), exposure to strong UV radiation, and temperature fluctuations in the field (Yang et al., 2007b). Conversely, BSCs formations are more appropriate for fixing sand and restoration of damaged soil in arid areas. However, it will take several years, or even a few decades, to induce BSCs formation in nature depending on environmental conditions during the initial stage of BSCs (Belnap and Eldridge, 2003). Chinese researchers developed a rapid induction method, the straw

checkerboard system, to stabilize mobile sand dunes under adverse environmental conditions (Li et al., 2006). The system can effectively entrap dust, leading to accumulation of soil organic matter and nutrients, but is labor-intensive, expensive, and impractical for large areas. As an alternative to the system, we first attempted combined application of chemical agents and cyanobacterial cells to bare sand particles for rapid and stable induction of artificial BSCs formation under laboratory conditions. Combined application of biological agents and chemical agents can accelerate BSCs formation under natural conditions when compared to single application of cyanobacteria as a biological agent or single application of SAP as a chemical agent. The chemical agent of SAP can physically fix fine sand particles in a short period, which is crucial to cyanobacterial settlement for BSC induction on bare sand soils, while also increasing water availability for cyanobacterial growth due to its high water absorbing capacity. Thus, we assume that the double action of SAP can enhance more rapid induction and stable formation of cyanobacterial BSCs than application of cyanobacterial cells alone under natural conditions. At the same time, combined application of SAP with cyanobacterial cells can overcome the defects associated with SAP during field trials.

The present study was conducted to induce biologically active soil crust through the use of cyanobacterial cells, which were the main biological agents of BSC induction in our trial. To facilitate stable and rapid induction of cyanobacterial BSC, a supplementary SAP was used as a soil fixing and water retention agent, which is necessary for cyanobacterial BSC induction in the early stages of BSC succession. To accomplish this, either 1) single application of *Nostoc* cells; 2) single application of SAP; and 3) combined application of *Nostoc* cells and SAP were conducted to investigate the effects of SAP on cyanobacterial BSCs formation. Cyanobacterial biomass, level of extracellular polysaccharide, and artificial BSC stability were analyzed and compared between single applications of algal cells and combined application of algal cells and SAP to evaluate the feasibility of combined application for promoting BSC induction.

Materials and Methods

Cyanobacterial Isolation and Cultures

Soil samples were collected from the Tengger Desert in Ningxia Province (N 37° 27′ 29.9″, E 105° 00′ 37.1″), Northern China. Soils were ground to pass through a 0.1-mm mesh sieve and then mixed with sterilized water. For cyanobacteria isolation, the BSC sample solution was transferred to BG-11 agar medium (Rippka et al., 1979) and then incubated for 2 weeks at 25°C under a light intensity of 100 μmol photons m^{-2} s^{-1} for 12 h per day. Following incubation, a single colony was selected under a dissecting microscope (SMZ645; Nikon, Tokyo, Japan) and then streaked for isolation on new BG-11 agar medium. Isolated cyanobacteria were examined under a compound microscope (ZEISS Scope, Al; Zeiss, Oberkochen, Germany), transferred to 500-mL Erlenmeyer flasks containing 200 mL of BG-11 liquid medium and then cultured with shaking at 150 rpm in a growth chamber (temperature 25°C, relative humidity 60%, and light intensity 100 μmol photons m^{-2} s^{-1} for 12 h per day) for 1 month. Stock cultures of the isolated cyanobacteria were kept in a freezer (4°C) and transferred to fresh medium every three months. Five cyanobacteria (*Nostoc* sp. Vaucher ex Bornet & Flahault, *Nostoc edaphicum* Kondrateva, *Anabaena* sp. Bory de Saint-Vincent ex Bornet & Flahault, *Phormidium* sp. Kützing ex Gomont, and *Microcoleus vaginatus* Gomont ex Gomont) were isolated by morphological and molecular identification.

Treatments

Sand material was purchased from a local market and the textural characteristics were determined (Table 1). Soil samples were then passed through a 1-mm sieve and sterilized by autoclaving at 121°C and 15 psi for 20 min, after which they were air dried at 100°C for 12 h. Next, 30 g of sterilized soil were placed in a petri dish (9 cm in diameter). Superabsorbent polymer (SAP, WCS-0907) with the properties described in Table 2 was purchased from Greenfield Co., Ltd., Korea. Due to the high viscosity of SAP at greater than 0.3%, the polymer was applied at a concentration of 0.05% to 0.3%, which is equivalent to 0.025 mg to 0.15 mg DW (dry weight)/cm^2 to determine the minimum effective dose for fixing soil particles. SAP was applied by spraying onto the soil surface. The soil particles treated with SAP were then incubated at 25°C for 3 days and analyzed for soil aggregate stability (fragment size distribution and mean weight diameter of soil aggregates). A SAP concentration of 0.025 mg DW/cm^2 was found to be the minimum effective dose (Figure 1) and was therefore employed for further investigations of BSC formation with algal cells. *Nostoc* sp. was used in this study due to the filamentous form, presence of heterocysts fixing atmospheric nitrogen and secretion of extracellular polysaccharides. Furthermore, many *Nostoc* sp. can occur in symbiotic associations with lichen-forming fungi to form lichens, which are one of the main biological components of BSCs during the later stages of their development in nature (Meeks, 1998). In this study, aseptically cultured *Nostoc* sp. was harvested during the exponential growth phase and centrifuged for 5 min at 3000 rpm. In a previous study (Wang et al., 2009), cyanobacterial cells of 1.6 g DW/m^2 were inoculated for artificial biological soil crust formation in desert areas. Based on this information, cyanobacterial cells were inoculated at an approximately 1.6 g DW/m^2 (8 mg FW(fresh weight)/cm^2).

Table 1. Characteristics of sand used in this study

Particle size fraction (%)						
Silt	Very fine sand	Fine sand	Medium sand	Coarse sand	Aggregate stability MWD (mm)	pH
2.9	7	27.8	47.5	14.7	0.35	6.4

Table 2. Specifications of superabsorbent polymer used in this study

Chemical product name	Superabsorbent Polymer
Appearance	Granule
Chemical formula	Sodium Polyacrylate
Average particle size	0.2–2.0 mm
Color	Black
Free absorbency of distilled water	200–500 g/g
Free absorbency of saline solution	$\geq$35 g/g
Nutritive component	Humic acid, mineral elements
pH	6.0–7.5

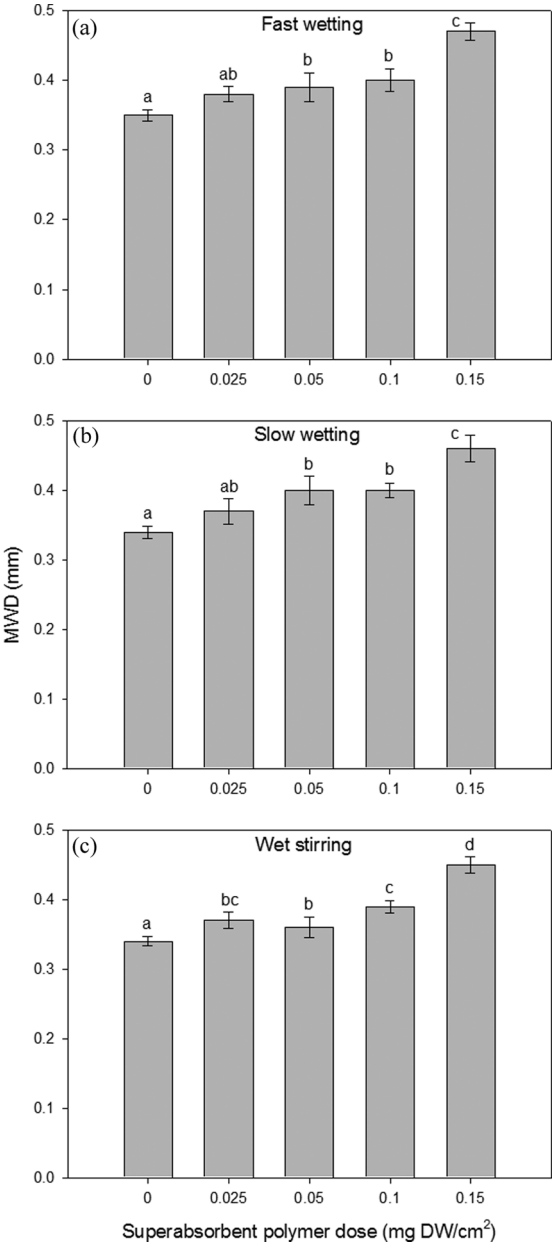

Figure 1. Values of the mean weight diameter (MWD) of sand particles treated with different levels of superabsorbent polymer. Values represent the mean of triplicate measurements. Values with the same letter are not significantly different according to the multiple comparison test (95% Tukey's HSD). Error bars represent standard errors.

For single application of algal cells, *Nostoc* sp. was weighted at 5, 10, 15, 20, and 25 mg FW/cm^2 and then homogenized with 3 mL of sterile distilled water, after which it was inoculated evenly to fine sand particles in each petri dish. For combined

application of algal cells and superabsorbent polymer (ACSAP), the same doses of algal cells were inoculated with SPA at 0.025 mg DW/cm^2. The inoculated soil particles were then incubated at 25°C under 100 μmol photons m^{-2} s^{-1} for 12 h per day for 3 mo to induce BSC. Each treatment was conducted in triplicate.

Aggregate Stability

Once BSCs started to form, their stability is important for development to maturity. During development, the types and amounts of rainfall are a primary cause of BSC destruction in nature. We investigated the effects of fast wetting, slow wetting and wet stirring on the stability of BSC. Aggregate stability was measured in triplicate according to the method described by Le Bissonnais (1996), which distinguishes the different destruction mechanisms that cause aggregate breakdown. Each test corresponds to different wetting conditions and energy inputs.

1. Fast wetting is useful for testing the behavior of dry soils under fast wetting events such as heavy rains in summer. For the slaking test, 5 g of aggregates were carefully submerged in 250-mL beakers containing 50 mL of Milli-Q water for 10 min. The water was then discarded by pipette, after which the soil was moved to sieves that had previously been submerged in ethanol to estimate fragment size distribution.
2. Slow wetting tests the behavior of dry or low water content soils under slow wetting events such as moderate rains. This test is less destructive than fast wetting and allows discrimination of unstable soils. Five grams of aggregates were placed on filter paper at a matric potential of −0.3 kPa for 30 min. Soil was then moved to sieves that had been submerged in ethanol to estimate the fragment size distribution.
3. Wet stirring is used to test the cohesion of moist material independently of slaking (mechanical breakdown test). This test is conducted by using a non-polar liquid miscible with water (ethanol) to prevent the breakdown of aggregates by slaking. For the mechanical breakdown test, 5 g of aggregates were carefully submerged in 250-mL beakers containing 50 mL of ethanol for 10 min, after which the ethanol was discarded by pipette. The soil was then moved to a 250-mL Erlenmeyer flask containing 50 mL Milli-Q water and then diluted to 200 mL with water. The Erlenmeyer flask was capped and stirred end over end 20 times, after which it was allowed to stand for 30 min to enable sedimentation of coarse fragments. The water was subsequently discarded by pipette and sediment material was moved to individual sieves previously submerged in ethanol to estimate fragment size distribution.

The stability for each test was described by using the resulting fragment size distribution in six granulometric classes ranging from >2, 1–2, 0.5–1, 0.2–0.5, 0.1–0.2, and >0.053 mm. Detailed descriptions of the method are provided by Attou et al. (1998), Le Bissonnais (1996), and Malam Isssa et al. (2001). The Mean Weight Diameter (MWD) is the total of the mass fraction of soil left on each sieve after sieving. MWD values were calculated as follows:

$$MWD = \frac{\sum_{i=1}^{6} \bar{x}_i w_i}{100}$$

with $\bar{x}_i$ being the mean diameter of the pores of two consecutive sieves, and w_i being the weight ratio of particles remaining on each sieve.

Polysaccharide Analysis

The soil extracellular polysaccharide (EPS) content was determined as carbohydrate according to the method described by Safařík and Šantrůčková (1992). Briefly, 5 mg of soil aggregates were scooped into a test tube (100 × 12 mm), after which 1 mL of distilled water and 5% phenol solution were added and vortexed. Next, 5 mL of concentrated sulfuric acid were added and the solutions were vortexed for 10 sec, then incubated at room temperature for 1 h. Additionally, blanks were prepared with distilled water instead of phenol solution. The tubes were subsequently centrifuged at 4600 rpm for 10 min, after which the absorbance of the supernatant was measured at 485 nm. A linear regression curve was obtained using glucose (Sigma-Aldrich, St. Louis, MO, USA) as a standard polysaccharide material (Figure 2), after which the polysaccharide concentration was calculated using a regression equation.

Chlorophyll a *Determination*

The biomass of *Nostoc* cells was determined by measuring the chlorophyll *a* contents. Briefly, 5 mL of ethanol (99.9%) were added to 2 g soil in a 50-mL cap tube, then allowed to stand for 5 min in a 80°C water bath, after which they were allowed to cool for 30 min and centrifuged at 4000 rpm for 5 min. The absorbance of the supernatant was then measured (A665) and the chlorophyll *a* concentration was estimated using the following equation (Ritchie, 2006):

$$\text{Chlorophyll } a\,(\mu\text{g/g}) = \frac{11.9035\,\text{A665} \times \text{ethanol(mL)}}{\text{sample weight(g)} \times \text{cell path length}}$$

Statistical Analysis

Differences in cyanobacterial cell dose and superabsorbent polymer (SAP) treatments with respect to MWD values, Chlorophyll *a*, and EPS contents were analyzed using one-way ANOVA (Tukey's HSD). Analyses were conducted using SPSS 18.0 and a $p \leq 0.05$ was considered significant for all analyses.

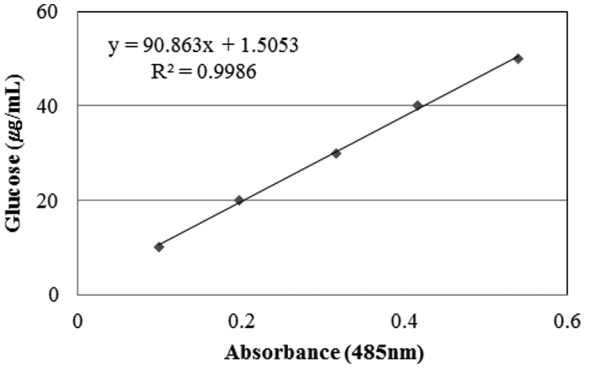

Figure 2. The linear regression curve that was used to calculate polysaccharide concentrations of the soils. The curve was obtained using glucose as a standard polysaccharide material.

Results

Aggregate Stability

Fragment Size Distribution

As shown in Figure 3, SAP had no noticeable effect on aggregate stability of sand particles (Figure 4) relative to *Nostoc* sp. treatment (AC). Although coarse fragments larger than 2 mm were not found in the no-superabsorbent treatment

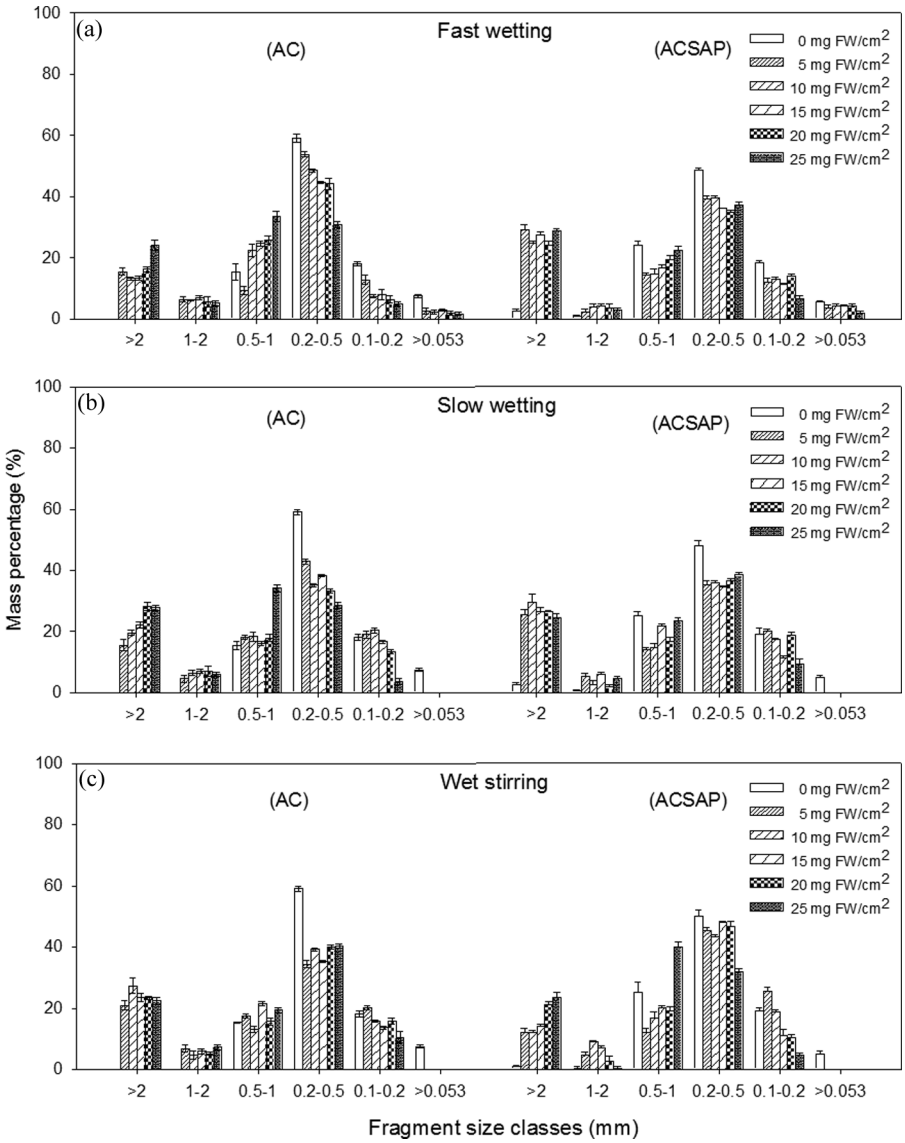

Figure 3. Fragment size distribution of soil aggregates treated with algal (*Nostoc*) cells (AC), and combined application of algal cells and superabsorbent polymer (0.025 mg DW/cm^2) (ACSAP) after three testing treatments. Values represent the mean of triplicate measurements. Error bars represent the standard errors.

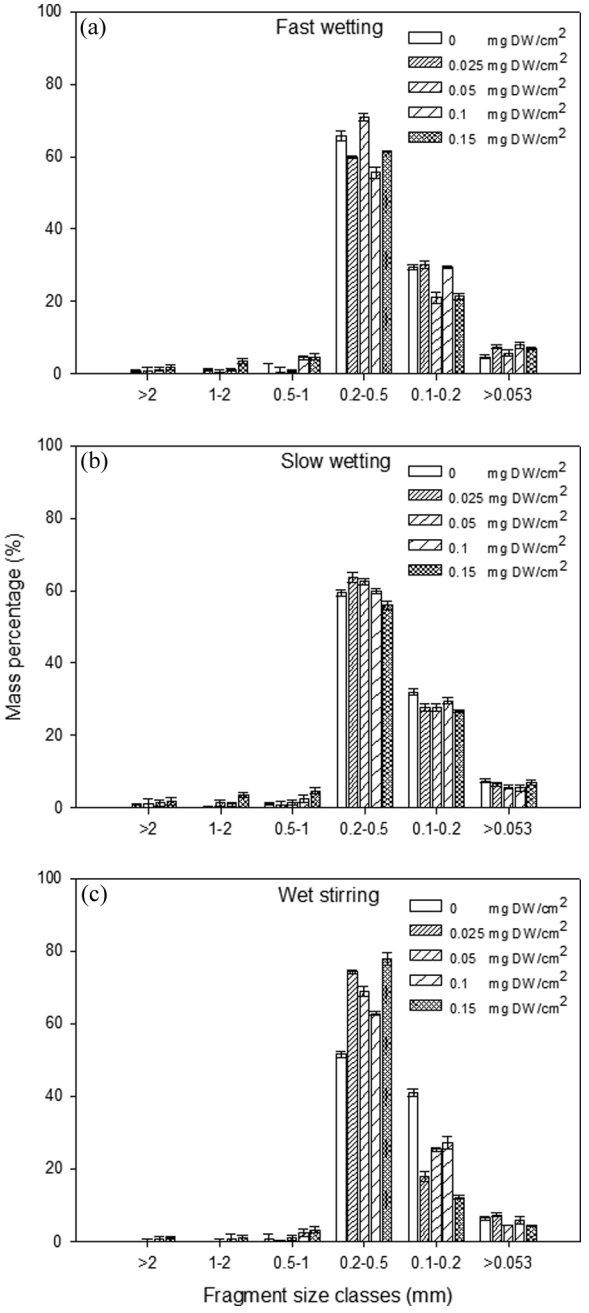

Figure 4. Fragment size distribution of soil aggregates treated with different doses of superabsorbent polymer. Values represent the mean of triplicate measurements. Error bars represent standard errors.

(0 mg DW/cm^2), only 0.8–1.9% of the mass formed coarse fragments larger than 2 mm in response to 0.025–0.15 mg DW/cm^2 SAP application. More than 60% of the mass formed small fragments ranging from 0.2 to 0.5 mm in response to the

SAP, and the formation of small fragments was apparent in the wet stirring test. However, *Nostoc* sp. treatment (AC) had a strong effect on soil aggregation (Figure 3), and combined treatment with *Nostoc* cells and superabsorbent (ACSAP) exerted a synergistic effect on soil aggregation that was evident in both the fast and slow wetting tests (Figure 3). Specifically, the fast wetting test revealed that the average mass percentage of coarse fragments of AC and ACSAP was 13.7% and 22.8%, respectively (Figure 3). These findings imply that ACSAP samples were more resistant to aggregate breakdown than AC as they produced less fine fragments and more coarse fragments. In the slow wetting test (Figure 3), the average mass percentage of coarse fragments of AC and ACSAP was 18.9% and 22.5%, respectively, and the results showed similar behavior to those observed in the fast wetting test. In the wet stirring test (Figure 3), the average mass percentage of coarse fragments of AC and ACSAP was 19.6% and 14.2%, respectively. In contrast, AC samples showed a higher percentage of coarse fragments than ACSAP. In these three tests, the mass percentage of coarse fragments in AC treatment became higher as *Nostoc* cell doses increased. However, the percentage of coarse fragments of the samples in the ACSAP treatment was similar to that observed at all test *Nostoc* cell dose. These results demonstrate that cyanobacterial cells can more effectively aggregate sand particles when applied in combination with SAP.

Mean Weight Diameter

SAP treatment increased the MWD values of aggregates in the three tests of aggregate stability (Figure 1). In the fast wetting test, MWD values of no SAP applications (0 mg DW/cm^2) ranged from 0.34–0.35 mm, while the SAP treatments showed MWD values of 0.37–0.47 mm and the MWD values were significantly higher than those of no SAP treatment. These results are consistent with those obtained from the other two tests. In general, MWD values increased as the SAP doses increased in all three tests of aggregate stability. However, the difference in MWD values was not statistically significant among SAP doses of 0.025, 0.05, and 0.1 mg DW/cm^2. As a result, the minimal dose of SAP required for soil aggregation was determined to be 0.025 mg DW/cm^2.

Nostoc sp. treatment (AC) also increased the MWD values in the three tests of aggregate stability (Figure 5), and the resulting MWD values were much higher than those of the SAP application (Figure 1). When compared with MWD values of 0.35–0.38 mm in the no *Nostoc* cell (0 mg FW/cm^2) treatment in the fast wetting test (Figure 5), *Nostoc* cell treated samples (5–25 mg FW/cm^2) showed much higher MWD values of 0.68–0.93 mm and 0.82–0.93 mm in the AC and ACSAP treatments, respectively. Moreover, the increase in MWD induced by *Nostoc* sp. amendment was stimulated by combined application of SAP (0.025 mg DW/cm^2) in the fast wetting test. The highest MWD value in the fast wetting test was 0.93 mm in both AC and ACSAP treatments when *Nostoc* cells were inoculated at 25 mg FW/cm^2. In the slow wetting test, MWD values of AC and ACSAP ranged from 0.69–1.0 mm and 0.84–0.89 mm, respectively (Figure 5). The MWD values of AC samples tended to gradually increase as *Nostoc* cell doses increased. In the wet stirring test, MWD values of AC and ACSAP ranged from 0.80–0.87 mm and 0.60–0.89 mm, respectively (Figure 5). Most AC samples showed higher MWD values than ACSAP samples, but the difference was not significant at high dose (20 and 25 mg FW/cm^2) of *Nostoc* sp. The MWD values of the fast and slow wetting test were more stable in the ACSAP

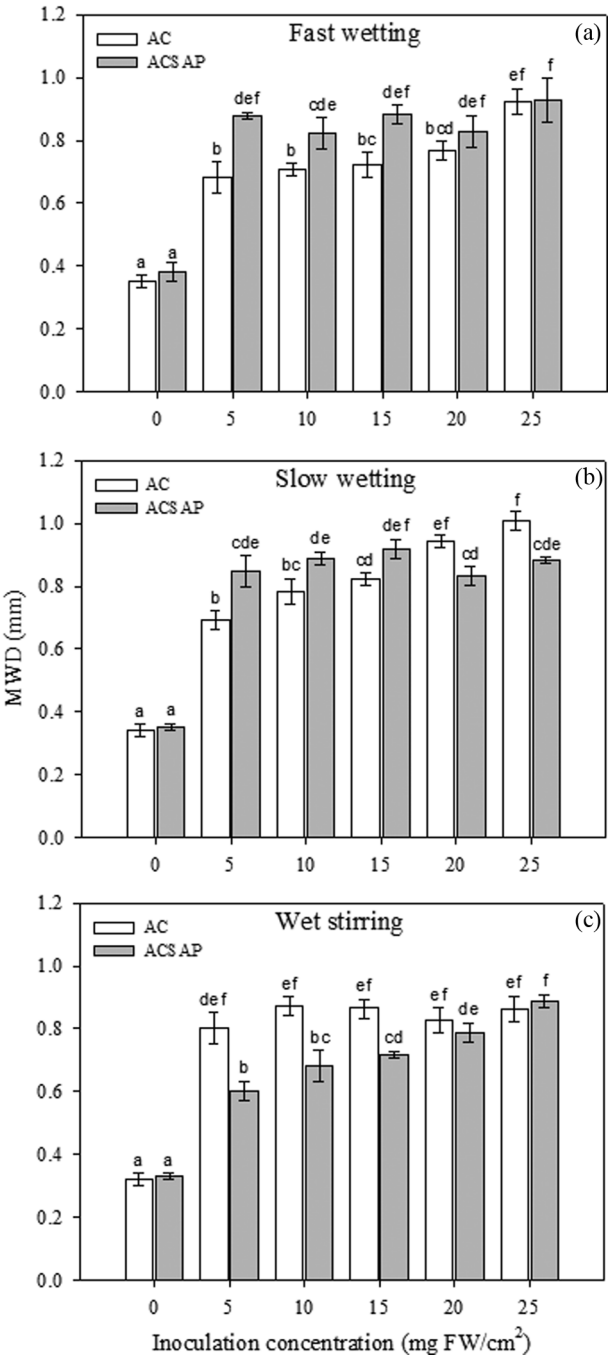

Figure 5. Values of the mean weight diameter (MWD) of sand particles treated with algal (*Nostoc*) cells (AC), and combined application of algal cells and superabsorbent polymer (0.025 mg DW/cm^2) (ACSAP) after three testing treatments. Values represent the mean of triplicate measurements. Values with the same letter are not significantly different according to the multiple comparison test (95% Tukey's HSD). Error bars represent standard errors.

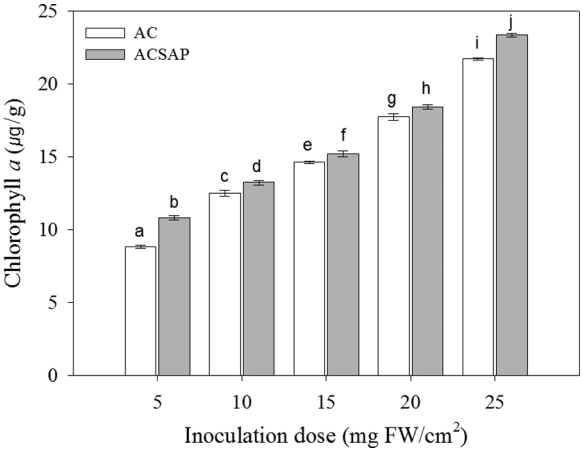

Figure 6. Chlorophyll *a* contents of soil treated with algal (*Nostoc*) cells (AC), and combined application of algal cells and superabsorbent polymer (0.025 mg DW/cm^2) (ACSAP). Values represent the mean of triplicate measurements. Values with the same letter are not significantly different according to the multiple comparison test (95% Tukey's HSD). Error bars represent standard errors.

treatment than the AC treatment at the tested cell dose of *Nostoc* sp. However, AC samples were more stable than ACSAP samples in the wet stirring test.

Biomass

Biomass of *Nostoc* sp. was measured by estimating the chlorophyll *a* content (Figure 6). As the *Nostoc* cell dose increased from 5 to 25 mg FW/cm^2, the biomass

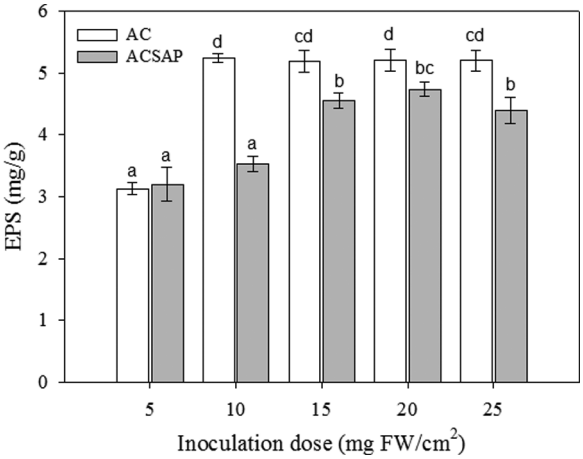

Figure 7. Extracellular polysaccharide (EPS) contents of the soil treated with algal (*Nostoc*) cells (AC), and combined application of algal cells and superabsorbent polymer (0.025 mg DW/cm^2) (ACSAP). Values represent the mean of triplicate measurements. Values with the same letter are not significantly different according to the multiple comparison test (95% Tukey's HSD). Error bars represent standard errors.

of both samples increased significantly. The final biomass of the ACSAP samples was significantly higher than that of the AC samples after 3 months of incubation. The biomass of the AC and ACSAP samples ranged from 8.8–21.7 μg/g and 10.8–23.4 μg/g, respectively, indicating that SAP was beneficial to *Nostoc* cell growth.

Extracellular Polysaccharide

The EPS contents of AC and ACSAP samples are presented in Figure 7. The EPS content of the AC samples was 3.13 mg/g at a dose of 5 mg FW/cm^2 of *Nostoc* cells, while those at the other dose (10–25 mg FW/cm^2) were very similar to each other (5.19–5.23 mg/g). Additionally, the EPS content of ACSAP samples was 3.20–4.73 mg/g, which was relatively lower than that of the AC samples at all tested *Nostoc* cell dose.

Discussion

Polyvinyl alcohol (PVA) and polyacrylamide (PAM) are hydrophilic polymers widely used as soil fixing agents or conditioners (Gokcen, 2010). Previous studies showed that these polymers significantly decrease runoff and soil erosion (Uysal et al., 1995). However, for combined application with cyanobacteria, polymers that have no harmful effects on cyanobacterial growth should be considered. Therefore, we attempted to identify alternative polymers that can fix sand particles and are not harmful to cyanobacterial cells. SAP has high water holding capacity, which would be beneficial to cyanobacterial cell growth, and its high viscosity can bind soil particles together. Therefore, in this study, SAP was prepared at different doses (0.025–0.15 mg DW/cm^2) to estimate the stability of soil aggregates formed in response to its application for determination of the minimal dose of SAP. The MWD values showed that SAP enhanced soil aggregation, and that the resulting aggregates were more stable against destructive factors than SAP untreated samples. Because SAP application at doses of 0.025–0.1 mg DW/cm^2 showed similar MWD values, we determined SAP 0.025 mg DW/cm^2 to be the minimal dose for the combined application of SAP with *Nostoc* cells in this study.

The aggregate stability of single application of cyanobacterial cells (AC) and combined application of cyanobacterial cells and SAP (ACSAP) were estimated by the fast, slow and wet stirring methods. The fast wetting method is good for comparison of the behavior of a large range of soils subjected to rapid wetting (heavy rains in summer) and slow wetting with controlled tension (gentle rains). It is well known that cyanobacterial cells make soil stable, and our results agreed well with those of previous reports (Wu et al., 2013; Xiao et al., 2011; Yu et al., 2011). Moreover, combined use of SAP with *Nostoc* cells (ACSAP) resulted in significantly stronger resistance against aggregate breakdown than use of *Nostoc* cell alone (AC). Soil particles were more aggregated by SAP in ACSAP treatment than AC treatment. As a result, increases in the percentage of coarse aggregates were more evident in ACSAP treatment than in AC. MWD values of ACSAP treatment were also higher than that of AC. As shown in Figure 6, the superabsorbent used in our study enhanced cell growth of *Nostoc* sp. The biomass of ACSAP treatment was significantly higher than that of AC at all *Nostoc* cells doses. This result may be due to mineral elements that originated from SAP and could be a result of nutritional factors that is consequently beneficial to *Nostoc* cell growth. Cyanobacteria supplemented with SAP grew very well, showed elongated filaments, and increased sand

fixing performance. Schulten (1985) demonstrated that increased biomass of cyanobacteria enhanced binding capacity with sand grains or direct contact with clay particles. Xie et al. (2007) also mentioned that, while filaments extend to clay particles for absorbing nutrition, they inevitably bind with sand grains and make larger fragments. Physical properties of soil might be improved by SAP because they have the following properties: increased water use efficiency, enhanced soil permeability and infiltration rates, reduced irrigation frequency, reduced compaction tendency, stopping erosion, and water run-off (Ekebafe et al., 2011). The biomass of algal crusts is closely related to its compressive strength, which was enhanced by the increase in algal biomass (Xie et al., 2007). In contrast to the fast and slow wetting tests, the wet stirring test revealed that the mass percentage and MWD values of AC samples were higher than those of ACSAP at all *Nostoc* cell doses (Figure 5). Although AC samples had less biomass than ACSAP samples (Figure 6), the EPS content was higher in the AC treatment than the ACSAP treatment (Figure 7). Measurement of the stability of soil aggregates by the fast and slow wetting test was conducted using the slaking test. Slaking is the breakdown of large, air-dried soil aggregates (>2–5 mm) into smaller sized microaggregates (<0.25 mm) when they are suddenly immersed in water. Slaking occurs when aggregates are not strong enough to withstand internal stresses caused by rapid water uptake. Slaking indicates the stability of soil aggregates and resistance to erosion, and suggests how well soil can maintain its structure to provide water and air for plants and soil biota when it is rapidly wetted. ACSAP treatment showed a higher increase in the biomass of *Nostoc* cells, which have elongated filamentous cells relative to AC treatment, and these filamentous cells bind with sand grains to form larger fragments. Because SAP has viscous properties, it might contribute in part to the stronger soil stability of ACSAP than AC by means of its gluing activity of sand grain. In contrast to the fast and slow wetting test, a wet stirring test was conducted by a mechanical breakdown test. As shown in Figure 7, AC treatment generated a higher content of EPS than ACSAP treatment. The ESP can function as a gluing material to hold sand grains by physical contact. Under natural conditions, BSC is more vulnerable to slaking mediated by heavy and moderate rains than to mechanical breakdown. Thus, this speculation suggests that combined application of cyanobacterial cells and SAP has a positive effect on soil aggregation stability and soil biota of the induced BSC by maintaining its structure relative to application of cyanobacteria alone.

The content of EPS was not directly related to the amount of cyanobacterial biomass in our study. However, it is well known that the ESP secreted by microorganisms are produced to protect cells from adverse conditions such as desiccation (Costerton et al., 1981; Whitfield, 1988). Because the SAP kept high moisture content for the cyanobacterial cells, *Nostoc* cells in ACSAP samples were exposed to less dry conditions than AC. Due to the high water absorbing capacity of the SAP, the cyanobacterial cells might produce less EPS under mild desiccation stress. The resulting aggregate stability of AC samples was comparatively stable against mechanical breakdown. Previous studies also suggested that increasing the EPS content improved aggregation of inoculated soils due to changes in the micromorphological characteristics of the aggregates (Belnap and Gardner, 1993; Malam Issa et al., 2001).

Although *Nostoc* cell dose is likely proportional to soil stability, no significant differences were observed in MWD values between AC and ACSAP, regardless of *Nostoc* cell dose, implying that the SAP might play a role in soil aggregation together

with *Nostoc* cells. The EPS contents were not significantly different among *Nostoc* cell dose of 10, 15, 20, and 25 mg FW/cm^2. The aggregate stability tests and EPS contents suggested that the minimal inoculation dose of *Nostoc* cells should be 10 mg FW/cm^2.

Synthetic polymer materials have caused significant environmental problems due to their non-biodegradable properties (Azahari et al., 2011). For these reasons, non-toxic, eco-friendly and biodegradable polymers have been used in recent years. Many studies (Azahari et al., 2011; Gokcen, 2010; Liu et al., 2012; Yang et al., 2007a) have investigated the application of polymers alone to fix the soil surface. The results of these studies showed that the chemicals could effectively fix soil surfaces in the short term, but that fixed soils could be degraded in nature within few years. This is the first study to investigate combined application of cyanobacterial cells with synthetic polymers. The chemicals facilitate BSC formation of artificially inoculated cyanobacteria with the aid of rapid fixation of soil particles at the initial stage of application. Combined application can be a novel approach for restoring damaged soil in arid areas. SAP positively influenced *Nostoc* cell growth and showed better performance of artificial BSC induction. Consequently, increased cyanobacterial biomass with SAP application will stimulate soil aggregation, and EPS will contribute to soil stability in the later stages of BSC formation.

Funding

This work was supported by a grant from the Forest Science & Technology Projects (Project No. S111212L030100) provided by the Korea Forest Service and the Chinese Academy of Sciences visiting professorship for senior international scientists (Grant No. 2013T2Z0007).

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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