

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

O. Malam Issa^a, L. J. Stal^b, C. Défarge^a, A. Couté^c and J. Trichet^a

^a UMR 6531 CNRS. Université d'Orléans, BP 6759, 45067 Orléans cedex 2, France

^b Netherlands Institute of Ecology, Centre of Estuarine and Coastal Ecology, PO Box 140, 4400 AC Yerseke, The Netherlands

^c Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, 12 rue Buffon, 75005 Paris, France

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	<i>Porphyrosiphon kaernbachii</i>	+ ^a	+	+
	<i>Schizothrix penicillata</i>	+	+	+
	<i>Microcoleus lacustris</i>	+	–	+
	<i>Microcoleus sociatus</i>	+	+	–
	<i>Lyngbya epiphytica</i>	+	–	–
Heterocystous forms	<i>Scytonema javanicum</i>	+	–	–
	<i>Nostoc</i> 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 mm² 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
Dark	Initial chlorophyll <i>a</i> content (mg m ⁻²)	42	41	57
	Final chlorophyll <i>a</i> 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 <i>a</i> 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 <i>a</i> content (mg m ⁻²)	115	102
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 metabolic 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.

Acknowledgements

We would like to thank J.-L. Rajot and C. Valentin for assisting with fieldwork. This project was supported by the FED program in Niger "Amélioration des jachères en Afrique occidentale" and IRD (called before ORSTOM). This is publication 2713 of NIOO-CEMO.

References

- Bergman, B., Gallon, J.R., Rai, A.N. and Stal, L.J., 1997. N₂ fixation by non-heterocystous cyanobacteria. *FEMS Microbiology Reviews* **19**, pp. 139–185.
- Decho, A.W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Reviews* **28**, pp. 73–153.
- De Winder, B., Matthijs, H.C.P. and Mur, L.R., 1989. The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. *Archives of Microbiology* **152**, pp. 458–462.
- Eldridge, D.J. and Greene, R.S.B., 1994. Assessment of sediment yield by splash erosion on a semi-arid soil with varying cryptogam cover. *Journal of Arid Environments* **26**, pp. 221–232.
- Isichei, O., 1980. Nitrogen fixation by blue-green algal soil crusts in Nigerian savannah. In: Rosswall, T., Editor, , 1980. *Nitrogen Cycling in West African Ecosystems*, Scope-UNEP International Nitrogen Unit, Royal Swedish Academy of Sciences, Stockholm, pp. 191–198.
- Jeffries, D.L., Klopatek, J.M., Link, S.O. and Bolton Jr, H., 1992. Acetylene reduction by cryptogamic crusts from a black brush community as related to re-saturation and dehydration. *Soil Biology & Biochemistry* **24**, pp. 1101–1105.
- Johansen, R.J., 1992. Cryptogamic crusts of semi-arid and arid lands of North America. *Journal of Phycology* **29**, pp. 140–147.
- Lange, O.L., Kidron, G.J., Büdel, B., Meyer, A., Kilian, E. and Abeliovich, A., 1992. Taxonomic composition and photosynthetic characteristic of the ‘biological soil crusts’ covering sand dunes in the western Negev Desert. *Functional Ecology* **6**, pp. 519–527.
- Lange, O.L., Meyer, A., Zellner, H. and Heber, U., 1994. Photosynthesis and water relations of lichen soil crusts: field measurements in the coastal fog zone of the Namib desert. *Functional Ecology* **8**, pp. 253–264.
- Malam Issa, O., Trichet, J., Défarge, C., Valentin, C. and Couté, A., 1999. Morphology and microstructure of microbiotic soil crusts on a tiger bush sequence (Niger, Sahel). *Catena* **37**, pp. 175–196.
- Pentecost, A., 1985. Investigation of variation in heterocyst numbers, sheath development and false-branching in natural populations of Scytonemataceae (Cyanobacteria). *Archiv für Hydrobiologie* **102**, pp. 343–353.
- Pérez, F.L., 1997. Microbiotic crusts in the high-equatorial Andes and their influence on paramo soils. *Catena* **31**, pp. 173–198.
- Potts, M., 1994. Desiccation tolerance of prokaryotes. *Microbiological Reviews* **58**, pp. 755–805.

Scherer, S., Ernst, A., Chen, T.-W. and Böger, P., 1984. Rewetting of drought-resistant blue-green algae: time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. *Oecologia* **62**, pp. 418–423.

Skujins, J. and Klubek, B., 1978. Nitrogen fixation and denitrification in arid soil cryptogamic crust microenvironments. In: Krumbein, W.E., Editor, , 1978. *Environmental Biogeochemistry and Geomicrobiology* **vol. 2**, Ann Arbor Science Publishers, Ann Arbor, Michigan, pp. 543–552.

Stal, L.J., 1988. Nitrogen fixation in cyanobacterial mats. In: Packer, L. and Glazer, A.N., Editors, 1988. *Methods in Enzymology, Cyanobacteria* **vol. 167**, Academic Press, San Diego, pp. 474–484.

Stal, L.J., 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist* **131**, pp. 1–32.

Stal, L.J., Grossberger, S. and Krumbein, W.E., 1984. Nitrogen fixation associated with the cyanobacterial mats of a marine laminated microbial ecosystem. *Marine Biology* **82**, pp. 217–224.

Verrecchia, E., Yair, A., Kidron, G.J. and Verrecchia, K., 1995. Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy soils, north-western Negev Desert, Israel. *Journal of Arid Environments* **29**, pp. 427–437.

Zaady, E., Groffman, P. and Shachak, M., 1998. Nitrogen fixation in macro- and microphytic patches in the Negev desert. *Soil Biology & Biochemistry* **30**, pp. 449–454.