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Nitrogen fixation by microbial crusts from desiccated Sahelian soils (Niger)

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Abstract

Cyanobacterial crusts developing on the sandy and loamy soils of fallow lands in the Sahel (Niger) were investigated for their potential to fix nitrogen. Three sites were selected in this arid environment, differing in sediment type and species composition. In the sandy sites heterocystous nitrogen-fixing cyanobacteria were present, whereas the loamy site did not contain such species. All sites showed light-dependent nitrogenase activity, starting within 2 h after re-wetting of the desiccated crust samples. Inhibition of photosystem II caused a decrease of nitrogenase activity in the samples with heterocystous cyanobacteria, but was stimulatory in the non-heterocystous crust. The results suggest that cyanobacterial crusts may be important for the improvement of the soil by enriching it with nitrogen.

Author Keywords: Nitrogen fixation; Cyanobacteria; Microbiotic crusts; Arid and semi-arid environments

Cyanobacterial crusts in desert ecosystems fulfil a number of important functions. The filamentous cyanobacteria form tough, entangled structures that render stability to the soil surface and protect it from erosion (De Winder et al., 1989). Mucilage produced by the cyanobacteria serves as water storage (Decho, 1990). Photosynthesis enriches the soil with organic matter, which improves its structure and biological activity (Lange et al., 1994). Because many cyanobacteria are capable of fixing nitrogen, the soil may be enriched with nitrogen, hence serving as natural fertiliser (Zaady et al., 1998).

This investigation was carried out to demonstrate the capacity of nitrogen fixation by microbial crusts developing in the Sahel (Niger) and to relate this to species composition and sediment morphological characteristics.

The samples were obtained from western Niger, between 13° 32′ N and 2° 42′ E. The climate is typical of the southern Sahel, with a single summer rainy season of 4 months and a mean annual average rain of about 560 mm. Average minimum and maximum temperatures are 22 and 34°C. Samples of the desiccated crusts were collected in 1995 from three different sites located in fields left fallow. The sites varied in their topographic and hydrological characteristics, particle size distribution, and microbial cover. Site 1 (Hama) contained microbial crusts covering 60–80% of the surface of residual sand dunes that developed on sandy loam soil material. At site 2 (Chef 2) microbial crusts covered more than 80% of the surface of a depressed zone of loamy soil material between sand dunes where water accumulated after rain events. Microbial crusts at site 3 (Mali-Djibo1) covered almost 100% of the border of a depressed zone of sandy to sandy-loam material. The microbial crusts were sampled at the beginning of the dry season and kept desiccated in the dark for 3 years until they were used for the experiments.

The biological composition of the microbial crusts revealed that *Porphyrosiphon kaernbachii* was by far the most abundant species, but other cyanobacteria were also encountered. These included other non-heterocystous filamentous species of the genera *Microcoleus*, *Schizothrix* and *Lyngbya*. Heterocystous cyanobacteria were represented by two species: *Nostoc* sp. and *Scytonema javanicum*. They were found at sites 1 (both species) and 3 (only *Nostoc* sp.), but were absent from site 2 (Table 1). All of these species appeared to be well suited to survival in long periods of drought as witnessed by the thick pigmented gelatinous sheaths (Potts, 1994).

Table 1. Species composition of cyanobacteria in Sahelian microbial crusts

Species		Site 1	Site 2	Site 3
Non heterocystous forms	*Porphyrosiphon kaernbachii*	+ [a]	+	+
	Schizothrix penicillata	+	+	+
	Microcoleus lacustris	+	−	+
	Microcoleus sociatus	+	+	−
	Lyngbya epiphytica	+	−	−
Heterocystous forms	*Scytonema javanicum*	+	−	−
	Nostoc sp.	+	−	+

[a] + = presence; − = lacking.

In all samples, nitrogenase activity (acetylene reduction) started within 24 h after re-wetting, but in some cases it appeared in less than 2 h. After this lag, activity increased exponentially until it reached a constant value. Dark incubated samples reached a constant but low rate of acetylene reduction after 3.5–31 h, depending on the site, while this took up to 163 h for samples incubated in the light. (Table 2).

Table 2. Nitrogenase activities and chlorophyll *a* contents at the 3 selected sites containing cyanobacterial desert crusts. Nitrogen fixation was measured by the acetylene reduction (AR) method (Stal, 1988). Briefly, crust samples of about 100–150 mm^2 and 2–3 mm thick were wetted with 0.5 ml distilled water, and subsequently incubated with 2 ml of acetylene in a sealed 22.5 ml glass vial. The vials were placed in a water bath at 30°C and under continuous illumination at 50 μmol m^{-2} s^{-1} by 2×50 W halogen lamps. Another set of samples was incubated in the dark by wrapping the vials in aluminium foil. The effect of oxygenic photosynthesis was investigated by adding 5 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosystem II. Chlorophyll *a* was determined by extraction with 96% ethanol. Absorption of the extracted solution was read at 665 nm using a spectrophotometer and the chlorophyll *a* content was calculated using an absorption coefficient of 72.3 ml mg^{-1} cm^{-1}

Incubation	Biomass and N_2-ase activity	Experimental sites		
		Site 1	Site 2	Site 3
	Initial chlorophyll *a*content (mg m^{-2})	42	41	57
Dark	Final chlorophyll *a* content (mg m^{-2})	72	60	40
	Time lag to constant AR (h)	5	31	3
	Areal rate of AR (nmol m^{-2} h^{-1})	30	10	100
	Chl-based rate of AR (nmol mg^{-1} h^{-1})[a]	0.53	0.20	2.06
Light + DCMU	Final chlorophyll *a* content (mg m^{-2})	87	66	88
	Time lag to constant AR (h)	139	127	37
	Areal rate of AR (nmol m^{-2} h^{-1})	25,150	40,070	1,280
	Chl-based rate of AR (nmol mg^{-1} h^{-1})[a]	390	749	17.7
Light	Final chlorophyll *a* content (mg m^{-2})	115	102	98
	Time lag to constant AR (h)	148	163	112
	Areal rate of AR (nmol m^{-2} h^{-1})	36,710	34,700	42,040
	Chl-based rate of AR (nmol mg^{-1} h^{-1})[a]	468	485	542

[a] Values of range limits were calculated from the average of initial and final chlorophyll contents.

The areal rate of acetylene reduction after full induction of nitrogenase was calculated by linear regression of ethylene formation plotted against time. This rate varied from 10 (dark) to 42,040 (light) nmol m^{-2} h^{-1}, depending on the incubation and origin of the sample. At all sites, the highest acetylene reduction rates were observed in the light varying from 34,700 to 42,040 nmol m^{-2} h^{-1}. Only small differences between the three sites were noted with respect to the potential rate of nitrogen fixation based on surface area. The addition of DCMU resulted in a strong inhibition (97%) of acetylene reduction in site 3. The effect of DCMU on acetylene reduction in the samples of sites 1 and 2 were less dramatic. A slight inhibition (31%) was observed in the samples from site 1 whereas those from site 2 acetylene were slightly stimulated (16%). Samples incubated in the dark showed low rates of acetylene reduction, ranging from 10 to 100 nmol m^{-2} h^{-1} (Table 2).

The chlorophyll contents of the crusts were very similar, ranging from 41 to 57 mg m^{-2}. During the course of the incubation in the light, chlorophyll *a* content increased with a factor 1.5–2.7. When the rates of acetylene reduction were normalised to chlorophyll *a* the same conclusions were drawn as when normalised to area. However, the stimulatory effect of DCMU on nitrogenase activity in samples from site 2 was much higher (54%), when normalised to chlorophyll *a*. (Table 2).

Our results have demonstrated that the crusts of the Sahelian soils possess the capacity of nitrogen fixation. The cyanobacterial crusts maintained a full metabolical potential, even after being kept desiccated for 3 years. Malam Issa (unpublished thesis, University of Orléans, 1999) demonstrated that respiratory activity started immediately after re-wetting the crusts and photosynthesis was already detected after 40 min. As was shown in the present investigation, nitrogenase activity appeared after photosynthesis, as early as 2 h after re-

wetting of the sample. Similar observations were made by Scherer et al. (1984) for communities of terrestrial *Nostoc* spp. and for crusts of Nigerian savannah (Isichei, 1980).

All three sites possessed light-dependent nitrogenase activity which was in the same order of magnitude whether expressed on an area basis or normalised to chlorophyll *a*. This was remarkable because heterocystous cyanobacteria were only encountered at sites 1 and 3 and were absent from site 2. Heterocystous cyanobacteria are specialists with respect to diazotrophic growth although also a few non-heterocystous species are known to fix nitrogen (Bergman et al., 1997). Nitrogenase in the samples with heterocystous cyanobacteria was inhibited by DCMU, which is typically for these organisms (Stal, 1995). In contrast, nitrogenase in samples of site 2 was stimulated by DCMU, which is typical for non-heterocystous nitrogen-fixing cyanobacteria. It is not clear which of the species found at site 2 was responsible for nitrogen fixation. However, considering the relatively low specific (chlorophyll-normalised) rate of nitrogenase activity, it seems unlikely that it is associated with *Porphyrosiphon kaernbachii*, which is the dominant species at all 3 sites.

The question arises why heterocystous species were absent from site 2. Differences between sites may be related to their sediment texture. Sites 1 and 3 were sandy and sandy loam, respectively, whereas site 2 was of a loamy texture, which may become inundated during the rainy season and retains water more efficiently. Pentecost (1985) found a significant correlation between the decrease of water availability and the frequency of heterocysts in natural populations of Scytonemataceae. Stal (1995) reported that mats of heterocystous and non-heterocystous cyanobacteria in coastal intertidal sediment occupy different areas. Mats of non-heterocystous cyanobacteria occurred in the lower parts and were more frequently inundated, while heterocystous cyanobacteria were found in the higher and dryer parts. He concluded that the different oxygen dynamics in these mats selected against heterocystous species. Although this was not measured in the present investigation, it seems possible that the same mechanism acts at site 2.

The surface covered by microbial crusts was 65–100%. Assuming that nitrogen fixation occurs during 12 h day^{-1} and during 100 day $year^{-1}$, we would estimate an annual nitrogen input of 3.5 kg ha^{-1} $year^{-1}$. This value is similar to those reported by Isichei (1980) for crusts of savannah regions in Nigeria and by Jeffries et al. (1992) in southern Utah (USA). Our estimates of nitrogen fixation are also in the same order of magnitude as those measured in highly active coastal cyanobacterial mats (Stal et al., 1984). However, our estimate is much lower than the values of 10–100 kg ha^{-1} $year^{-1}$ that were reported for arid soils by Skujins and Klubek (1978). The relatively low specific rates of nitrogenase activity were caused by the small contribution of diazotrophic cyanobacteria. This might easily become an order of magnitude higher, when the community composition would shift towards diazotrophic species. This suggests that microbial crusts in the Sahelian soils of Niger represent an important source of nitrogen and may serve as a natural fertiliser for crop production.

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