Soil Biology & Biochemistry 41 (2009) 926–929

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area

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ARTICLE INFO

Article history:
Received 24 December 2007
Received in revised form 23 June 2008
Accepted 1 July 2008
Available online 27 December 2008

Keywords:
Cyanobacterial field inoculation
Biological soil crusts formation
Soil physicochemical characteristics
Vascular plants

ABSTRACT

Practical testing of the feasibility of cyanobacterial inoculation to speed up the recovery of biological soil crusts in the field was conducted in this experiment. Results showed that cyanobacterial and algal cover climbed up to 48.5% and a total of 14 cyanobacterial and algal species were identified at the termination of inoculation experiment; biological crusts' thickness, compressive and chlorophyll *a* content increased with inoculation time among 3 years; moss species appeared in the second year; cyanobacterial inoculation increased organic carbon and total nitrogen of the soil; total salt, calcium carbonate and electrical conductivity in the soil also increased after inoculation. Diverse vascular plant communities composed of 10 and 9 species are established by cyanobacterial inoculation on the windward and leeward surface of the dunes, respectively, after 3 years. The Simpson index for the above two communities are 0.842 and 0.852, while the Shannon–Weiner index are 2.097 and 2.053, respectively. In conclusion, we suggest that cyanobacterial inoculation would be a suitable and effective technique to recover biological soil crusts, and may further restore the ecological system.

Biological soil crusts occur in semiarid and arid regions throughout the world, and they have been proved to play an important role in maintaining or improving the state of the environment (Belnap, 2003). However, due to their sensitivity to anthropogenic and natural disturbances, they are in a degraded state in many areas throughout their range (Maestre et al., 2006). Many studies were conducted to estimate the recovery rates of the biological soil crusts under natural conditions. The results indicated that there existed a range of decades to millennia for full recovery of all components (Belnap, 1993; Belnap and Eldridge, 2003). To speed up recovery, inoculation of soils with biological crust components has been recommended in degraded arid and semiarid ecosystems (St Clair et al., 1986; Belnap, 1993).

Cyanobacteria are generally the first photosynthetic species to appear on disturbed soils (Booth, 1941). Much interest has been focused on the effects of cyanobacterial inoculation on arid and semiarid soils (Tiedemann et al., 1980; Belnap, 1993; Buttars et al., 1994, 1998; De Caire et al., 2000; Acea et al., 2001, 2003; Davidson et al., 2002; Maestre et al., 2006; Nisha et al., 2007). However, the large majority of these studies were carried out on experimental conditions while field applications have not yet been successful; it was presumed that using inoculants for even moderately large disturbances is not feasible now, or in the near future (Belnap, 2003).

In this study, traditional approaches using inoculants alone were improved by combining mixed cyanobacterial inoculation with sand barriers of straw checkboards and automatic sprinkling microirrigation techniques.

The objective of this study was to test the feasibility of cyanobacterial inoculation for biological crust formation in desert areas. Therefore, we evaluated the effect of inoculation on: (i) colonization and development of biological soil crusts; and (ii) physicochemical characteristics of the topsoil. In addition, the germination and establishment of vascular plants after cyanobacterial inoculation were examined.

A factorial experiment was carried out under field conditions from 2003 to 2006. The design of the experiment consisted of two types of slope (windward and leeward) and four levels of recovery times (0, 1, 2, and 3 years), resulting in eight treatment combinations. One adjacent dune without checkboards, which was not inoculated and irrigated, served as control (Table 1).

The research was conducted on a degraded area in Dalateqi county, Inner Mongolia (40°21′ N, 109°51′ E). Two cyanobacteria strains, *Microcoleus vaginatus* Gom. and *Scytonema javanicum* Born et Flah, were isolated from desert algal crusts. Mass cultivation was conducted in a greenhouse.

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0038-0717/$ – see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.soilbio.2008.07.001

Table 1
Ten treatment combinations designed in the experiment.

Recovery times	Inoculation time	Sampling time	Slopes	Sampling sites	Treatment numbers
Control	–	2006	Windward	A	1
Control	–	2006	Leeward	A′	2
18 d(0 year)	2005	2005	Windward	B	3
18 d(0 year)	2005	2005	Leeward	B′	4
1 year	2005	2006	Windward	C	5
1 year	2005	2006	Leeward	C′	6
2 years	2004	2006	Windward	C	7
2 years	2004	2006	Leeward	C′	8
3 years	2003	2006	Windward	D	9
3 years	2003	2006	Leeward	D′	10

Both cultures and liquid medium were pumped into a tank ($2 \times 2 \times 1.5$ m^3) and then transferred to the moving dunes where sand barriers of straw checkboards (1×1 m^2) had been previously constructed. After mixing two cultures in the ratio of 10 to 1 (*M. vaginatus*:*S. javanicum* (dry weight)), inoculation was performed by spraying the mixture (ca. 1.6 g dry weight m^{-2}) over the sand surface as homogeneously as possible. Sand was intermittently irrigated with 20 mm of groundwater per day by automatic sprinkling micro-irrigation facilities from 9:00 to 16:30 for 15–18 d until man-made cyanobacterial crusts formed.

Sampling and investigation took place on September 25–27, 2006 (except 3 and 4, which were conducted on September 17, 2005), 2 days after rain. For each of 10 treatments (10 sampling sites, Table 1), three plots (3×3 m^2) were chosen subjectively. For each plot, five quadrats (1×1 m^2) were placed randomly. At each quadrat within plots, a small amount (ca. 20 g) of soil was collected to a depth of about 1 cm and composited into a composite soil sample ($n = 5$ quadrats). Subsamples of ca. 300 g were carried to the laboratory for chemical analyses. From each quadrat, the cyanobacterial crusts were collected as approximately 1 cm diameter cores with a glass ring, packed in the sterilized aluminium boxes, and carried to laboratory with the soil samples.

The soil physicochemical characteristics were measured by the standard methods, described by Bo (2000). The content of chlorophyll *a* was measured with spectrophotometer according to the method of Brostoff (2002) and compressive strength was measured using a soil sclerometer as described by Xie et al. (2007). Algal species diversity was measured using dilution plate cultures and the moistened soil methods (Johansen et al., 1993).

All results were obtained from three replicates of each treatment. One-way analysis of variance (ANOVA) and Tuckey's post hoc test were carried out to separate the means of different treatments. The Pearson's correlation coefficients (r) were obtained to evaluate the relationships between the variables. All analyses were performed using the program SPSS 11.5.

The soils of the control samples were unconsolidated sand and did not contain any type of biological soil crust (Table 2). Eighteen days after cyanobacterial inoculation, cyanobacterial crusts with 100% cover formed. The cover decreased to 41.5% one year later and then increased to 45.5% and 48.5% after 2 and 3 years recovery, respectively. Two years later, moss crusts colonized the inoculated soils in the leeward face of sand dunes. It shortened colonization time by 100% compared with the results of Li et al. (2004). No lichen crusts were found in all inoculated soils. Table 2 showed a time-dependent increase in the biological crusts' thickness, compressive strength and chlorophyll a content. Biological crusts' thickness and chlorophyll *a* content in the windward face were significantly lower than the leeward face, whereas compressive strength in the windward face was significantly higher than the leeward face. Algal species showed the highest number in treatment 10 (Table 3). Li et al. (2004) reported the same 14 species number using sand-binding vegetation after 20 years. The numbers followed the order $10 > 8 > 9 = 7 = 6 = 5 > 4 > 3$. *Phormidium tenue*, *Schizothrix friesii* and *Chloroccum humicola* gradually became the dominant species and sub-dominant species with increasing inoculation time. *M. vaginatus* and *S. javanicum* still were the dominant species and sub-dominant species, respectively. Tiedemann et al. (1980) noted that one chlorella was the most prominent species and *Phormidium* sp. was dominant on the soil surface at the end of the 20 weeks' cyanobacterial inoculation experiment. The reason for these differences may be because of different soils and environmental parameters.

Table 3
Variations of cyanobacterial and algal species diversity after inoculation on moving dunes (+++, indicates dominant species; ++, sub-dominant species; +, rare species).

Species	3	4	5	6	7	8	9	10
Cyanobacteria (8)								
Aphanocapsa sp.						+		+
Calthrix sp.				+	+	+	+	+
Lyngbya martensiana Men.						+		+
Microcoleus vaginatus Gom.	+++	+++	+++	+++	+++	+++	+++	+++
Nostoc commune Vauch.						+	+	+
Phormidium tenue (Men.) Gom.			+	+	++	+++	++	+++
Schizothrix friesii (Ag.) Gom.			+	+	++	++	+++	++
Scytonema javanicum Born et Flah	++	++	++	++	++	++	++	++
Chlorophyta (3)								
Chlamydomonas sp.				+		+		+
Chlorella vulgaris Beij.			+		+	+	+	+
Chloroccum humicola (Naeg.) Rab.			+	+	+	++	+	++
Bacillariophyta (3)								
Gomphonema constrictum Ehr.		+	+		+			+
Hantzschia amphioxys (Her.) Grun.						+		+
Navicula cryptocephala Kütz.	+	+	+	+		+		+

The control's soils were low in organic C (0.33–0.49 g kg^{-1}) and total N (0.18–0.19 g kg^{-1}) (Table 4). However, in all inoculation

Table 2
Changes in the characteristics of the biological soil crusts over time in different cyanobacterial inoculation treatments (mean ± SD).

Characteristic	1	2	3	4	5	6	7	8	9	10
Cyanobacterial cover (%)	0a	0a	100d	100d	40.33 ± 3.79b	42.67 ± 5.03b	39.00 ± 6.56b	49.67 ± 5.03bc	40.00 ± 3.61b	57.00 ± 7.21c
Lichen cover (%)	0	0	0	0	0	0	0	0	0	0
Moss cover (%)	0a	0a	0a	0a	0a	0a	0a	2.4 ± 1.22b	0a	4.6 ± 1.51c
Thickness (mm)	0	0	0.99 ± 0.04a	1.02 ± 0.09a	1.31 ± 0.20a	1.75 ± 0.27b	2.18 ± 0.26b	2.78 ± 0.13c	2.34 ± 0.20b	3.38 ± 0.23d
CS (kg cm^{-2})	0	0	0.36 ± 0.04a	0.34 ± 0.04a	1.34 ± 0.38b	1.22 ± 0.17b	3.81 ± 0.19c	2.47 ± 0.21c	3.90 ± 0.12d	3.83 ± 0.07c
Chl *a* (mg g^{-1})	0	0	2.97 ± 0.07b	3.03 ± 0.08b	1.59 ± 0.08a	2.33 ± 0.22a	3.00 ± 0.18b	3.56 ± 0.38b	3.42 ± 0.74b	5.31 ± 0.24c

For a given parameter, values with the same letters are not significantly different ($p < 0.05$) according to Tukey's post hoc test. CS, compressive strength; Chl *a*, chlorophyll *a* content.

Table 4
Changes in the soil characteristics in different cyanobacterial inoculation treatments (mean ± SD).

Characteristic	1	2	3	4	5	6	7	8	9	10
Bulk density ($g\,cm^{-3}$)	1.54 ± 0.04ab	1.56 ± 0.04b	1.56 ± 0.10b	1.55 ± 0.06ab	1.56 ± 0.05b	1.52 ± 0.04ab	1.54 ± 0.02ab	1.49 ± 0.04ab	1.53 ± 0.06ab	1.41 ± 0.03a
Organic C ($g\,kg^{-1}$)	0.49 ± 0.24a	0.33 ± 0.13a	2.71 ± 0.43b	2.67 ± 0.44b	3.95 ± 1.15bc	3.05 ± 0.96b	5.65 ± 0.61 cd	7.01 ± 0.80d	6.23 ± 0.72d	8.83 ± 1.17e
Total N ($g\,kg^{-1}$)	0.19 ± 0.02a	0.18 ± 0.07a	0.43 ± 0.04bc	0.45 ± 0.04bc	0.45 ± 0.13bc	0.37 ± 0.07b	0.47 ± 0.06bc	0.61 ± 0.07 cd	0.66 ± 0.07de	0.79 ± 0.09e
C/N	2.55	1.85	6.35	5.97	8.89	8.09	11.88	11.43	9.49	11.14
Total salt ($g\,kg^{-1}$)	0.28 ± 0.05a	0.31 ± 0.07a	0.36 ± 0.05a	0.39 ± 0.06a	0.27 ± 0.04a	0.31 ± 0.04a	0.32 ± 0.04a	0.37 ± 0.06a	0.29 ± 0.03a	0.60 ± 0.05b
$CaCO_3$ ($g\,kg^{-1}$)	4.31 ± 0.41a	4.65 ± 0.13a	4.12 ± 0.39a	4.67 ± 0.71a	9.79 ± 1.48b	9.30 ± 1.18b	15.50 ± 0.77c	15.04 ± 1.42c	17.33 ± 0.86 cd	19.14 ± 1.98d
Soil pH	8.58 ± 0.1abc	8.54 ± 0.22abc	8.95 ± 0.15bc	8.98 ± 0.14c	8.52 ± 0.26ab	8.64 ± 0.11abc	8.41 ± 0.09a	8.49 ± 0.19ab	8.48 ± 0.21a	8.47 ± 0.14a
EC($s\,m^{-1}$)	0.03 ± 0.01a	0.02 ± 0.01a	0.06 ± 0.01a	0.07 ± 0.02a	0.06 ± 0.01a	0.06 ± 0.02a	0.06 ± 0.02a	0.12 ± 0.03b	0.07 ± 0.01a	0.14 ± 0.03b

For a given soil parameter, values with the same letters are not significantly different ($p < 0.05$) according to Tukey's post hoc test. $CaCO_3$, calcium carbonate; EC, electrical conductivity.

treatments, organic C and total N ranged from 2.67 to 8.83 g organic C kg^{-1} soil and from 0.37 to 0.79 g N kg^{-1} soil, respectively. The bulk density of treatment soils varied from 1.41 to 1.56 g cm^{-3} and they were generally lower than control's soils (1.54–1.56 g cm^{-3}). The concentrations of total salt and $CaCO_3$ in inoculation soils varied from 0.27 to 0.60 g salt kg^{-1} soil and from 4.12 to 19.14 g $CaCO_3$ kg^{-1} soil, respectively, whereas in control's soils they ranged from 0.28 to 0.39 g salt kg^{-1} soil and from 4.31 to 4.65 g $CaCO_3$ kg^{-1} soil, respectively. The soil pH showed the highest values in treatments 3 and 4, which were inoculated for 18 d. The highest EC occurred in the leeward surface of the sand dunes which were inoculated for 3 years. Detailed analyses revealed that organic C, $CaCO_3$ and EC increased with increasing recovery time. On the contrary, the value of pH decreased with increasing recovery time. Results that total N and total salt decreased after 1 year inoculation and increased subsequently were unexpected. In general, the values of organic C, total salt and EC in the windward surface were significantly lower than the leeward surface, while bulk density had the opposite result in all cyanobacterial inoculation treatments. Our results agree with those of experimental results obtained in greenhouse studies (Acea et al., 2003; Nisha et al., 2007), which showed that algal inoculation increased organic C and total N of the soil, but decreased soil bulk density. However, Tiedemann et al. (1980) reported that cyanobacterial inoculation had no effect on the total C and total organic N. Changes of values of total N and C/N are not consistent with those of Nisha et al. (2007), who suggests these two parameters continuously change with the recovery time.

It can be seen from Table 5 that herbaceous and subshrub cover increased with increasing recovery time among the eight cyanobacterial inoculation treatments. Total species numbers and the diversity indices (Simpson index and Shannon–Weiner index) increased similarly. For these four Characteristics control treatments showed a significant difference compared to treatments 5–10. No shrub species were found in any cyanobacterial inoculation treatments. Table 6 shows the relative importance value (relative height + relative coverage) of subshrub and herbaceous species in different treatments. The diverse vascular plant communities composed of 10 and 9 species are established by cyanobacterial inoculation in windward and leeward surfaces, respectively, after 3 years. These communities are more likely to support and foster ecosystem resilience than a community dominated by one or a few species (Elmarsdottir et al., 2003). The Simpson index for above two communities is 0.842 and 0.852, while the Shannon–Weiner index is 2.097 and 2.053, respectively. With the exception of treatment 8, the dominant species was *Artemisia ordosica* Krasch. in cyanobacterial inoculation treatments from 5 to 10. No vascular plants were found in the soils of the control.

No significant correlation has been found between cyanobacterial cover and total N ($r = -0.23$, $p = 0.584$); however, the correlation coefficient between vascular plants cover and total N was significant ($r = 0.847$, $p < 0.01$). There was also a significant correlation between chlorophyll *a* content and total N ($r = 0.860$, $p < 0.01$). Moss crusts occurred when vascular plants cover reached a fairly high level, and it only occurred on the leeward side of the dunes. Perhaps it needs sufficient shading and moisture to survive (Fenton and Frego, 2003). Why no lichens were found in the biological soil crusts is unknown and needs further study in the future.

Table 5
Changes in the characteristics of vascular plants in different cyanobacterial inoculation treatments.

Characteristic	3	4	5	6	7	8	9	10
Herbaceous cover (%)	1	2	4.5	5	10	26	26	33
Subshrub cover (%)	0	0	12	15	17	0	27	22
Shrub cover (%)	0	0	0	0	0	0	0	0
Total species number	1	2	4	2	5	4	10	9
Simpson index (*D*)	0	0.471	0.657	0.452	0.734	0.628	0.842	0.852
Shannon–Weiner index (*H′*)	0	0.664	1.213	0.644	1.470	1.174	2.097	2.053

Acknowledgements

This work was supported by the project for the Study of Basic Biology of Desert Algae and Crusts Forming Techniques by Desert Algae, financially supported by the State Key Laboratory of Freshwater Ecology and Biotechnology of China.

Table 6
The relative importance value (relative height + relative coverage) of subshrub and herbaceous species in different cyanobacterial inoculation treatments.

Species	3	4	5	6	7	8	9	10
Agriophyllum squarrosum (Linn.) Moq.	1.000					0.168	0.095	0.057
Agropyron mongolicum Keng		0.620						
Artemisia ordosia Krasch.			0.504	0.655	0.427		0.324	0.275
Artemisia sphaerocephala Krasch.							0.088	0.088
Astragalus adsurgens Pall.					0.113	0.172	0.082	0.106
Calamagrostis epigeios (Linn.) Roth.					0.132		0.079	
Carex duriuscula C. A.								0.033
Elymus dahuricus Turcz.			0.235	0.345	0.161	0.550	0.086	0.108
Ixeris dentata (Thunb.) Nakal.							0.036	
Leymus chinensis (Trin.) Tzvel.							0.066	0.106
Leymus secalinus (Georgi) Tzvel.					0.168			
Pycnosteluma laterif lorum			0.127					
Salsola collina Pall.		0.380				0.110		
Setaria viridis (L.) Beauv.							0.076	0.127
Sophora alopecuroides L.								0.100
Taraxacum mongolicum Hand. –Mazz.							0.068	
Thermopsis lupinoides (Linn.) Link			0.134					

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