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## Cyanobacteria in alkaline soil and the effect of cyanobacteria inoculation with pyrite amendments on their reclamation

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**Abstract** The succession of cyanobacteria was studied in a usar (alfisol, solonetz, alkaline) soil, located in a tropical region of upper Gangetic plain, following the first rainfall for a period of 10 months (i.e., July–April). A dozen cyanobacteria were identified to grow on the soil surface and their appearance was in the following order: *Microcoleus* sp., *Calothrix brevissima*, *Scytonema* sp., *Cylindrosprum licheniformae*, *Cylindrosprum fertilissima*, *Nostoc calcicola*, *Nostoc punctiformae*, *Aphanothece parietina*, *Nostoc commune*, *Aulosira fertilissima*, *Phormidium* sp., and *Oscillatoria* sp. Among these cyanobacteria, *N. calcicola* was the dominant species. *N. calcicola* was inoculated on the alkaline soil and incubated under ambient conditions in the light for 2 years in the laboratory. Changes in soil properties were more rapid after the incorporation of pyrite (FeS<sub>2</sub>). Recovery was monitored by using a filamentous heterocystous cyanobacterium *N. calcicola* and its bicarbonate-resistant (HCO<sub>3</sub><sup>-R</sup>) mutant. The mutant strain showed better response to modification of soil pH following growth in soil.

**Keywords** Cyanobacteria · pH · Pyrite · Reclamation · Alkaline soil

### Introduction

Usar soils (Alfisol, solonetz, alkaline, sodic, saline) extensively distributed in the northern part of India (2.5 million ha) are characterized as alkaline and/or saline depending on the salt content. These soils are unproductive, impermeable, and hard due to the presence of undesirable salts on the surface. Solonetz soils are characterized by exchangeable Na<sup>+</sup> (more than 15%), low quantities of Ca<sup>2+</sup>,

and pH values that usually range between 8.5 and 10.5 (Dhar and Mukherji 1936; Singh 1950, 1961).

Physical and chemical methods such as addition of gypsum, sulfur, or excessive irrigation usually used in the reclamation of alkaline soils do not completely remove the soluble salts and exchangeable sodium in soil. Singh (1961) suggested that cyanobacteria could be used to reclaim alkaline soils because they form a thick stratum on the surface of the soil during the rainy season and the winter months. The algal material incorporated in the soil conserves organic C, organic N, and organic P as well as moisture, and converts Na<sup>+</sup> clay to Ca<sup>2+</sup> clay. Organic matter and N added by cyanobacteria bind the soil particles, and thus improve soil permeability and aeration (Singh 1961). They are capable of solubilizing microbial nutrients (Fritsch 1945) and dissolving insoluble carbonates nodules through the secretion of oxalic acid (Fritsch 1945; Singh 1961). Improvement of soil aggregation by lowering the pH and electrical conductivity and by increasing the hydraulic conductivity of saline and alkali soils by cyanobacteria was documented by Kaushik and Subhashini (1985) and Kaushik (1987). A significant role of cyanobacteria and associated moss community in the soil formation of Schirmacher Oasis, Antarctica, was also described (Pandey et al. 1992).

Although the occurrence of cyanobacteria in saline/alkaline soil has been described (Singh 1950, 1961; Ali and Sandhu 1972), the sequence of appearance of cyanobacteria in an alkaline (saline) (Whitton and Potts 2000) soil having monsoon type of character has not been studied. For this reason, we followed the sequence of appearance of cyanobacteria in a usar soil area and showed that some of them constitute dominant flora. Since the cyanobacterial growth markedly decreased the pH of the alkaline soils under natural condition (Singh 1961) and pyrite has been used to reclaim such soils (Verma and Abrol 1980), we conducted an experiment to study if the cyanobacteria and pyrite could show a cumulative effect in the reclamation of alkaline soils. The other scope of the work was to study the effect of the inoculation of dominant cyanobacterium *Nostoc calcicola* and its bicarbonate-resistant (HCO<sub>3</sub><sup>-R</sup>)

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mutant on soil properties, and to compare it with treatment using FeS<sub>2</sub> (pyrite).

## Materials and methods

### Site description

The investigation site is located approximately 4 km south of Banaras Hindu University campus (latitude 25°18'N, longitude 80°1'E, 76 m above sea level) and lies in the upper Gangetic plains. Annual rainfall was approximately 983.4 mm and the soil moisture content of alkaline soil varied between 3.2 and 24.7% throughout the year, with the maximum level recorded during the rainy season (July–September) and the minimum in June. The alkaline soil was not subjected to cultivation for the last 25 years or so and remained fallow during that period.

### Collection of soil samples and determination of physicochemical characteristics

Soil cores (10 cm<sup>3</sup>) were collected during the driest period of the year, when no growth of cyanobacteria was evident on the soil surface. Sampling was replicated three times at least 50 m apart, and samples were placed in aluminum cans and transported to the laboratory for physicochemical analysis. After the first rain, collection of soil samples was made at 15-day intervals over a period of 3 months.

Texture, water holding capacity, and pH of the soil sample were determined as described by Piper (1966). Soil pH was measured in a soil/water suspension (1:5, w/v) with pH meter. Organic C and total N content (%) of soil were determined by Walkley and Black rapid titration method and micro-Kjeldahl techniques, respectively (Piper 1966). Exchangeable Ca<sup>2+</sup> and Na<sup>+</sup> (%) were determined after soil leaching as described by Jackson (1968). Total P was estimated by the ignition method (Jackson, 1968).

### Collection of cyanobacterial samples and identification of strains

Cyanobacteria growing on alkaline soils and water were collected in sterile polythene bags and specimen tubes using clean implements. They were brought to the laboratory and examined microscopically. The strains were identified as reported by Desikachary (1959) and Rippka et al. (1979).

### Cyanobacterial strains, isolation of mutant, culture conditions, and growth measurements

*N. calcicola*, a heterocystous cyanobacterium and the dominant constituent of alkaline soil, was enriched and grown in Allen–Arnon nutrient agar medium (Allen and Arnon 1955) in Petri dishes. Clones were isolated and an axenic

population was grown following standard microbiological techniques.

The exponentially growing culture of *N. calcicola* was harvested via centrifugation (10,000×g; 10 min) and washed with sterilized distilled water. Cells (1×10<sup>5</sup>) were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; 100 µg ml<sup>-1</sup>) (Pandey and Singh 1984) in phosphate buffer (0.01 M; pH 8.0) for 1 h in light. The treated population was inoculated on bicarbonate (1 M) containing nutrient agar and incubated under growth conditions. Healthy clones were picked up in 5 ml nutrient broth. One of such clones tolerating 1 M HCO<sub>3</sub><sup>-</sup> in nutrient broth was selected for further study.

The cultures were grown photoautotrophically at 25±1°C and 25 µE m<sup>-2</sup> s<sup>-1</sup> light intensity. Growth was measured by protein content (Herbert et al. 1971) or by population size (Alexander 1965; Roger and Kulasoorya 1980).

### Monitoring cyanobacterial growth in alkaline soil under natural conditions

Alkaline soil patches (10 cm<sup>3</sup>) with thick growth of cyanobacteria were randomly sampled from three to four places and flooded in glass with double-distilled water and incubated for 2 years under natural conditions.

Alkaline soil (5 cm thick) was heated with distilled water in a 500-ml culture vessel (200 ml), sterilized at 1.05 kg cm<sup>-2</sup> pressure for 15 min, and then cooled at room temperature for 24 h. Exponentially growing cyanobacterium *N. calcicola* was inoculated, flooded with sterilized double-distilled water at 7 days interval, and then incubated under natural conditions for 2 years.

### Preparation of soil extract and its effect on the growth of *N. calcicola*

Dried and sieved alkaline soil (500 g) was treated with 500 ml deionized water in a flask (1 l). The flask was steamed in an autoclave for 30 min and cooled overnight; the procedure was repeated two to three times. The clear supernatant that appeared upon cooling was filtered through Whatman filter paper (No. 42), and soil extract thus obtained was sterilized in an autoclave at 1.05 kg cm<sup>-2</sup> pressure for 15 min. This soil extract was supplemented in Allen–Arnon medium at different concentrations (% v/v) while keeping constant the pyrite level 2 mg ml<sup>-1</sup>. *N. calcicola* was inoculated at an inoculum density of 20 µg protein ml<sup>-1</sup> and the cultures were incubated under growth conditions for 20 days.

### Quantitative estimation of cyanobacteria

Soils were taken randomly from the Petri dish and the cyanobacterial mass was scrapped, suspended in the culture medium, and shaken on a gravitory shaker to separate the cyanobacterial growth into individual filaments. Quantita-

tive estimations of the cyanobacteria were made by plating technique (Roger and Kulasooriya 1980).

#### Inoculation of cyanobacterial strains and pyrite

Powdered alkaline soil (40 g) was spread into Petri dish (diameter 95 cm; height 2 cm), to obtain a 1-cm soil layer and was mixed with pyrite (25–250 mg 100 g<sup>-1</sup> soil). Two experiments were performed: in the first one, the Petri dish with soil and pyrite was sterilized, whereas in the second it was kept unsterilized. After flooding with sterilized distilled water for 15 days, the Petri dishes were inoculated with exponentially growing cyanobacterium and incubated at 30±2°C in an incubator for 24 months to observe their growth on alkaline soil. The soil in each Petri dish was flooded with sterilized distilled water at 7 days interval.

#### Statistical analysis

Cyanobacterial samples were collected from ten to 15 places to study succession, while soils and soil blocks were sampled in three to four replicates randomly. Each experiment was performed in triplicate and the mean values (with standard deviation) of the data are presented in tables and figures.

Analysis of variance (ANOVA) and *t* test were performed with the help of a computer and using Sigma stat, version 2.0.

## Results

#### Succession of cyanobacteria in alkaline soils under natural conditions

During summer (April–June), alkaline soil in the field becomes dry and cyanobacterial flakes are easily blown away by the wind. Following the first rain shower (July), small patches of *Microcoleus* sp., along with *Calothrix brevisissima* and *Scytonema* sp., appeared on the soil surface. Some low-lying areas of the alkaline soil became waterlogged due to heavy rains that lasted for 10–15 days. After about a month, *Nostoc punctiforme* grew as small patches on the soil and water surface. Nearly 2 months after the appearance of *Microcoleus* sp., colonization by *Cylindrospermum licheniforme*, *C. fertilissima*, *Nostoc calcicola*, *N. punctiforme*, and *Aphanothece parietina* was observed. Nevertheless, growth of *Microcoleus* and *C. brevisissima* still persisted. Among them, *N. calcicola* appeared as a dominant N<sub>2</sub> fixer, although *Microcoleus* remained throughout the rainy and winter seasons. After this stage, it was observed that the growth of *Nostoc commune* formed small flakes on the soil surface with plastic-type stiffness, and *Aulosira fertilissima* formed a thick mat on shallow water and moist soils. During the later part of cyanobacteria growth, some non-N<sub>2</sub> fixers such as *Phormidium* and *Oscillatoria*

**Table 1** Chronological sequence of appearance of cyanobacterial genera on alkaline soil following rain fall

Cyanobacteria	Duration
<i>Microcoleus</i> sp. <sup>a,b</sup>	July–March
<i>Calothrix brevisissima</i> <sup>a,c</sup>	July–March
<i>Scytonema</i> sp. <sup>a,c</sup>	July–March
<i>Nostoc punctiforme</i> <sup>a,c</sup>	August–January
<i>Aphanothece parietina</i> <sup>a,d</sup>	September–November
<i>Cylindrospermum fertilissima</i> <sup>a,c</sup>	September–October
<i>Cylindrospermum licheniforme</i> <sup>a,c</sup>	September–October
<i>Nostoc calcicola</i> <sup>a,c</sup>	September–April
<i>Nostoc commune</i> <sup>a,c</sup>	October–April
<i>Aulosira fertilissima</i> <sup>a,c</sup>	October–January
<i>Phormidium</i> sp. <sup>b</sup>	November–April
<i>Oscillatoria</i> sp.	November–April

<sup>a</sup>Aerobic diazotroph

<sup>b</sup>Non-heterocystous filamentous

<sup>c</sup>Heterocystous filamentous

<sup>d</sup>Unicellular

*illatoria* were intermingled with the diazotrophic forms (Table 1).

The succession of cyanobacteria population on the same alkaline soil, but under controlled laboratory conditions, was similar to that observed in situ. However, under laboratory conditions, *N. calcicola*, *N. punctiforme*, *N. commune*, *Phormidium*, and *Oscillatoria* were the dominant flora. Indeed, even if *Microcoleus* appeared on the soil surface, the frequency of its occurrence was low.

#### Soil properties

The color of alkaline soil was dirty white to grayish, and was of clay-type soil texture. The pH of soil ranged from 10

**Table 2** Physico-chemical characteristics of alkaline soil

Properties	Characteristics
Colour	Whitish gray
Compactness	Soil particles loosely arranged
pH	10.40
Sand <sup>a</sup>	44.60
Silt <sup>a</sup>	33.60
Clay <sup>a</sup>	21.80
Water holding capacity <sup>a</sup>	45.00
Na <sub>2</sub> CO <sub>3</sub> <sup>a</sup>	0.04
Organic C <sup>a</sup>	0.12
Total N	0.02
C/N ratio	6.00
Exchangeable Ca <sup>2+a</sup>	0.35
Na <sup>a</sup>	0.78
Total P <sup>a</sup>	0.03

Soil samples were collected in the month of June, before the onset of rain

<sup>a</sup>Percent on dry weight

**Table 3** Changes in alkaline soil characteristics following growth of mixed population of cyanobacteria (natural inoculum) and *Nostoc calcicola* under natural conditions after 2 years

Soil properties	Pre-cyanobacterial growth	Post-cyanobacterial growth <sup>a</sup>	Post <i>Nostoc calcicola</i> growth
Total N <sup>b</sup>	10.40	8.80 <sup>&lt;</sup>	5 <sup>&lt;</sup>
Organic C <sup>b</sup>	0.02	0.17 <sup>&lt;</sup>	0.18 <sup>&lt;</sup>
C/N ratio	0.12	0.66 <sup>&lt;</sup>	0.59 <sup>&lt;</sup>
Na <sup>b</sup>	6.00	4.00 <sup>&lt;</sup>	3.28 <sup>&lt;</sup>
Total P <sup>b</sup>	0.78	0.6 <sup>&lt;</sup>	0.6 <sup>&lt;</sup>
	0.03	0.03 <sup>&lt;</sup>	0.3 <sup>&lt;</sup>

Inoculum size of *N. calcicola* was  $0.5 \times 10^3$  filaments  $g^{-1}$  dry wt soil and incubated for 2 years

<sup>a</sup>Both  $N_2$ -fixing and non-diazotrophic cyanobacteria

<sup>b</sup>Percent of dry weight

<sup><</sup>Significant at  $p < 0.001$ , based on  $t$  test results

<sup><<</sup>Significant at  $p < 0.05$ , based on  $t$  test results

to 10.5 and the water holding capacity was 45%. The soil with no apparent growth of cyanobacteria or algae contained 0.036%  $Na_2CO_3$  and the exchangeable  $Ca^{2+}$  and  $Na^+$  were 0.35 and 0.78%, respectively. The soil was poor in organic C, N, and P contents (Table 2).

#### Effect of cyanobacterial growth on the physicochemical properties of alkaline soil

Since tropical climate is characterized by strong seasonal variations, the visible growth of cyanobacteria can be observed during the rainy season. The soil is apparently dry during rest of the year and cyanobacteria undergo perennation. The soil moisture seems to regulate the appearance and disappearance of cyanobacterial flora. Because *N. calcicola* was the dominant species, it was used in various experiments conducted for 2 years (Table 2). The experiments were performed in glass with soil under natural conditions with naturally occurring cyanobacteria inocu-

lated with *N. calcicola*; it was observed that soil pH decreased from 10.5 to 8.8 and 8.5, respectively (Table 3). Increase in total N as well as organic C and P contents were also observed, whereas  $Na^+$  content declined following cyanobacterial growth either in mixed population or in monoculture.

#### Effect of alkaline soil extract on the growth of *N. calcicola*

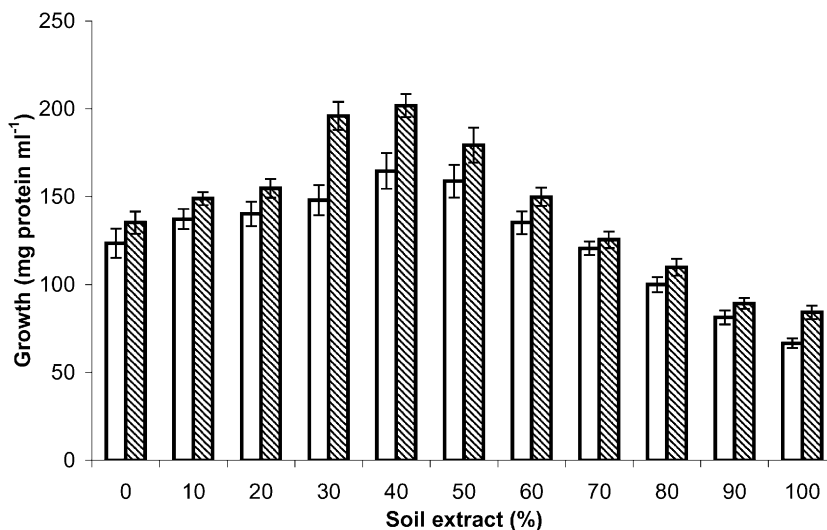
We focused on *N. calcicola*, isolated from the solonetz alkaline soil, to study the effect of alkaline soil extract on cyanobacterial growth in the presence and absence of pyrite. The preliminary experiment showed that pyrite in culture medium supported the growth of cyanobacterium up to  $200 \mu g ml^{-1}$  as growth was nearly 20% higher in the presence than in the absence of  $FeS_2$ . The increasing percentage of soil extract in the medium enhanced the growth measured as protein content up to 40%. Further increase in soil extract in the medium inhibited growth, and in the presence of 100% soil extract with or without pyrite, it accounted for nearly 60% of control (Fig. 1). Two-way ANOVA indicated that concentration of soil extract had a significant effect on growth ( $F_{10,21}=28.53$ ;  $p < 0.001$ ) and pyrite addition ( $F_{1,21}=17.695$ ;  $p < 0.002$ ).

Results revealed that *N. calcicola* could tolerate alkaline soil salt stress; therefore, this cyanobacterium was used in further experiments to remediate the alkaline soil.

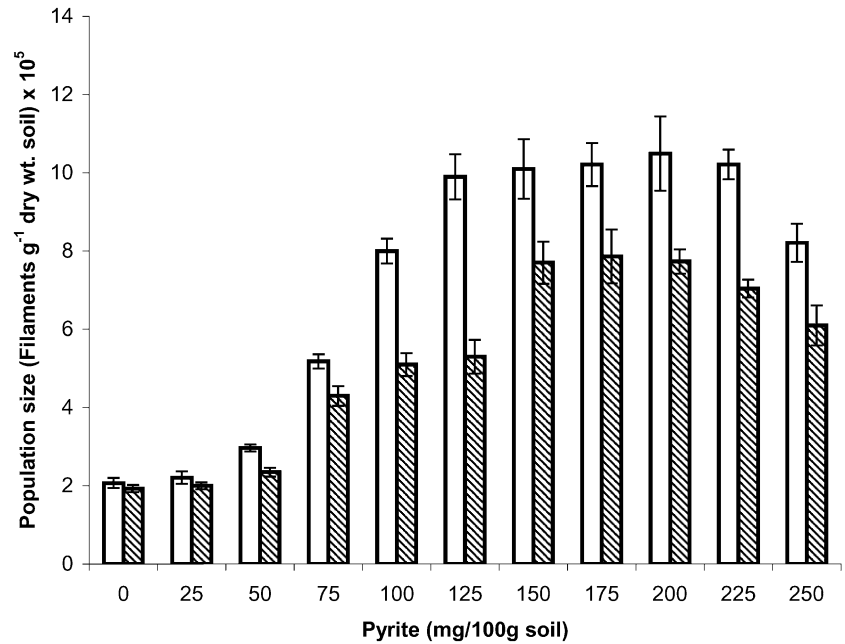
#### Effect of pyrite and *N. calcicola* on alkaline soil

Pyrite increased the cyanobacterial population, expressed as number of filaments per square centimeter, to a level of  $200 mg 100 g^{-1}$  sterilized soil and  $175 mg 100 g^{-1}$  unsterilized soil (Fig. 2). Two-way ANOVA indicated that pyrite addition had a significant effect on population size ( $F_{10,21}=15.95$ ;  $p < 0.001$ ) and the effect of sterilization was also statistically significant ( $F_{1,21}=28.54$ ;  $p < 0.001$ ).

**Fig. 1** Effect of alkaline soil extract on the growth of *Nostoc calcicola* in the absence (open bar) and presence (hatched bar) of pyrite ( $2 mg ml^{-1}$ ) in the medium when soil extract was added before the inoculation of cyanobacterium ( $10 \mu g$  protein  $ml^{-1}$ ). The cultures were incubated under photoautotrophic condition. Growth was monitored at the end of 20 days of incubation



**Fig. 2** Effect of pyrite on *Nostoc calcicola* population size in sterilized soil (open symbol) and unsterilized (closed symbol) alkaline soil. *N. calcicola* was inoculated at  $0.5 \times 10^3$  filament  $\text{g}^{-1}$  dry wt soil and incubated for 2 years in the laboratory



#### Role of *N. calcicola* and $\text{HCO}_3^{-\text{R}}$ mutant in the reclamation of alkaline soil

The previous experiment conducted with *N. calcicola* clearly indicated that soil properties were modified by the growth of cyanobacteria on alkaline soil. To test whether a bicarbonate-resistant ( $\text{HCO}_3^{-\text{R}}$ ) mutant of cyanobacterium also has the ability to grow and lower the pH of alkaline soil, we used *N. calcicola* (wild type) and its mutant ( $\text{HCO}_3^{-\text{R}}$ ). Results of this experiment are presented in Table 4. Inoculation with *N. calcicola* decreased the pH value, and the decrease was more pronounced in sterilized soil. The decrease in pH of alkaline soil inoculated with  $\text{HCO}_3^{-\text{R}}$  mutant was higher than that with wild-type cyanobacterium. No growth of any cyanobacteria was observed in the uninoculated alkaline soil. The cyanobacterial strains amended with pyrite were more effective in lowering the pH of alkaline soil compared to cyanobacterium inoculated on soil without pyrite. Also, pyrite enhanced the population size of strains (Table 4).

#### Discussion

The reclamation of solonetz soils (alkaline soils), which are extensively distributed in India, is important so as to make these soils fertile. Depending on the degree of soil alkalinity, these soils can be reclaimed by water leaching and by adding chemical correctives such as gypsum (raw  $\text{CaSO}_4$ ) to replace  $\text{Na}^+$  with  $\text{Ca}^{2+}$  (Singh 1961).

Soil pH is an important factor which determines the biodiversity and dominance of cyanobacteria on a given soil surface. Fogg (1956) reported that, under natural conditions, most of the cyanobacteria grow in neutral to alkaline conditions and sometimes the growth of diazotrophic cyanobacteria in rice fields is limited by low pH (Whitton and Potts 2000). Alkaline soil with high pH values and  $\text{Na}^+$  content favor the growth of diazotrophic cyanobacteria with a consequent decrease in pH. Indeed, cyanobacteria can decrease soil pH from 9.2 to 7.5 (Singh 1961). The role of  $\text{Na}^+$  in cell uptake of different nutrients and in  $\text{N}_2$  fixation is well documented (Thomas and Apte 1984).

**Table 4** Growth (population size) of wild-type *Nostoc calcicola* and its mutant,  $\text{HCO}_3^{-\text{R}}$ , in alkaline soil and effect of their growth on soil pH

Strains	Pyrite ( $2 \text{ mg g}^{-1}$ soil)	Soil pH		Population size $\times 10^5$ filaments $\text{g}^{-1}$ dry weight soil	
		Sterilized	Unsterilized	Sterilized	Unsterilized
<i>Nostoc calcicola</i>					
Wild type	–	9.8 $\pm$ 0.1	9.6 $\pm$ 0.3	3.6 $\pm$ 0.1	4.0 $\pm$ 0.2
Wild type	+	9.0 $\pm$ 0.1	9.2 $\pm$ 0.2	4.5 $\pm$ 0.5	4.3 $\pm$ 0.2
$\text{HCO}_3^{-\text{R}}$ mutant	–	8.9 $\pm$ 0.3	9.1 $\pm$ 0.2	12.8 $\pm$ 0.8	10.7 $\pm$ 0.5
$\text{HCO}_3^{-\text{R}}$ mutant	+	8.7 $\pm$ 0.2	8.9 $\pm$ 0.3	20.3 $\pm$ 1.1	18.8 $\pm$ 1.3

The experiment was conducted in Petri dishes containing 40 g usar soil (initial soil pH 10.7) and inoculated with  $0.5 \times 10^3$  filaments of wild-type or  $\text{HCO}_3^{-\text{R}}$  mutant. The Petri dishes were incubated for 2 years under natural conditions. Data analyzed (*t* test) were statistically significant at  $P < 0.05$ .

Pyrite used as a chemical corrective to reclaim sodic soil lacks complete oxidation and may cause toxicity by releasing ferrous and sulfide ions. However, cyanobacteria growing in soils utilize iron and sulfide for their growth. Nitrogenase induction by sulfide in nonheterocystous cyanobacteria is optimal at high pH (Villbrandt and Stal 1996). The sulfide acts as a reducing agent and its addition to the diazotrophic *Plectonema boryanum* enhanced nitrogenase activity (Kashyap et al. 1996). Cyanobacterial inoculation significantly reduced the oxidizable matter, sulfide, phosphorous, and iron in soils (Aiyer et al. 1972).

Iron is seldom limiting for cyanobacterial growth and its deficiency may be due to the chelating properties of cyanobacteria extracellular products (Whitton 1965). In microbial mats, iron may participate as iron sulfide (FeS) or pyrite (FeS<sub>2</sub>). Iron reacts with oxygen and keeps the partial pressure of oxygen sufficiently low to allow efficient photosynthesis and N<sub>2</sub> fixation by cyanobacteria (Whitton and Potts 2000). Changes in the properties of the upper 0.7 cm of the brown earth silt loam were attributed to an increase in the cyanobacterial population (Rao and Burns 1990). The organic metabolites produced by cyanobacterial growth and released in the extracellular soil environment can be decomposed. However, some metabolites and their degradation products can accumulate and increase the organic N content, an improvement which subsequently maintains the fertility of soil year after year (Roger and Kulasooriya 1980; Ladha and Reddy 1995). Growth of cyanobacteria in soil decreases the C/N ratio due to N<sub>2</sub> fixation (Watanabe et al. 1977). An increase in C/N ratio in the experiment conducted under natural conditions with natural inoculum compared to *N. calcicola* inoculation may be due to the growth of many non-N<sub>2</sub>-fixers. Soil properties such as soil structure can be improved via the production of extracellular substances by cyanobacteria (Whitton and Potts 2000).

The presence and succession of diazotrophic cyanobacteria in alkaline soil was reported by Singh (1961). The low nutrient content in our soil may have supported the growth of diazotrophic cyanobacteria, which can fix both inorganic C and N. The nutrient-depleted soil and aquatic environment of Schirmacher Oasis, Antarctica, also supported the growth and diversity of diazotrophic cyanobacteria (Pandey et al. 1995). The occurrence of *Microcoleus* sp. and *N. commune* on alkaline soil supported the view of Whitton (1990), who reported the widely spread *Microcoleus* on saline surface. The above discussions revealed that chemical correctives like pyrite may be used with cyanobacteria to prevent iron or sulfur pollution in alkaline soil. A long-term fertility experiment indicated that the use of chemical fertilizers is not sufficient to restore soil fertility—organic matter supplementation is also required. Under such conditions, biofertilizers like cyanobacteria could provide essential nutrient and organic matter to the soil (Singh and Bisoyi 1993).

The solonetz soil contains huge amounts of carbonate. Addition of pyrite in such soils may result in the formation of sulfurous and sulfuric acid, which may react with carbonate and eventually (and predictably) produce CO<sub>2</sub>. The

growth of cyanobacteria in such soils increased soluble CO<sub>2</sub> from 0.23×10<sup>-4</sup> to 8.1×10<sup>-4</sup> (Singh 1961). The inoculation of HCO<sub>3</sub><sup>-R</sup> mutants of *N. calcicola* in pyrite-treated alkaline soil led to better reclamation results as determined by pH coupled with growth (population) compared to the same treated soil inoculated with the wild-type. Since the HCO<sub>3</sub><sup>-R</sup> mutant strain grew optimally at 250 μM bicarbonate, the presence of high HCO<sub>3</sub><sup>-</sup> concentration in alkaline soil can facilitate its growth and thus its photosynthesis (data not shown) with the release of greater amounts of organic C to soil than at low HCO<sub>3</sub><sup>-</sup> concentration. In conclusion, these experiments were conducted with cyanobacteria only, and the role of other microbes in the reclamation and fertility of solonetz alkaline soil cannot be ignored. For these reasons, further investigation on the subject is required.

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