THE INFLUENCE OF CYANOBACTERIAL AMENDMENTS ON SOIL AGGREGATE SIZE, SOIL NITROGEN CONTENT AND SEEDLING ESTABLISHMENT IN A GRAVEL PIT

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ABSTRACT

Cyanobacteria are amongst the first colonizers of disturbed lands and their activities are reported to improve soil conditions by the addition of fixed atmospheric nitrogen, by aggregation of soil particles, and by the enhancement of seed germination and establishment. Seedling establishment may also be improved by the presence of soil crust disturbances such as cracks and irregularities in the microtopography. In this study, we report the results of experiments in a field situation and in controlled greenhouse conditions in which a bulk culture of the nitrogen-fixing cyanobacterium, *Nostoc commune*, was sprayed onto the soil surface. Three replicates of four treatments were used: soil surface inoculated with *Nostoc* and surface disturbed by trampling, soil surface inoculated and surface undisturbed, soil surface not inoculated and disturbed, and soil surface not inoculated and undisturbed.

After a period to allow for growth of *Nostoc*, seeds of *Poa compressa,* Canada bluegrass, (field study) and *Schizachyrium scoparium* Little bluestem, (greenhouse study) were sown on all surfaces. Results from the field and greenhouse studies differed greatly. Soil aggregate size and nitrogen content were not significantly different among treatment groups in the field plots, however, in the greenhouse significantly larger aggregates were produced in inoculated trays, and a significant interaction was found among all treatments for the NH_4^+ form of soil nitrogen. In the field, disturbance enhanced seedling emergence and early establishment significantly, though this effect was not evident in the greenhouse trials.

Introduction

Human dependency on sand, gravel and crushed stone resources requires the removal of surface soil and vegetation over large expanses of land at licensed extraction sites. In Ontario, the 1990 Aggregate Act was established to ensure that members of the aggregate industry have an acceptable plan for the restoration of the extracted area prior to breaking ground. Restoration plans typically include alteration of the physical topography after the completion of extraction and the re-establishment of vegetation to prevent soil losses by erosive forces. Frequently aggregate companies select non-native rapidly germinating ground cover species that require high initial maintenance, including fertilizer and topsoil, and which can slow natural succession for many years.

It may be possible to achieve faster rates of native seedling and plant community establishment by utilizing pioneer colonizers of disturbed lands. Pioneer colonizers condition the soil and prepare it for vascular plant colonization. Algae and cyanobacteria are known to fulfill this role in arid and semi-arid environments where they are integral components in structures known as cryptogamic crusts (Campbell *et al*. 1989; Ashley and Rushforth 1984). Many of the properties commonly associated with cryptogamic crusts may be attributed to the algal/cyanobacterial component, even though lichens, mosses, fungi and bacteria are often present..

Beneficial properties typically associated with cryptogamic crusts include: reduction of aeolian and water erosion (McKenna-Neuman *et al.* 1996; Maxwell and McKenna-Neuman 1994; Campbell *et al.* 1989; Forster and Nicholson 1981), increased size of soil aggregates, increased water infiltration and retention rates, and increased organic and inorganic nutrient content of the surrounding soil (Johansen 1993; St. Clair *et al.* 1984; Booth 1941). These factors result in improved conditions for seedling germination and establishment (Johansen 1993; St. Clair *et al* 1984; Booth 1941). In addition, St. Clair *et al* (1984) report enhanced seedling germination on intact crust surfaces compared to surfaces damaged by simulated trampling, indicating that surface microtopography may be an important factor.

Cyanobacteria are prime candidates for use in restoration practices due to the beneficial properties associated with cryptogamic crusts. The innoculant used in this study was *Nostoc commune* Vaucher, a heterocystous nitrogen-fixing cyanobacterium commonly found in cryptogamic crusts throughout the world. The purpose of the study was threefold: 1) to determine the effect of *Nostoc commune* on soil aggregation and soil nitrogen content; 2) to determine whether crust formation enhanced seedling germination and establishment; and 3) to examine the influence of crust disturbance on seedling establishment.

Crust and Seedling Establishment in a Field Situation

Site Description

Pinewood Aggregates Site: 44°18.1′ north; 78° 4.1’ west

The field location used in this study, Pinewood Aggregates, is located approximately 23 km east of Peterborough, Ontario. This site is an active sand and gravel pit. A section of the pit, which had not been extracted for approximately three years, was designated as the study area by Pinewood Aggregates management. At the time of this study, there had been no restoration activity in the study area, as it is the intention of the company to use the area in future extraction operations. As a result, very little vegetation was present in the area prior to the initiation of this study; however, all quadrats were cleared of existing vegetation by hand before treatments began.

Twelve quadrats (1m x 1m) were set up using 17m by 17m gridlines along the north-south and east-west axes of the allotted study area. The topography of the site was level. Plots were randomly chosen by means of a Random Number Table. Treatments were randomly assigned. The 12 study plots were separated into 4 treatments groups: not inoculated/undisturbed; not inoculated/disturbed; inoculated/undisturbed; and inoculated/disturbed.

The cyanobacterial amendment used to inoculate the plots consisted of a solution of 2.8 L of *Nostoc commune* (University of Toronto Culture Collection, UTCC 314) cultured in Bold Basal Medium (Nichols and Bold 1965) and 8.9L of de-ionized water. Prior to spraying, *Nostoc* masses were blended in a glass blender for approximately 1 minute to allow for easy passage through the nozzle of the spray applicator.

Two litres of cyanobacterial cutlure were applied to each of the six experimental plots on June 10, 1996. Uninoculated plots were watered with two litres of deionized water. All test plots were watered on a daily basis with 2L of tap water, except on days when a significant rainfall occurred, but the amount was reduced to 1L between July 02 and August 13. To further encourage crust development, 0.25L of Bold Basal Medium was applied to each test plot on three occasions, and clear plastic sheets were placed over each plot to reduce excessive evaporation.

Soil sampling was conducted randomly on June 10 and August 13 by taking 3 sub-samples from each plot. Plots designated as "disturbed" were trampled by two individuals, each of whom took 25 evenly distributed steps per plot. Following disturbance treatments, seed were dispersed to all plots by hand. Each plot was seeded with 2g of

Poa compressa seeds by hand. This amount is based on the standard seeding rate of 20kg/ha per species recognized by most aggregate companies. Each plot was given 1 L of tap water following seeding, after which all watering activities were terminated.

Seedling emergence and establishment were assessed by counting the total number of seedlings present in each plot on October 25.

Seedling Establishment under Greenhouse Conditions

A similar study was conducted in greenhouse conditions. Sand was collected from the Pinewood Aggregates field site, and autoclaved at 120 C for 20 minutes to remove any existing biota. It was then divided between twelve plastic-lined aluminum trays (29.9cm x 23.5cm x 6.4cm). Three replicates of each treatment were assigned randomly to the trays. The treatment groups were the same as for the field component.

550ml of a bulk culture of *Nostoc commune* was sprayed onto each of the test trays, while uninoculated trays were watered with an equivalent amount of deionized water. Trays were watered on a demand basis (approximately every 2 to 3 days) with 0.25L of water per tray, however, all trays were given the same amount of water on the same day to retain a consistent watering level. Although a surface growth was visible at the beginning of the experiment, it soon vanished and no signs of growth were visible for the following 2 months.

Some problems were experienced with both crust dessication and contamination. At this time a tent, constructed of clear plastic sheeting, was placed over the experimental trays in an effort to reduce contamination and to maintain adequate temperature and moisture levels for cyanobacterial growth.

On February 24 all trays assigned to disturbance treatment were subjected to 25 blows to the soil surface with an Erlenmeyer flask. This action was used to simulate trampling. Following disturbance, 0.141 g of *Schizachyrium scoparium* (Little Bluestem) seeds were distributed by hand to each tray. The seeding rate used is based on the same 20 kg/ha standard seeding rate applied to the field study. Trays were watered every 2 days with 150 ml. of de-ionized water until March 08. After which the amount of water was increased to 200 ml. per tray. In addition to watering, trays were moved every 2 days to avoid a position effect. Watering rates were increased to daily on March 22, and each tray was given 150 ml. of de-ionized water on a daily basis until April 07.

Seedling emergence data was statistically analyzed with a 3-way ANOVA (Zar 1996).

Soil Nitrogen Content

Soil samples from both the field and the greenhouse were analyzed for soil nitrogen content in the bio-available forms of NO_3^{2-} and NH_4^+. For each study site the initial soil nitrogen content was compared to the soil nitrogen content after cultivation of a cyanobacterial crust. Soil extractions were completed with 2M KCL solution according to the methods of Kalra and Maynard (1991). Soil extractions were filtered with a vacuum filtrator. Levels of NO_3^{2-} and NH_4^+were measured on a Technicon Autoanalyzer II. Soil nitrogen results were analyzed with a 3-way ANOVA (Zar 1996).

Soil Aggregation

Soil samples stored at 5°C prior to sieving. Several days prior to testing soil samples were dried on U-cut paper bags. Soil aggregate size was examined on the Wentworth scale with sieve trays ranging from -2.0 to +3.0 phi units. Each sieve tray was shaken 50 times by hand. The mean particle size for each plot was analyzed by a 3-way ANOVA (Zar 1996).

Results

Soil Nitrogen Content

ANOVA results for the Westwood field site indicate that there is no significant difference among any of the treatment groups in soil nitrogen content in the forms of NH_4^+ and NO_3^{2-} (see table 1). In addition, no significant interactions were found among any of the groups. Soil nitrogen results from greenhouse soil samples indicate that inoculated trays had significantly higher levels of NH_4^+ ($F=32.35$; $p<0.001$). There were also significant interactions among all factors for NH_4^+ ($F=7.93$; $p<.01$).

Table 1 Soil nitrogen and ammonium content

Treatment	Pinewood field site					
	Start (10-06-96) NO_3 ± s.d. (mg/g) (n = 3)	Finish (13-08-96) NO_3 ± s.d. (mg/g) (n = 3)	Difference ± s.d.	Start (10-06-96) NH_4 ± s.d. (mg/g) (n = 3)	Finish (13-08-96) NH_4 ± s.d. (mg/g) (n = 3)	Difference ± s.d.
Control / undisturbed	1.02 ± 0.92	3.97 ± 5.27	-2.95 ± 4.36	1.54 ± 0.95	0.39 ± 0.29	1.15 ± 1.08
Control / disturbed	0.49 ± 0.15	2.06 ± 0.95	-1.57 ± 0.98	0.86 ± 0.66	0.75 ± 0.27	0.11 ± 0.52
Nostoc / undisturbed	0.50 ± 0.21	1.19 ± 1.43	-0.70 ± 1.63	1.27 ± 0.96	1.36 ± 0.41	-0.09 ± 0.97
Nostoc / disturbed	0.55 ± 0.37	1.16 ± 0.94	-0.61 ± 0.71	1.21 ± 0.81	0.37 ± 0.29	0.84 ± 1.09
	Greenhouse					
	Start (04-10-96) NO_3 ± s.d. (mg/g) (n = 3)	Finish (04-04-97) NO_3 ± s.d. (mg/g) (n = 3)	Difference ± s.d.	Start (04-10-96) NH_4 ± s.d. (mg/g) (n = 3)	Finish (04-04-97) NH_4 ± s.d. (mg/g) (n = 3)	Difference ± s.d.
Control / undisturbed	0.68 ± 0.80	0.44 ± 0.01	0.24 ± 0.01	0.59 ± 0.83	0.1 ± 0.01	-0.48 ± 0.01
Control / disturbed		0.44 ± 0.07	-0.24 ± 0.07		1.15 ± 1.43	0.57 ± 1.42
Nostoc / undisturbed		1.20 ± 1.20	0.52 ± 1.19		2.23 ± 1.24	1.64 ± 1.24
Nostoc / disturbed		0.54 ± 0.08	-0.14 ± 0.08		1.83 ± 1.17	1.24 ± 1.17

Soil Aggregation

ANOVA results of soil aggregation data from the field site at Pinewood Aggregates indicate that there is no significant difference among any of the treatment groups in the size of soil aggregates (see table 2). In contrast, results from the greenhouse soil samples indicate that inoculated trays contain significantly larger aggregates than control trays ($F=8.90$; $p<0.01$) (see table 2). The mean aggregate size of inoculated trays was 0.877 phi, while the mean aggregate size of control trays was only 0.640 phi. No significant differences were found for before/after inoculation and disturbed/undisturbed trays for the greenhouse soil samples.

Table 2 **Soil aggregation**

Treatment	Pinewood field site		Greenhouse	
	Start (13-06-96) ($\phi \pm$ s.d.) (n = 3)	Finish (14-08-96) $\phi \pm$ s.d.) (n = 3)	Start* ($\phi \pm$ s.d.) (n = 3)	Finish ($\phi \pm$ s.d.) (n = 3)
Control /undisturbed	0.49 ± 0.14	0.23 ± 0.20	0.98 ± 0.16	1.29 ± 0.38
Control / disturbed	0.72 ± 0.05	0.51 ± 0.82		1.29 ± 0.65
Nostoc / undisturbed	0.70 ± 0.32	0.74 ± 0.34		0.75 ± 0.01
Nostoc / disturbed	0.64 ± 0.28	0.62 ± 0.45		0.80 ± 0.60

Seedling Emergence and Early Seedling Establishment

ANOVA results of seedling counts from Pinewood Aggregate plots indicate that disturbance is the most important factor out of the treatments tested in promoting seedling germination and establishment ($F=13.050$; $p<0.01$) (see table 3). The mean number of seedling from disturbed plots was 485, whereas the mean number of seedlings from undisturbed plots was 137. Inoculation did not have a significant effect on seedling emergence and seedling establishment at the Pinewood Aggregates field site.

ANOVA results of greenhouse plots indicate that inoculation is the only significant treatment ($F=6.84$; $p<0.03$). The mean number of seedlings in inoculated trays was 8.5, compared to 2.5 mean seedlings in control trays.

Table 3 **Seedling emergence**

Treatment	Pinewood field site Seedling no. ± s.d.	Greenhouse Seedling no. ± s.d.
Control / undisturbed	204 ± 185	1 ± 1
Control / disturbed	421 ± 124	4 ± 2
Nostoc / undisturbed	70 ± 15	11 ± 6
Nostoc / disturbed	449 ± 70	9 ± 5

Discussion:

Soil Nitrogen Content

Soil nitrogen results for the Pinewood Aggregates field site and for the greenhouse differed greatly. At the field site there was no significant difference in the soil content of NH_4^+ or NO_3^{2-}among any of the treatment groups. Results from the greenhouse study indicate a significant interaction among the treatment groups. Several factors may explain the discrepancy in the results. Possibly the greatest difference between the sites is that the soil in the greenhouse was autoclaved prior to usage to remove all existing soil biota. Soil at the Pinewood field site contained viable algal and cyanobacterial communities, which during the cultivation period formed an endemic crust. Samples of the endemic crust were taken, cultured in Bold Basal medium, and algal and cyanobacterial components were identified to the genus level. The endemic crust consisted of: cyanobacteria *Lyngbya, Nostoc,* and *Chroococcus,* the chlorophytes *Ulothrix* and *Stichococcus*, and a euglenoid.

The endemic crust was found in all but one of the quadrats at Pinewood. It seems likely that the lack of difference among the treatment groups resulted from the presence of an endemic crust. All but one of the plots contained nitrogen-fixing cyanobacteria. It is possible that all but one of the plots were experiencing higher soil nitrogen levels compared to soil that does not contain any cyanobacterial amendments, however, there may not have been a significant difference in the levels of soil nitrogen between the plots.

In the greenhouse, the difference in soil nitrogen was seen in a significant interaction among all the treatment groups for NH_4^+. It is likely that the difference was only seen in NH_4^+. and not NO_3^{2+} because NO_3^2 is a more mobile form of nitrogen. When nitrogen is present in the soil as NO_3^2 it tends to volatize or is leached or taken up rapidly by plants thus it may not be a good indicator of soil nitrogen content (Hausenbuiller 1985).

Soil Aggregation

Results of soil aggregation tests on Pinewood field site soil samples indicate that there is no significant difference in aggregate size among the treatment plots. It is likely that this is result of the endemic crust as well. The endemic crust contained several mucilaginous and filamentous algal and cyanobacterial genera. The same binding properties associated with *Nostoc* are also observed in other genera. Soil aggregation by cyanobacteria and/or algae is thought to be both mechanical and chemical in origin. Mechanical aggregation results from filamentous algal and cyanobacterial forms grasping and attaching onto soil particles (Campbell *et al*. 1989; Forster and Nicholson 1981, Fletcher and Martin 1948). Chemically, soil aggregation is strengthened by the excretions of polysaccharides and polypeptides (Isichei 1991), and by sheath production in filamentous cyanobacteria which when wet acts as a gluing agent (Campbell *et al.* 1989; Campbell 1979). Since both the endemic crust and the cyanobacterial amendment contained genera that exhibit binding properties, the lack of difference in soil aggregate size is not surprising.

The results of the analysis of soil aggregate size from the greenhouse indicate that trays which have been inoculated with the cyanobacterial amendment contain significantly larger soil aggregates than plots which have not been inoculated. The larger soil aggregate size is likely a direct result of the *Nostoc* mechanically and chemically binding the soil particles. The disparity between the greenhouse and field results can be attributed to the lack of crust forming organisms in uninoculated greenhouse trays..

Seedling Emergence and Early Establishment

Significant differences in seedling emergence and early seedling establishment were observed in both the greenhouse and the Pinewood field site, however, the treatment plots in which the differences were observed differed between the field and the greenhouse. In the field, significantly greater seedling emergence and early establishment were observed in plots subjected to disturbance treatment. Since almost all the plots at the field site contained some form of crust, this result is not surprising. Johansen (1993) suggested that crust surface microtopography may be an important factor in seedling establishment. In this study, the crust surfaces were relatively smooth compared to the pinnacled crusts described by Johansen (1993). It seems likely that the sown seeds risk being blown off such surfaces or be subject to predation. Cracks in the surface produced as a result of disturbance may provide more suitable "safe sites" for germination and establishment, with increased nutrient content, increased water availability and soil stability.

In the greenhouse, inoculated trays had significantly greater seedling emergence and seedling establishment. Trays which received no cyanobacterial amendments in the greenhouse were also devoid of all cryptograms due to autoclaving the soil prior to other treatments. Therefore, the results from the greenhouse confirm that the presence of a cyanobacterial crust can enhance seedling emergence and seedling establishment. Greenhouse samples were only tapped on the surface 25 times with the side of an Erlenmeyer flask; this may not be comparable to trampling by two individuals.

Conclusions

This study provides some preliminary evidence that the application of cyanobacterial amendments to gravel pit soil surfaces may aid in restoration practices, although further testing is necessary on a larger scale to determine the degree. Results from the field site did not reveal a significant beneficial effect of cyanobacterial amendments, however, the interference of an endemic crust may be masking the true differences. Promising results were found in the greenhouse where soil aggregates were found to be significantly larger, levels of soil nitrogen in the form of NH_4^+ were higher, and seedling emergence and early establishment was better on soils treated with a cyanobacterial amendment. Field results indicate that disturbance may have an important influential role, and hints that perhaps another avenue to examine is the utilization of endemic crusts in restoration practices.

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