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Rewetting of drought-resistant blue-green algae: Time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation

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Summary. The response of the terrestrial blue-green algae *Nostoc flagelliforme*, *Nostoc commune*, and *Nostoc spec.* to water uptake has been investigated after a drought period of approximately 2 years. Rapid half-times of rewetting (0.6, 3.3, and 15.5 min, respectively) are found. The surface-to-mass ratio of the three species is inversely correlated to the speed of water uptake and loss. The ecological relevance of these different time courses is discussed.

Respiration starts immediately after a 30-min rewetting period, whereas photosynthetic oxygen evolution reaches its maximum activity after 6 and 8 h with *N. commune* and *N. flagelliforme*, respectively. In the dark, recovery of oxygen uptake by *N. commune* is somewhat impaired, while slightly stimulated with *N. flagelliforme*. With both species, recovery of photosynthesis is inhibited by darkness.

Using colonies kept dry for two years, nitrogenase activity of *N. commune* attains its maximum 120 to 150 h after rewetting, while only 50 h were needed with algal mats kept dry for two days.

Thus, after a 2-year drought period, the physiological sequence of reactivation is respiration – photosynthesis – nitrogen fixation. Respiration and photosynthesis precede growth and are exhibited by existing vegetative cells, whereas recovery of nitrogen fixation is dependent on newly differentiated heterocysts.

Introduction

Resistance towards extreme water stress, i.e. desiccation, is widely observed in the plant kingdom, preferably in lichens and algae. Davis (1972) reported on more than 400 species of drought-tolerant green and blue-green algae (for general aspects of desiccation see reviews by Bewley 1979; Bewley and Krochko 1982).

Terrestrial blue-green algae participate in soil erosion and production of organic matter, especially in extreme environments (Cameron 1960; Fogg and Stewart 1968; Lund 1967). Furthermore, *Nostoc* species are used as food and medicine in China.

Little is known about physiological responses following rewetting of blue-green algae (comp. Fogg et al. 1973). One of the earliest reports was published by Correns (1889) on water uptake by dried *Gloeocapsa* and *Nostoc* colonies. Apparently, blue-green algae have a long surviving time. Lipman (1941) showed that at least desiccated *Nostoc commune* can survive for 87 years, and Cameron (1962), using the same herbarium collection, reported a longevity record of 107 years for this specimen.

No data are available as yet on time courses of respiration and photosynthesis of blue-green algae together with water loss during and after rewetting. Few reports have been published on the resistance of nitrogen fixation against drought (Jones 1977; Paul et al. 1971; Wang et al. 1981; Whitton et al. 1979), which has been claimed as the essential factor to limit nitrogen fixation by blue-green algae in natural habitats (Stewart 1974). Interestingly, the nitrogenase enzyme system of *N. commune* is highly frost-tolerant, if no desiccation occurs (Coxson and Kershaw 1983).

This paper contributes some data on recovery of physiological activities of some blue-green algal species after rewetting.

Material and methods

1. Species

The three *Nostoc* species used in this study were all collected at their natural habitats in China. Rarely, endospores were observed in the samples. Heterocysts were present either as single cells in the mats or at the ends of the filaments.

Nostoc commune Vauch. (Gollerbach et al. 1953) is a terrestrial species widespread in China where it is called "earthy ear". It occurs mainly on the surface of soil of hilly limestone regions in South China, south of the Yangtze River. It is dominant at pH 7.5 to 7.8, but rare in acid soil and absent in paddy fields. In the rainy season, *N. commune* forms macroscopic gelatinous colonies often occupying small depressions of limestone. The fresh samples of *N. commune* were collected during the rainy season in 1981 in Hunan province (Henyong district) and Guanshi Province (Guiling district) of Central China and stored in plastic bags after desiccation for 5 days at 50% relative humidity at 18–20° C.

Nostoc flagelliforme (Berk. et Curt.) [= *N. commune var. flagelliforme* (Berk. et Curt., Born et Flah.)] (comp. Hu Hong-Jaun 1980; Geitler and Pascher 1925) was collected during 1980 in the Gansu and Lingshia Province (Zongling and Haiyuang County) in North-west China. It grows on soil surfaces of arid regions at pH 7.5 to 8.0.

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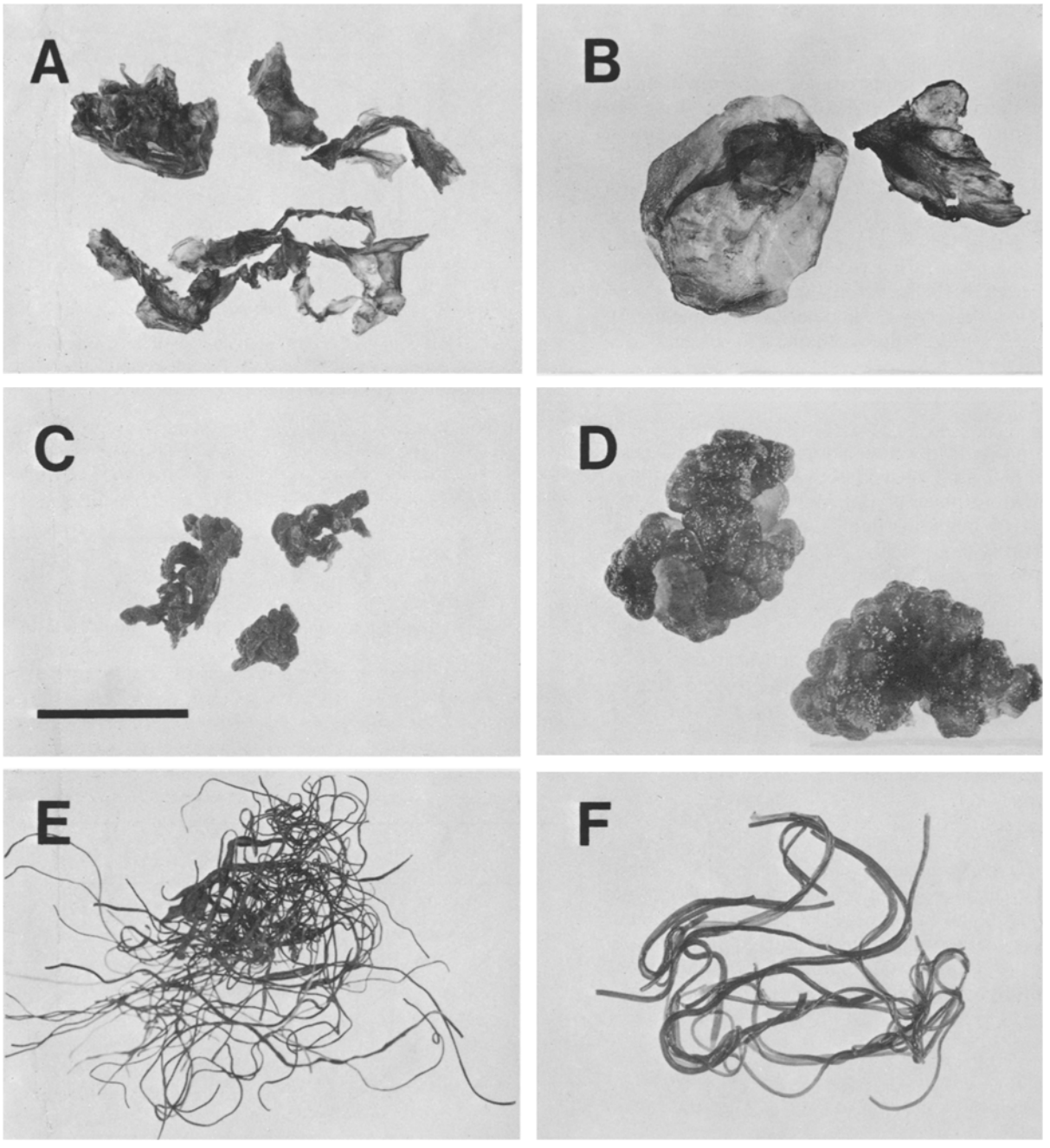

Fig. 1 A–F. The blue-green algal species investigated in this study are shown in the desiccated state (left) and 1 to 2 h after rewetting (right). **A, B** *Nostoc commune*, **C, D** *N. spec.*, **E, F** *N. flagelliforme*. Scale bar: 1 cm

Nostoc spec. was collected in small quantities as spherical colonies together with *N. commune*. Due to lack of material, gas exchange measurements were not carried out besides the rewetting experiment. Presumably we have been dealing with *Nostoc sphaericum* Vauch. (Hu Hong-Jaun 1980).

Figure 1 demonstrates the macroscopic morphology of the three species in dry and wet state. The surface-to-mass ratio was estimated by assuming a plain (*N. commune*), a spherical (*N. spec.*), or filamentous (*N. flagelliforme*) geometry of the colonies.

2. Water exchange

For rewetting, air-dried pieces of algal mats were submersed in BG-11_0 growth medium (Stanier and Cohen-Bazire 1977) for 20 to 30 min at room temperature, and the weight increase was determined after removing excess water by filter paper. For desiccation wet colonies were exposed to air in the laboratory (about 40% humidity) until a constant weight was reached. It should be noted that after rewetting and during the experiments the algal mats preserved a solid consistency which facilitated handling. Bacterial cont-

amination was negligible during rewetting and incubation time.

After rewetting, colonies were incubated in Petri dishes at 27° C, containing filter paper soaked with growth medium and illuminated with 500 lux white light (tungsten bulbs), unless noted otherwise. The length of this incubation is indicated in Figs. 4 to 6 and Table 2.

3. Oxygen gas exchange

Oxygen gas exchange was measured with a Clark-type electrode at 24° C and saturating red light (>610 nm) as described (Scherer et al. 1980); beforehand, the colonies were cut to pieces (less than 1 mm diameter) and suspended in MES buffer [2-(N-morpholino)-ethanesulfonic acid] adjusted with NaOH (10 mM, pH 6.9).

4. Nitrogenase activity

It was determined with rewetted samples (Sect. 2) as acetylene reduction in 7.8-ml glass vessels, stoppered by rubber seals, under an atmosphere of air including 12% C_2H_2 (v/v) at 30° C and 4500 lux white light. Gases were determined as described (Ernst et al. 1983). 0.2 g wet weight (*N. commune*) were used per assay vessel.

5. Chlorophyll

Methanol was added to wet colonies and heated to 60° C for 20 min, using an absorption coefficient according to Mackinney (1941) for the clear supernatant. Possible pheophytin content (see Whitton et al. 1979) was not considered, since no shoulder at 660 nm was detectable in the methanolic extracts.

6. Measurements

In case of *N. flagelliforme* and *N. spec.* material was limiting. However, it was possible to repeat the experiments 3 to 6 times. Data given represent a typical experiment. Unless mentioned otherwise in the legends, deviation from the mean was ±7% for rewetting and desiccation; ±10% for photosynthesis and respiration.

Results

1. Rewetting and desiccation

A rapid water uptake, completed after 10 min, was measured with *Nostoc flagelliforme*, that of *Nostoc spec.* was completed after 3 to 4 h (Fig. 2). A double-reciprocal plot of the data from *N. spec.* exhibited a straight line, which was not seen with the other two species. With *N. commune*, a biphasic behavior is evident, the first phase being similar to *N. flagelliforme*, whereas the second phase is similar to *N. spec.* (see Table 1, 3rd col., for half-times of water uptake).

The kinetics of water loss in air is shown in Fig. 3. Straight lines were seen at first with all three species. Later on, the time course was non-linear. A fast water uptake was correlated to a rapid loss of water under drought conditions.

Apparently, the surface-to-mass ratio is negatively correlated with the half-times of both desiccation and rewetting. No relationship was seen between the kinetics of water exchange and the total water uptake per unit of dry weight (Table 1).

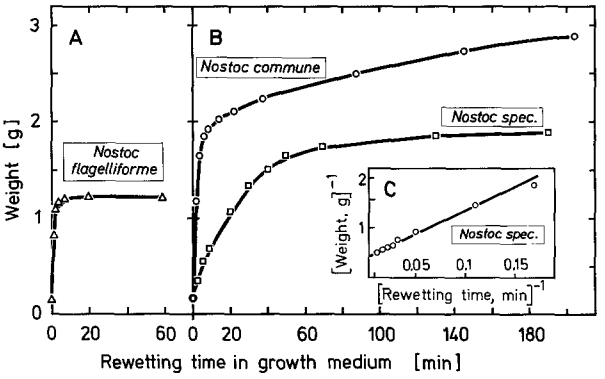

Fig. 2 A, B. Time course of water uptake by air-dried *Nostoc flagelliforme* **A**, *N. commune* and *N. spec.* **B**. Double-reciprocal analysis of the water-uptake kinetics of *N. spec.* is shown in **(C)**

Table 1. Relationship between surface-to-mass ratio and water exchange in three species of terrestrial *Nostoc*. Average values of 4 to 8 experiments are given

Species	Surface/mass (cm^2/g)	Half-time of desiccation (min)	Half-time of rewetting (min)	Total water uptake (cm^3H_2O/g dry weight)
Nostoc flagelliforme	82	135	0.6	5.8
Nostoc commune	37	290	3.3	15.0
Nostoc spec.	12	510	15.5	9.7

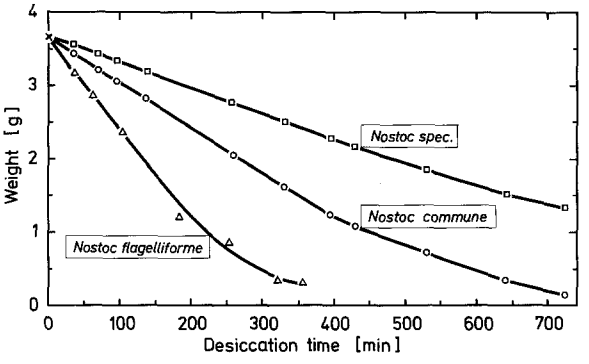

Fig. 3. Time course of water loss in air of fully wetted *Nostoc flagelliforme*, *N. commune* and *N. spec*

2. Reactivation of photosynthesis and respiration

On a fresh-weight basis, photosynthesis and respiration were higher in *Nostoc flagelliforme* than in *N. commune*, due to the lower water content of the former (see Table 1). Recovery of photosynthetic oxygen evolution of *N. commune* exhibited a lag phase and was completed after about 5 h (Fig. 4). A small activity was apparent already after 20 min. A lag period, though less pronounced, was seen

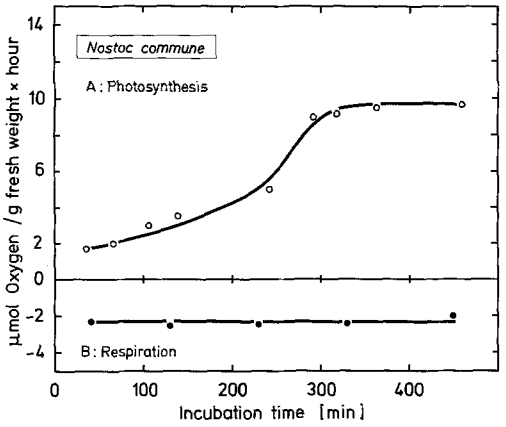

Fig. 4A, B. Recovery of photosynthesis and respiration of *Nostoc commune* after rewetting and transfer to filter paper soaked with growth medium (see Methods, Sect. 2). Oxygen evolution was not corrected for dark respiration

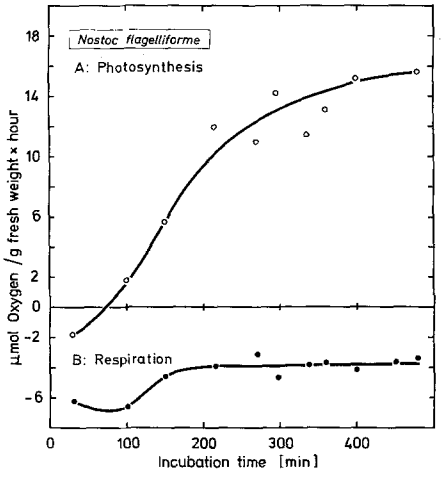

Fig. 5A, B. Recovery of photosynthesis and respiration of *Nostoc flagelliforme* after rewetting (for details see legend of Fig. 4)

with *N. flagelliforme* (Fig. 5), activity was at its maximum 8 to 9 h after rewetting.

High respiration rates were observed immediately after rewetting, remaining constant over the incubation time with *N. commune*. *N. flagelliforme* showed an even higher respiration during the first two hours of incubation, which subsequently slowed down to about 60% of the initial value. Oxygen uptake in the dark was completely abolished by 0.5 mM KCN (assayed with *N. commune* after a 3- and 5-h incubation period; data not shown).

After rewetting of *N. commune* in a buffer, a 23-h dark period markedly inhibited recovery of photosynthesis and impaired recovery of respiration as compared to the corresponding light period. This effect is barely seen after a 7-h incubation time (Table 2). With *N. flagelliforme* dark incubation slightly stimulated recovery of respiration. The fast recovery of photosynthesis and respiration is indicative of existing vegetative cells being activated. As checked by filament length, no growth occurred during the experimental times used here. We have evidence that additional slime material starts being produced 1 to 2 days after rewetting, as is seen by the decreasing ratio of chlorophyll to fresh weight (Table 3).

Table 2. Influence of light on reactivation of photosynthesis and respiration. Rewetted colonies were incubated on wet filter paper in the light (500 lux) or in the dark for 7 or 23 h, as described in Methods. Average values of 8 experiments (*Nostoc commune*) and 2 experiments (*N. flagelliforme*) are given. Data are mean values with ±20% tolerance

Time after rewetting	Incubation in the light (L) or in the dark (D)	Respiration	Photosynthesis
		(µmol O_2/g fresh weight × h)	
Nostoc commune			
7 h	L	2.7	8.3
	D	2.1	6.6
23 h	L	3.2	10.0
	D	2.0	2.5
Nostoc flagelliforme			
7 h	L	3.6	12.9
	D	4.9	6.0
23 h	L	5.2	15.5
	D	5.6	8.4

Table 3. Chlorophyll content and heterocyst frequencies in rewetted *Nostoc commune*, dependent on the incubation time

Incubation time (days) and additions	Chlorophyll content (µg/g fresh weight)	% Heterocysts total[a]	% Heterocysts intercalar[a]
0	98	2.7	0.4
2	77	2.8	1.4
4	64	4.2	2.1
5	60	6.4	2.6
5(+)Chloramphenicol, 25 µg/ml	not determined	3.4	0.1
5(+)Rifampin, 1 µg/ml	not determined	3.3	0.7

[a] Percent of cell number; each Figure was obtained by counting at least 1,000 cells

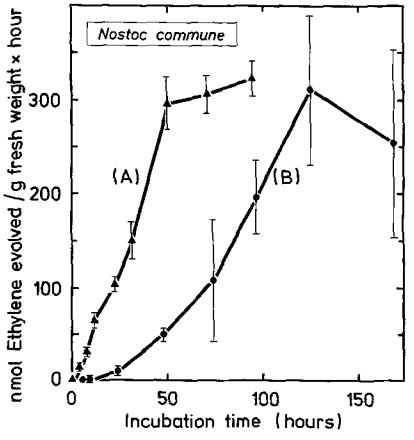

Fig. 6A, B. Nitrogenase activity (ethylene formation) of *Nostoc commune* after rewetting. After a 2-day dry period (**A**) and a 2-year dry period (**B**) both rewetted colonies were exposed to room temperature for one week (see Methods, Sect. 2 and 4)

3. Reactivation of nitrogen fixation

After a 2-year period of dryness, no nitrogenase activity was measured in *Nostoc commune* during the first 5 h after rewetting (Fig. 6B). After 4 to 5 days, nitrogenase activity reached its maximum. Reactivation after a 2-day drying period was complete already after 2 days (Fig. 6A); the lag phase was 2 to 3 h. In all cases, incubation in the dark barely restored nitrogenase activity. Nitrogenase recovery was strictly correlated with heterocyst differentiation, as is seen by the corresponding time course of formation of intercalar heterocysts, which is abolished by chloramphenicol and rifampin, both inhibitors of protein synthesis (Table 3).

Discussion

Only little attention has been directed towards quantitating desiccation tolerance and water uptake of blue-green algae. Hess (1962) found Scytonemataceae, Rivulariaceae, and Nostocaceae to be resistant to dry periods of one year and longer, but not Oscillatoriaceae. In contrast to our findings, however, the species investigated by Hess did not survive in the vegetative state, but as endospores. The only report dealing quantitatively with water uptake of blue-green algae was published by Whitton et al. (1979). *Nostoc commune* and *N. sphaericum* were found to absorb water with half-times of approximately 7 and 25 min, respectively, which is slower than we observed with our Chinese strains. No further data on time course of desiccation or geometry of the colonies were given.

The time courses of desiccation and water uptake correlated well with the surface-to-mass ratio. Therefore, the consistence of the slime seems to be less important for the kinetics of water uptake. At the moment, however, we are unable to functionally evaluate the time courses (cf. Fig. 2). They may reflect different ecological strategies to cope with drought. *Nostoc flagelliforme* grows on the surface of the soil in hot arid areas, where water must be taken up quickly in short rainy periods, since it flows off immediately. Due to the geometry of the colony, desiccation is fast. On the other hand, *N. commune* and *N. spec.* grow in limestone depressions in temperate climates, frequently in the shadow of higher plants (see also Whitton and Sinclair 1975; Whitton et al. 1979). Due to longer wet periods and water-retaining depressions, these species are not forced to take up water as fast as *N. flagelliforme*.

Maximum respiration is observed right after the short rewetting period, while photosynthesis needs 300 to 400 min to attain a plateau. The cell number did not increase during experimental time (data not shown), that is *vegetative* cells were reactivated in contrast to Hess (1962) reporting no survival of vegetative cells. At the moment, we can only speculate about the mechanisms, i.e. whether reactivation is related to build-up of the redox machinery or whether existing structures are used. Noteworthy, Potts et al. (1983) demonstrated with desiccation-resistant *Chroococcus* that internal structures were retained apparently without damage during dryness. The availability of ATP and reduced pyridine nucleotides may be decisive for recovery as suggested by the marked light effect on photosynthesis reactivation (Table 2). Data on nucleotide pools should be provided in future.

Nitrogenase activity was shown to be drought-resistant in the lichen symbiosis (e.g. Kershaw and Dzikowsky 1977; Henriksson and Simu 1971). After short periods (some days) of drought, *Nostoc* mats resume nitrogenase activity within one hour, whereas longer dry periods (e.g. 1 year) delay restoration markedly (24 h; Whitton et al. 1979; comp. also Paul et al. 1971). These reports are in accordance with our findings (Figure 6). The note on fast recovery (30 min) of 11-month old dry material appears to be exceptional (Wang et al. 1981). After a 2-day drying period, nitrogenase activity started to increase immediately after rewetting. Recovery of nitrogenase was paralleled by formation of intercalar heterocysts (Table 3) indicative of recovery being due to synthesis of nitrogenase. Of course, this *de novo* formation has to be proven, but it is consistent with the rapid turnover of nitrogenase and its light-induced activity in blue-green algae reported e.g. by Bone (1971), Scherer et al. (1980), and Ernst (unpubl. results). Future work should clarify the limiting factors of nitrogenase activity after short- and long-term desiccation.

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Note added in proof

Most recently, DS Coxson and KA Kershaw, Can J Bot 61: 2658–2668 (1983) have published similar data on rewetting. However, they used *Nostoc* subjected to shorter drought periods, and CO_2-exchange measurements.