Symbiosis, 37 (2004) 169–187
Balaban, Philadelphia/Rehovot

Associations between Arctic Cyanobacteria and Mosses

B. SOLHEIM[1*], H. WIGGEN[1], S. RØBERG[1,2], and H.P. SPAINK[3]
[1]*Department of Biology, University of Tromsø, N-9037 Tromsø, Norway, Tel. +47-776-44424, Fax. +47-776-46333, Email. bjorn.solheim@ib.uit.no;*
[2]*The University Centre in Svalbard, N-9171 Longyearbyen, Norway;*
[3]*Institute of Molecular Plant Sciences, Leiden University, 2333 AL Leiden, The Netherlands, Tel. +31-71-5275055, Fax. +31-71-5275088, Email. Spaink@rulbim.leidenuniv.nl*

Received October 8, 2003; Accepted December 19, 2003

Abstract

Biological fixation of atmospheric nitrogen by cyanobacteria in association with mosses is important for the nitrogen economy of terrestrial polar environments. Several microscopy techniques were used to resolve the questions of specificity of the interaction and the diversity of cyanobacteria taking part. By confocal laser scanning microscopy it was possible to work with large living samples and create three-dimensional images. The use of 2-photon laser excitation made visualisation of deeper cell layers possible and the resolution was high enough to determine possible types of cyanobacteria. The leaves of *Sanionia uncinata* (Hedw.) formed grooves almost completely enclosing the cyanobacteria. Intracellular colonisation of dead leaf cells was also found. In *Calliergon richardsonii* (Mitt.) Kindb. the cyanobacteria were found in the space between stem and leaves and between leaves. The matrix entrapping the cyanobacteria, together with the structure and growth pattern of leaves, was probably the main reason for the high number of cyanobacteria associated with *S. uncinata* and *C. richardsonii*.

Presented at the 4th International Symbiosis Congress, August 17–23, 2003, Halifax, Canada

*The author to whom correspondence should be sent.

0334-5114/2004/$05.50

Our study of *Hylocomium splendens* (Hedw.) B. S. G. was restricted to epifluorescence and scanning electron microscopy and of *Bryum pseudotriquetrum* (Hedw.) Schwaeger. to confocal laser scanning microscopy. A wide variety of microorganisms was found associated with the surface of the mosses studied. Filamentous cyanobacteria of the *Nostoc* type dominated, but other possible genera such as *Microchaete, Gloeocapsa* and *Asterocapsa* together with fungal mycelia and heterotrophic bacteria could also be seen. An epiphytic green alga, possibly *Kentrosphaera* sp., was found on *B. pseudotriquetrum.*

Keywords: Arctic ecology, cyanobacteria, cytology, epiphytes, microscopy, mosses, plant/microbe interactions

1. Introduction

Biological fixation of atmospheric nitrogen by cyanobacteria is important for the nitrogen economy of terrestrial polar environments (Fogg and Stewart, 1968; Horne, 1972; Croome, 1973; Alexander, 1974; Kallio, 1974; Davey, 1983; Henry and Svoboda, 1986; Lennihan and Dickson, 1989). Cyanobacteria constitute not only the dominant microbial phototrophs and nitrogen fixers, but also most of the microbial ecosystem biomass (Vincent, 2000). Cyanobacteria exist both in a free-living state and closely associated with other organisms such as bryophytes.

In the Arctic, bryophytes dominate the vegetation in wet and mesic areas and are often abundant in drier areas together with vascular plants. The nitrogen input in the rain and snow that fall over the Arctic are generally less than or equal to 30 mg m^{-2} yr^{-1} (Barsdate and Alexander, 1975), and Rempfler (1989) found the input of nitrogen on Spitsbergen in the Kongsfjord area to be 40 mg m^{-2} yr^{-1}. Based on available published data Chapin and Bledsoe (1992) calculated annual nitrogen fixation rates in different arctic and alpine ecosystems to be inside a range of 19–255 mg m^{-2} yr^{-1} with a mean of 127 mg m^{-2} yr^{-1}. Calculated as percent of plant uptake the range was between 0.7–20.5 percent with a mean of 7.1 percent. With a few exceptions, such as the ornithogenic tundra, the amount of nitrogen available in the soil is one of the major factors limiting plant growth in the Arctic (Shaver and Chapin, 1980; Nadelhoffer et al., 1992).

We have previously described biological nitrogen fixation on Spitsbergen, the largest island in Svalbard (Solheim et al., 1996) and found that cyanobacteria in association with mosses were by far the most important source of nitrogen in vegetated areas. In a study of nitrogen fixation in different vegetation types on Spitsbergen (Zielke et al., 2002) the importance of moss-

associated cyanobacteria was confirmed. Nitrogen fixation by moss-associated cyanobacteria has also been found to be of importance in Sub-Arctic and Alpine regions (see Solheim and Zielke, 2002) and in boreal forests (DeLuca et al., 2002). Broady (1979) and Smith (1984) found an uneven distribution of cyanobacteria along the shoot of moss plants and Smith (1984) found good correlation between abundance and acetylene reduction. Basilier et al. (1978) found a similar distribution of acetylene reduction along the shoot of *Sphagnum riparium* Ångstr. and *Drepanocladus exannulatus* (B. S. G.) Warnst. in a subarctic mire.

In all cases the highest activity was found in the green living part of the moss, even if significant activity often was found in the brown mainly dead part of the plant. Usually the activity close to the apex was lower than that further down but still in the green part of the moss. The distribution of *Nostoc muscorum* along mosses was maintained by a motile stage, called the hormogonium, in the life cycle of the cyanobacterium (Broady, 1979).

In the symbioses between *Nostoc* and the liverworts *Blasia pusilla* L. and several species of *Anthoceros* (Duckett et al., 1977; Rodgers and Stewart, 1977) the *Nostoc* colonies develop in slime cavities on the lower surface of the gametophyte. There is an effective transfer of fixed nitrogen from the cyanobacteria to the liverworts and fixed carbon in the other direction (Stewart and Rodgers, 1977).

Certain moss species seem to be especially well adapted to form associations with cyanobacteria (Granhall and van Hofsten, 1976; Solheim et al., 1996; 2002). For some *Sphagnum* sp. a possible mechanism for this adaptation is the presence of empty hyaline cells that become colonised by cyanobacteria (Granhall and van Hofsten, 1976). Jordan et al. (1978) found mainly *Nostoc* sp. on mosses studied, although *Anabaena* sp. and *Ocillatoria* sp. were also present. Large globular colonies of *Nostoc* sp. were often found in the leaf axils of the moss. The epiphytic microflora of *Polytrichum commune* Hedw. (Scheirer and Dolan, 1983) and *Funaria hygrometrica* Hedw. (Scheirer and Brasell, 1984) has been studied by different microscopic techniques, but without relating it to the role of moss morphology in harbouring cyanobacteria on their leaf surface. Solheim et al. (1996) found in the Arctic that *Sanionia uncinata* (*Drepanocladus uncinatus*) and *Calliergon richardsonii* were especially adapted to associate with nitrogen fixing cyanobacteria. High nitrogen fixation activity was also found associated with *Hylocomium splendens* growing in a subarctic birch forest (Solheim et al., 2002).

This paper describes the association between cyanobacteria and the mosses *S. uncinata*, *C. richardsonii*, *Bryum pseudotriquetrum* and *H. splendens* by different microscopic techniques. The goals of the study are to understand why these mosses harbour more nitrogen fixing cyanobacteria than other mosses growing in the same habitat and the diversity of the cyanobacteria involved.

2. Materials and Methods

Sample and site descriptions

Samples of the mosses *S. uncinata*, *C. richardsonii* and *B. pseudotriquetrum* were collected close to Ny-Ålesund in the Kongsfjord area, Spitsbergen, Svalbard (79°N,12'E) early July and mid August over a period of several years. The site was on a marine terrace around Lake Solvatnet (78°56'N, 11°57'E) in an area with dense moss and moss tussocks which was heavily grazed by barnacle geese (Loonen and Solheim, 1998). Samples of *H. splendens* were collected in August 2000 in Abisko, Swedish Lapland (68.35°N, 18.82°E, 360 m a.s.l.) in subarctic forest with an open canopy of *Betula pubescens* ssp. *Czerepanovii* (Orlova) Hämet-Ahti, a dense dwarf shrub layer. The vegetation had a prominent ground layer of *H. splendens* (Solheim et al., 2002).

Nitrogen fixation activity

Nitrogen fixation activity was measured as acetylene reduction activity and was determined along the moss plants by cutting the plants into 5 mm segments starting from the apex and well into the brown dead, part of the moss. The acetylene reduction activity in each sample of segments was measured by gas chromatography and calculated as described previously (Solheim et al., 1996).

Epifluorescence microscopy

Individual moss plants were selected randomly from moss samples and cut into segments of approximately 10 mm. Each segment was transferred to a drop of water on a microscope slide and covered by a coverslip before being studied in a Leitz Laborlux K microscope (Leica Mikroskopie und System GmbH, Wetzlar, Germany) by bright field, phase contrast and epifluorescence microscopy. For epifluorescence microscopy N2.1 filter was used.

Light microscopy

Samples with a large number of cyanobacteria and a high acetylene reduction activity, determined by epifluorescence microscopy and the acetylene reduction assay respectively, were selected for microscopy. The samples were immediately prefixed in McDowells solution at pH 7.2 containing 1% glutaraldehyde, 4% formaldehyde in 0.1 M phosphate buffer with 0.08 M sucrose and stored at 4°C. After prefixation the individual plants were cut into 3–5 mm segments and washed twice in 0.1 M phosphate buffer pH 7.4. The segments were then fixed in 1% OsO_4 for 2 h, washed again twice with buffer

and finally in Milli-Q-water, then submerged for 1.5 h in 2% uranyl acetate, dehydrated in increasing concentrations (30–100%) of ethanol, treated three times with propylene oxide and embedded in Epon/Araldite [6 ml Epon 812, 11 ml 2-Dodecenyl succinic acid (DDSA), 3 ml Araldite 502 and 0.4 ml 2,4,6-Tris (dimethyl-amminomethyl) phenol (DMP 30)]. Embedded material was sectioned with a glass knife on a Reichert Ultracut E microtome (Reichert, Vienna, Austria). Sections approximately 1 µm thick were transferred to a drop of water on a microscope slide and dried on a hot-plate, stained with 1% toluene blue in 2.5% (v/v) $NaCO_3$, washed with distilled water, dipped in 95% ethanol and a fresh wash in distilled water before drying on a hot plate. Then the slide was dipped in xylene, a drop of the mounting medium Histokitt (Assistent No. 1025/250, Germany) was added and the specimen was covered by a coverslip.

Confocal laser scanning microscopy

Confocal laser scanning microscopy was carried out with a Leica-DMIRBE inverted fluorescence microscope (Leica, Bensheim, Germany) equipped with a Leica SP confocal scan head. 2-photon excitation at 800 nm was established by direct coupling of a TiS Mira 900F laser (Coherent Inc., Sunnyvale, CA) to the Leica SP scan head, resulting in pulse lengths of around 150 femtoseconds and power of up to 400 mW. Multicolor images were acquired by sequential scanning with settings for excitation at 568 nm and emission detection between 650 and 720 nm (channel 1), followed by 2-photon excitation at 800 nm and emission detection between 450 and 650 nm (channel 2) and 650 and 750 nm (channel 3).

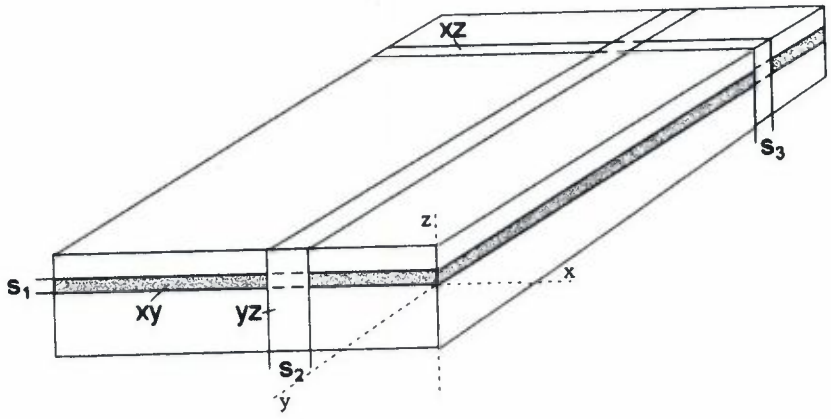

Figure 1. Illustration of how the 3D-computer generated image is section in three planes along x, y, z coordinates creating xy, xz and yz planes of thickness s1, s2 and s3, respectively.

In some cases, out of focus information in the original data was removed by deconvolution using the maximum likelihood estimate method as offered by the Huygens software package (SVI, Hilversum, The Netherlands). Output files of images were produced using Imaris software (Bitplane AG, Zurich, Switzerland). The images for this paper were created by sectioning the 3D-computer image in xy, xz and yz panels. Each of the panels are shown in two dimensions (Fig. 1). The thickness of the sections depended on how many optical sections were projected on top of each other forming a stack (s). The thickness of the three panels is given as s_1, s_2 and s_3, respectively.

Transmission electron microscopy (TEM)

Samples for TEM were treated as for light microscopy, but embedded material was sectioned with a diamond knife on the Reichert Ultracut E microtome and sections were collected on 100 mesh copper grids supported with formvar. The sections were post-stained with 5% (w/v) Uranyl acetate followed by Reynolds lead citrate (Weakley, 1981). A Jeol JEM-1010 transmission electron microscope (Jeol, Tokyo, Japan) was used at an accelerating voltage of 80 kV.

Scanning electron microscopy (SEM)

Samples for SEM were selected and prefixed as samples for light microscopy and TEM, but were dehydrated in increasing concentrations of ethanol (30–100%) directly after prefixation. Samples of *S. uncinata* and *C. richardsonii* were transferred to a Balzers Union Chamber (Baltech, Balzers, Lichtenstein) for critical point drying while samples of *H. splendens* were submerged in hexamethyldisilizane and dried in a desiccator. Samples from both treatments were mounted on stubs and coated with gold in a SEM coating unit E5000 (Quorun Technologies, New Haven, UK). The scanning electron microscopes used were Jeol JSM-5300 and Jeol JSM 840 (Jeol, Tokyo, Japan) at an accelerating voltage of 10 and 20 kV.

3. Results

The length of the bright green gametophytic part of the mosses *S. uncinata* and *C. richardsonii* around Lake Solvatnet varied with growing location, but for both species an average between 1.5 and 2.0 cm was found. Below this part was a transition zone of about 1 cm where the moss cells started to turn brown before only dead cells could be found. Usually the highest activity of acetylene reduction was found between 5 and 10 mm below the apex (Fig. 2).

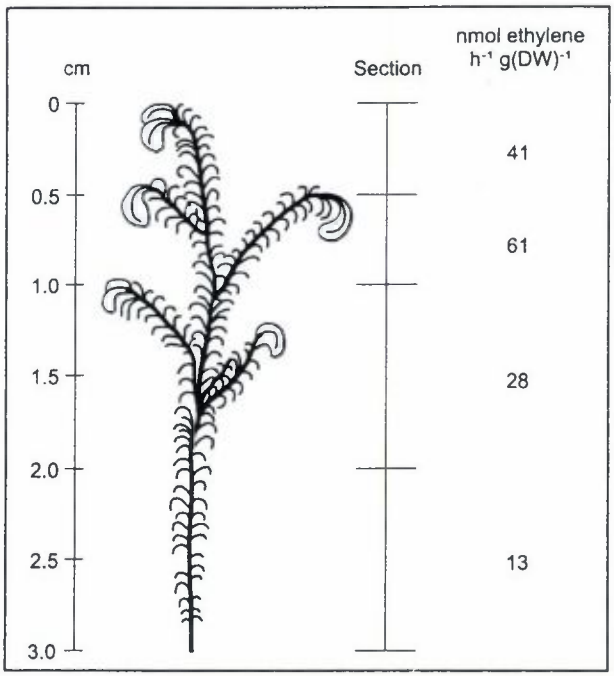

Figure 2. Acetylene reduction activity in segments of *Sanionia uncinata* collected around Lake Solvatnet. Enzyme activity is given as nmol ethylene h^{-1} g $(DW)^{-1}$.

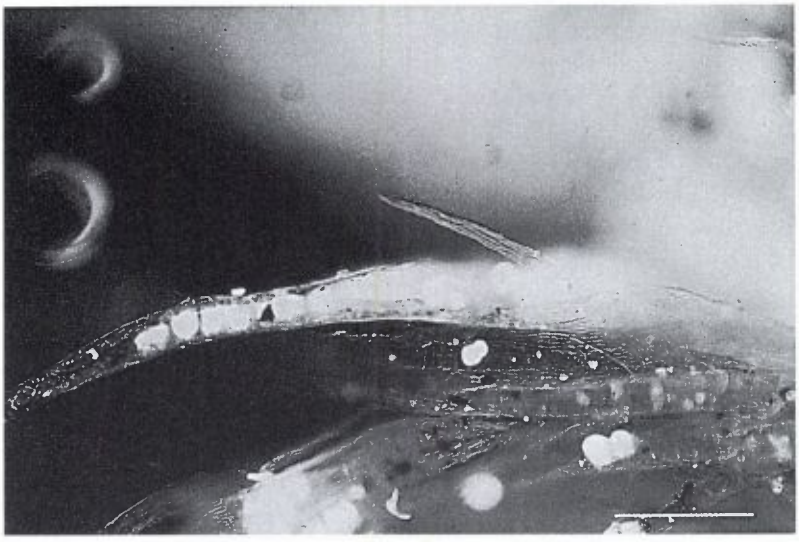

Figure 3. Epifluorescence microscopy of cyanobacteria on leaves of *S. uncinata*. Aseriate packages of cyanobacteria (grey spheres) fill the curved leaf of *S. uncinata*. Bar = 20 µm.

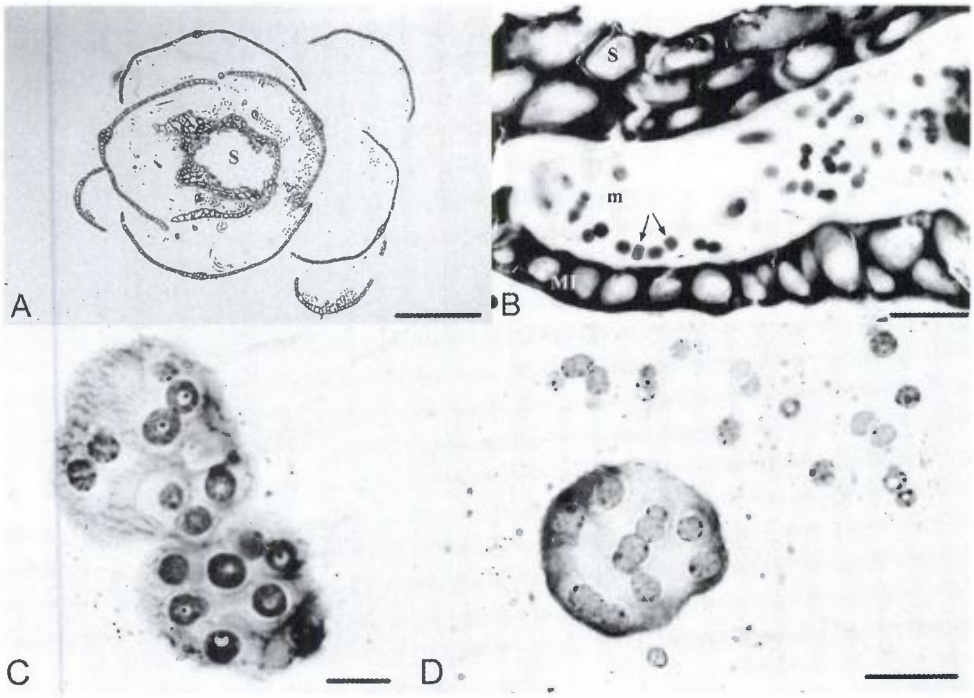

Figure 4. Light microscopy of sections perpendicular to the stem of *C. richardsonii*. A. Cyanobacteria are located on the inside of leaves. The hole in the central part of the stem (S) is an artefact. Bar = 100 µm. B. At higher magnification of A filamentous cyanobacteria with several heterocysts (arrows) can be seen embedded in a matrix (m) between a moss leaf (ML) and the stem (S). Bar = 10 µm. C. Single cells of cyanobacteria without heterocysts inside capsular material, possibly *Gloeocapsa* sp. Bar = 10 µm. D. Cells released from capsule. Bar = 10 µm.

In heavily grazed areas the highest activity was closer to the apex of the moss (results not shown). In *S. uncinata* the leaves frequently curled around the cyanobacteria, almost concealing them between host cells (Fig. 3). The cyanobacteria were clearly seen by epifluorescence microscopy and formed round balls typical of the aseriate stage of *Nostoc* (Fig. 3). In stained samples sectioned perpendicular to the stem of *C. richardsonii* the cyanobacteria were found in the space between stem and leaves and between leaves always associated with the surface of the stem or the surface of the leaves facing towards the central part of the moss shoot (Fig. 4A). At higher magnification of Fig. 4A it could be seen that the cyanobacteria were entrapped in a matrix, most of them forming strings of cells with frequent heterocysts (Fig. 4B). The frequency of heterocysts in relation to vegetative cells in filamentous cyanobacteria associated with *C. richardsonii* varied between 1:10 and 1:20. In

sections of *C. richardsonii* short chains of cells inside a common capsular structure were frequently found (Fig. 4C), and in some cases it seemed that these cells could be released to the outside (Fig. 4D).

In SEM images large numbers of cyanobacteria may be seen adhering to the stem close to leaf bases (Fig. 5). The fibrous structure of the matrix around the cyanobacteria is clearly visible by TEM (Fig. 6). Confocal laser scanning microscopy images revealed cyanobacteria both on the surface of the leaves of *S. uncinata* and within the lumen of dead cells (Fig. 7). Under the SEM short chains of cyanobacteria and numerous bacteria as well as fungal hyphae were found on the surface of the stems of *H. splendens* (Fig. 8A). At higher magnification it was obvious that bacteria associated both with the cell-surface of the moss and with the cyanobacteria (Fig. 8B). Typical *Nostoc*-type cyanobacteria with heterocysts were frequent in all samples and could be seen by all the methods used. Only confocal laser scanning microscopy gave the depth of field and resolution to visualise the diversity of organisms in moss samples. On *S. uncinata* the aseriate stage of *Nostoc* type of cyanobacteria had a prominent sheath around the cell packet that was absent around the seriate stage. Thick trichomes of cylindrical cells with terminal heterocysts, lemon shaped single cells with prominent capsules, very thin trichomes and micro-colonies of small single cells could also be seen (Fig. 9A). On *B. pseudotriquetrum* the epiphytic organisms were light blue (Fig. 9B). The membrane structure enclosing the cells was different from the types observed on *S. uncinata*.

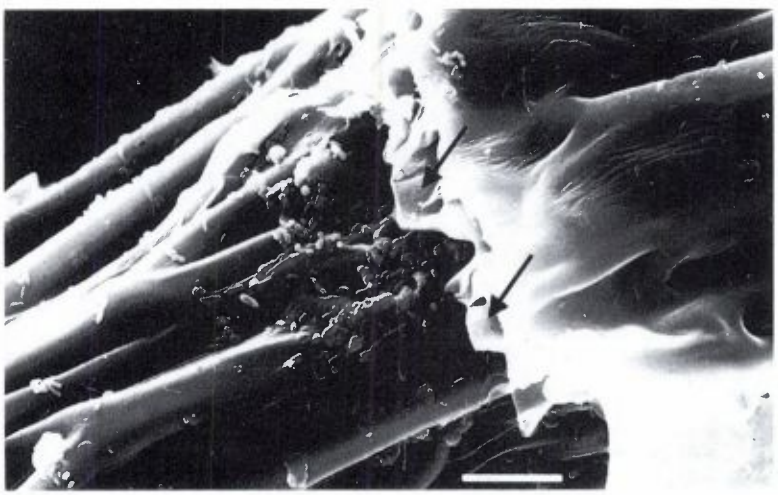

Figure 5. Scanning electron microscopy of epiphytic microorganisms on the stem close to the base of a leaf of *C. richardsonii*. Most of the leaf has been removed to expose the base. Arrows indicate where the leaf has been removed. Bar = 10 µm.

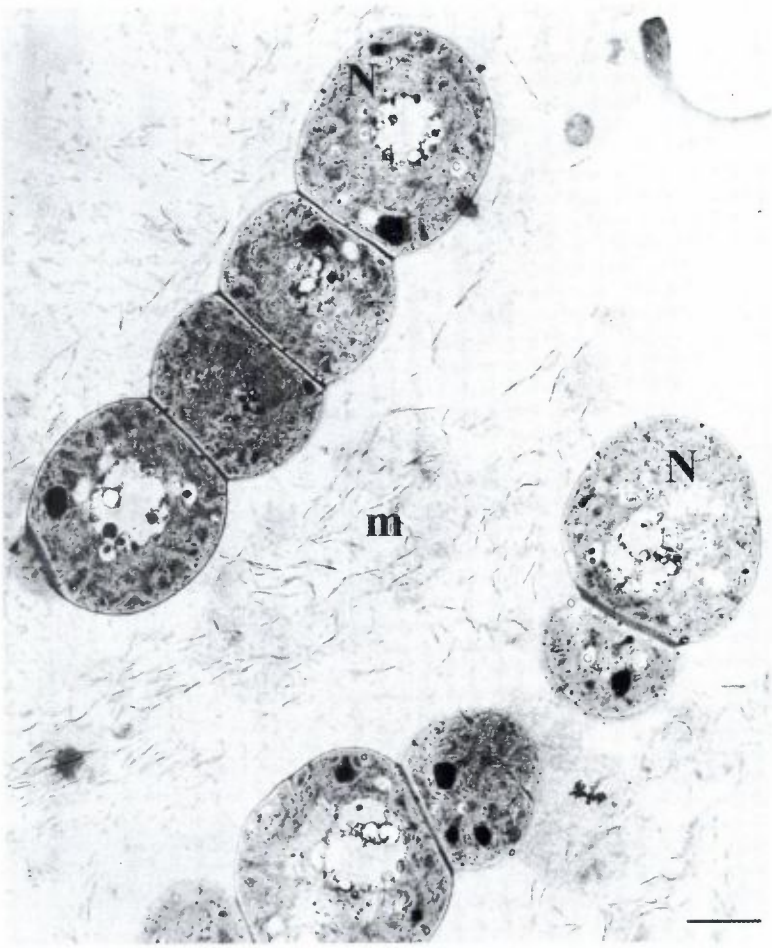

Figure 6. Transmission electron microscopy of typical *Nostoc*-type filaments (N) in association with moss (not visible). Cells embedded in matrix (m). Bar = 2 µm.

4. Discussion

Distribution and activity of cyanobacteria along the moss plants

The nitrogen fixation rates, measured as acetylene reduction, along the shoot of the plant varied in the same way as reported for other species of mosses (Basilier et al., 1978; Smith, 1984). To study the distribution of cyanobacteria along the moss, epifluorescence microscopy was found to be a fast and simple method (Scheirer and Brasell, 1984). There was a good correlation between the number of cyanobacteria and the rate of acetylene reduction in the upper 1 cm

with green, living moss cells. The number of cyanobacteria was often highest in the transition zone (1.5–2.5 cm) between green, living and dead, brown moss, but the acetylene reduction rate was normally lower than in the zone directly above. This indicated that cyanobacteria associated with living moss cells fixed nitrogen more actively than cyanobacteria associated with dead or dying moss cells.

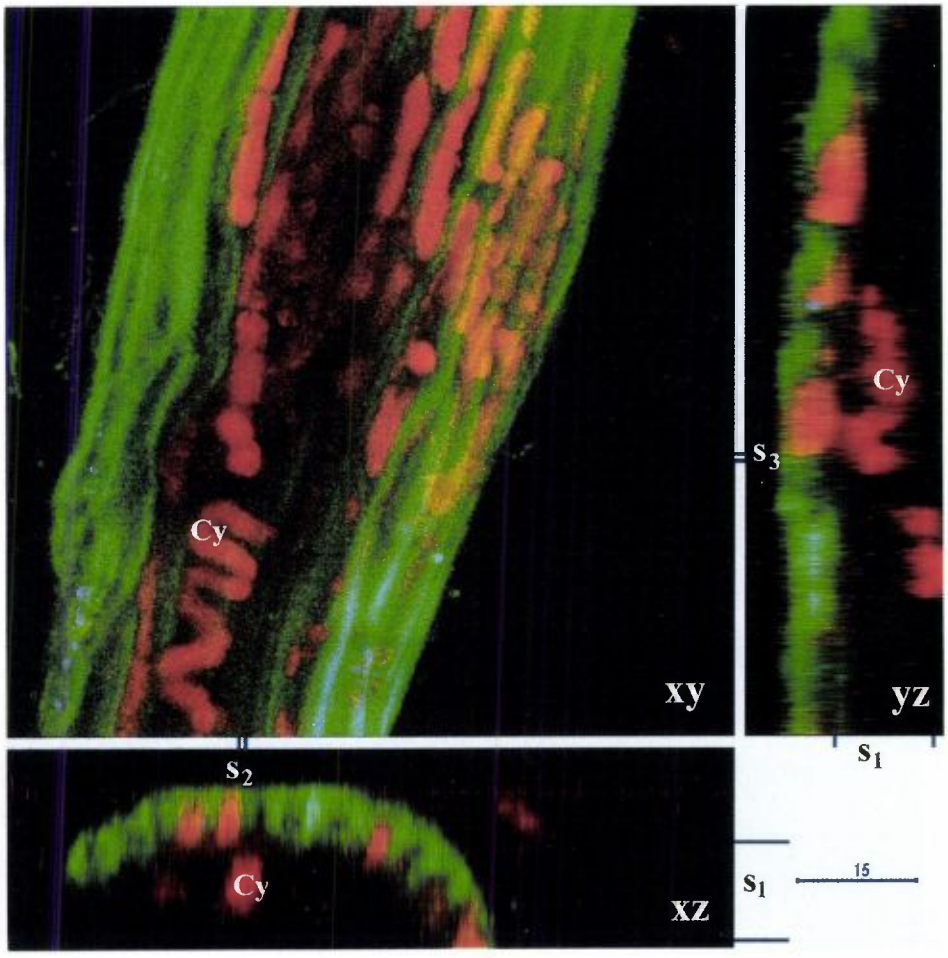

Figure 7. Confocal laser scanning microscopy of a leaf of *S. uncinata*. The three dimensional computer image has been sectioned in xy, xz and yz planes. In the xy plane cyanobacteria (Cy, red colour) can be seen to have colonised most of the dead moss cells (green colour, probably due to fluorescence of remaining cell content along the cell walls). In the xz and yz planes cyanobacteria can be seen both outside and inside moss leaf cells. Total image field 90x90x20 micrometer. Voxel size xy: 0.175; voxel size z: 1.459. The projected stacks (s_1; s_2; s_3) of images in each plane are indicated with black lines at the border of the shown xy, xz and yz planes. Bar = 15 µm.

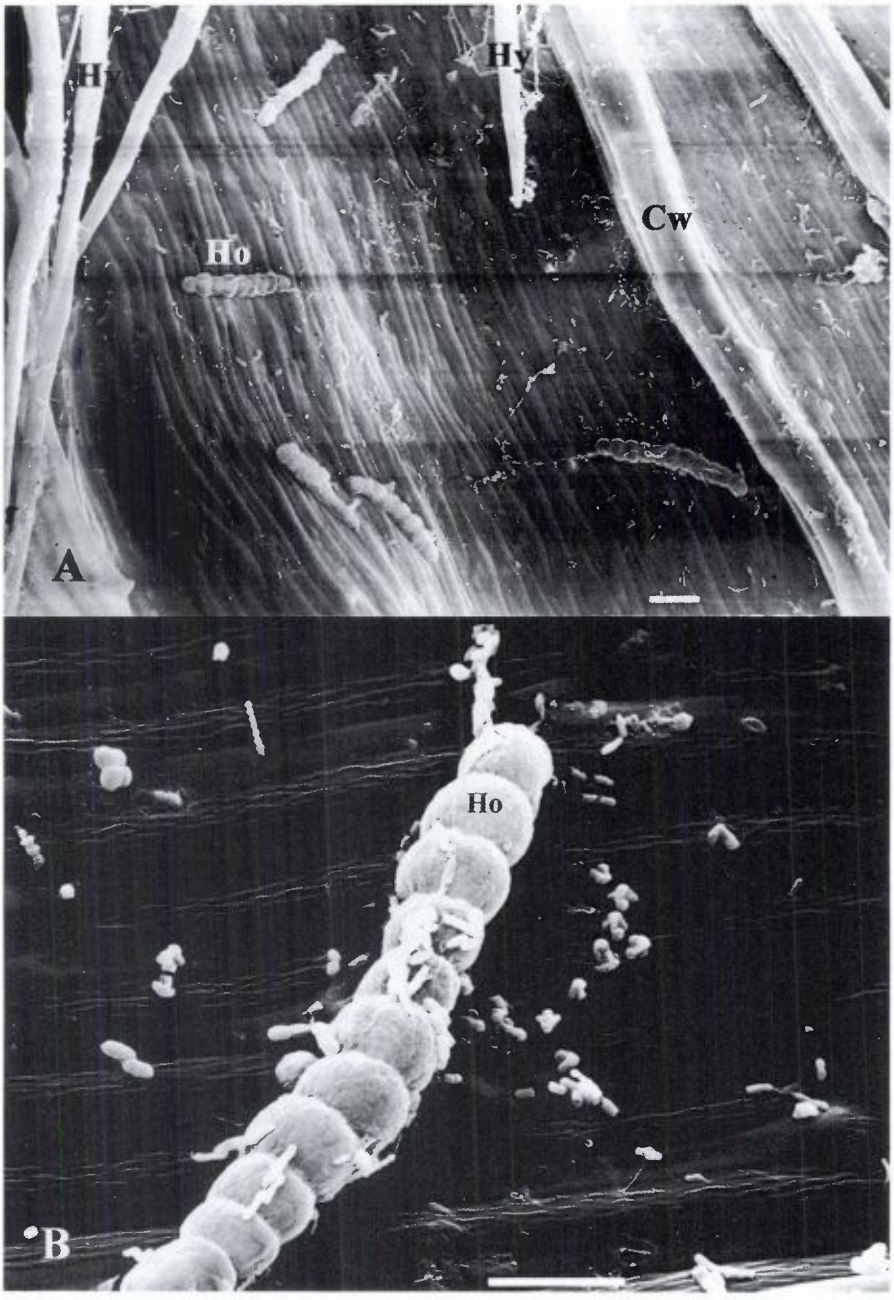

Figure 8. Scanning electron microscopy of epiphytic microorganisms on *H. splendens*. A. Fungal hyphae (Hy), bacteria and hormogonia (Ho) of cyanobacteria can be seen on the surface of the leaf. The moss cell wall (CW) forms ridges between moss cells. Bar = 10 µm. B. Bacteria are both associated with moss cell walls and a hormogonium (Ho). Bar = 5 µm.

Physical structures facilitating the associations

The shape of the leaves may make them especially suitable for the association with cyanobacteria. The characteristic colonisation by the cyanobacteria of the tubular leaf of *S. uncinata* encloses the cyanobacteria between moss cells of a single leaf. The morphological structure of the leaves may maintain high humidity around the cyanobacteria resulting in a longer growth period during the summer. One µm thick sections stained for light microscopy gave a good overview and were suitable for studies of the distribution of cyanobacteria in relation to the moss plant. Most of the cyanobacteria on *C. richardsonii* seen in sections in the light microscope were filamentous with heterocysts. All the cyanobacteria were associated with the side of leaves facing the central part of the moss or between the leaves and the stem. The side facing outwards of leaves was never colonised. The location of the cyanobacteria on *C. richardsonii* may protect the cyanobacteria from direct sunlight and desiccation. A preference by epiphytic microorganisms for the base of the leaves of *C. richardsonii* was seen when the leaf was removed and the stem exposed. This is probably the best protected location along the moss stem. No special structures such as the "slime cavities" found in liverworts (Duckett et al., 1977; Rodgers and Stewart, 1977) were found in *S. uncinata* or *C. richardsonii*, but the matrix holding the cyanobacteria in the space between leaves and stem, or closely to the inside of the leaves, probably stabilised the association and made transfer of nutrients between the organisms more efficient (Alexander et al., 1978). The matrix was probably extra-cellular polysaccharides (EPS) containing proteins of cyanobacterial origin (Potts, 1994). The high frequency of heterocysts found in "slime cavities" in liverworts or in the glands of *Gunnera* was not found for epiphytic cyanobacteria. Beside colonisation by cyanobacteria of empty dead moss cells the only "symbiotic structure" is probably the matrix embedding the cyanobacteria.

Several functions have been suggested for the EPS/protein matrix around cyanobacteria including, adhesion and immobilisation of the organisms, protection from grazing, protection from desiccation and protection against UV radiation (Potts, 2000; Stal, 2000). One principal function of the EPS is that it provides a repository for water (Potts, 1994). The matrix may restrict the cyanobacteria to the close vicinity of moss cells and prevent released nutrients being transported away by moving water, thereby facilitating nutrient exchange by diffusion between the organisms. The matrix, together with the water-holding capacity of the layers of leaves of the moss plant, may preserve enough humidity to prolong the period for biological activity during the summer season. This protection of the function of cyanobacteria is important for the nitrogen budget of polar ecosystems. Even if free-living terrestrial cyanobacteria have a high potential for nitrogen fixation their contribution

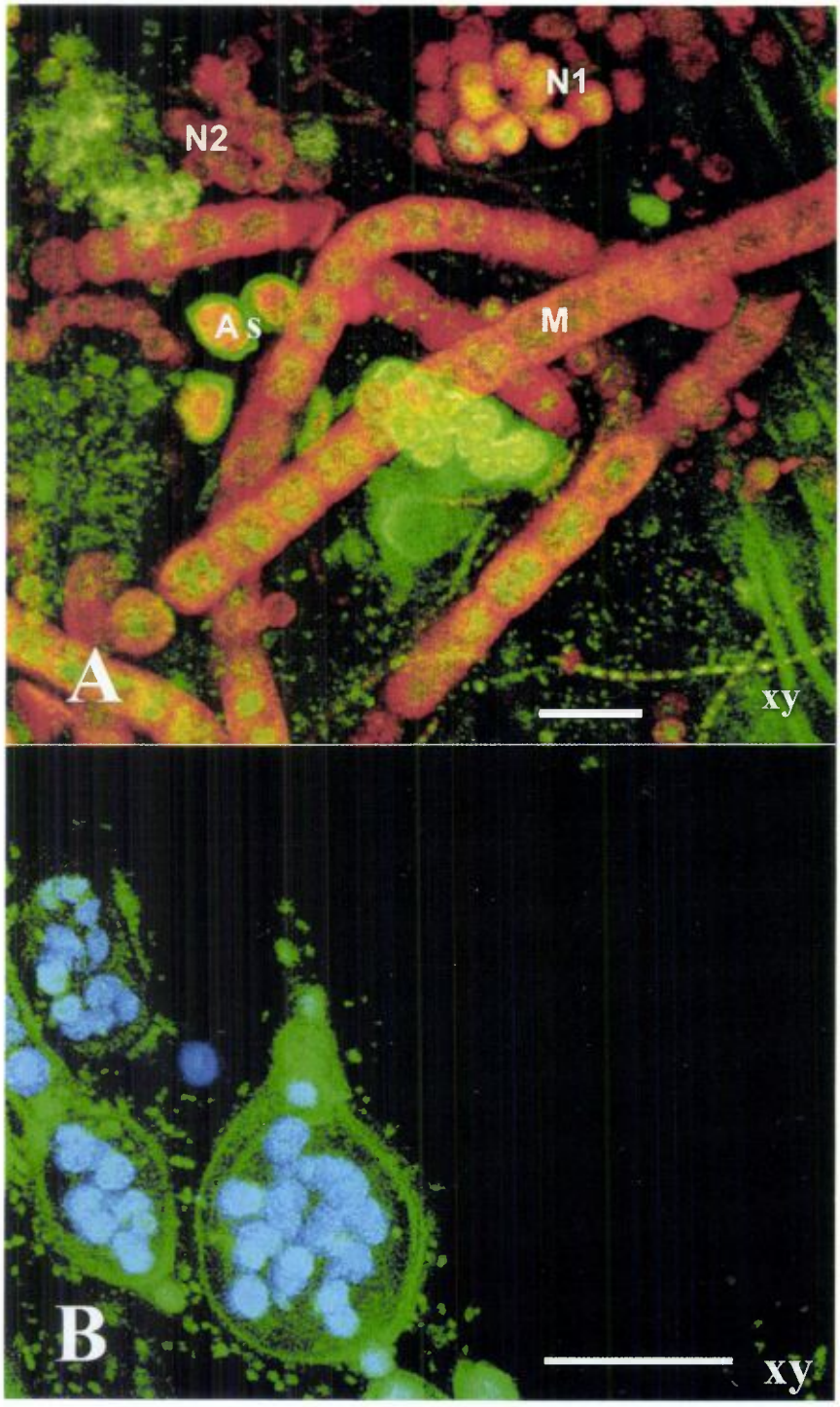

Figure 9. See legend on next page.

might be strongly reduced due to lack of water. In most areas they are found in the upper layer of bare ground or on top of the vegetation and are prone to desiccation. Moss-associated cyanobacteria provide 2–58% of nitrogen input to arctic ecosystems and 42–84% to antarctic moss vegetation (Dodds et al., 1995).

The leaves of *S. uncinata* seemed well adapted to colonisation by cyanobacteria by forming grooves almost completely enclosing the cyanobacteria. The intracellular colonisation of dead cells on leaves of *S. uncinata* may protect the cyanobacteria from environmental stress. However, contrary to the hyaline cells of *Sphagnum* mosses (Granhall and van Hofsten, 1976) these moss cells seem not to be in contact with living neighbouring cells. In *C. richardsonii* the cyanobacteria were found associated with the inner surface of the leaves or in the space between leaves and stem. The matrix entrapping the cyanobacteria close to the surface of the moss, together with the growth pattern of leaves close to the stem forming a relatively protected environment, are probably the main reason for the high number of cyanobacteria associated with *C. richardsonii* compared with other moss species (Solheim et al., 1996; 2002). Our study of *H. splendens* was restricted to epifluorescence and SEM, and we were not able to find evidence of structures or growth patterns favouring the high number of cyanobacteria present. Cyanobacteria were observed in low numbers on *B. pseudotriquetrum* (not shown), but on this moss we demonstrated that eukaryotic micro-algae grow epiphytically on arctic moss.

Diversity of epiphytic cyanobacteria

In SEM a wide variety of epiphytic microorganisms could be seen. On the leaves of *H. splendens*, in addition to fungal hyphae and heterotrophic bacteria, several hormogonia could be seen. The close association between hormogonia and heterotrophic bacteria is probably an indication of nutrients leaking out of the cyanobacteria. Hormogonia were also observed on the other two species of mosses studied, often in the same area as seriate and aseriate

See figure on previous page.

Figure 9. Confocal laser scanning microscopy. A. A leaf of *S. uncinata*. Several types of fluorescing bacteria, mainly cyanobacteria, can be seen. Possible cyanobacteria are, M: *Microchaete* sp., As: *Asterocapsa* sp., N1: Aseriate stage of *Nostoc* sp., and N2: Seriate stage of *Nostoc* sp. Total image field 91x91x24 micrometer. Voxel size xy: 0.177; voxel size z: 1.74. Bar = 10 µm. B. A leaf of *B. pseudotriquetrum* with an epiphytic organism, possibly a green alga of the genus *Kentrosphaera* Borzi in the stage of zoospore formation. Total image field 90x90x45 micrometer. Voxel size xy: 0.175; voxel size z: 1.459. Bar = 20 µm.

stages of *Nostoc* types of cyanobacteria. The role of the motile hormogonia has been suggested to be the colonisation of new-grown moss tissue (Broady, 1979). Frequently, short chains or individual cells of cyanobacteria could be seen inside spherical dense capsular material which was trapped inside the general matrix between leaves and stem of the moss. In some cases it seemed that the cells could be released from the capsular material. From their size and morphology the cyanobacterium might possibly be *Gloeocapsa* sp. This is supported by the report of *Gloeocapsa* spp. being epiphytic on mosses in Antarctica (Ohtani and Kanda, 1987).

By confocal laser scanning microscopy it is possible to work with large living samples and create three-dimensional images. The use of 2-photon laser excitation makes visualisation of deeper cell layers possible. Furthermore, the broad spectrum of excitation wavelengths makes distinctions between various autofluorescing structures possible. Pictures with high depth of view can be created by stacking optical sections on top of each other. With a three-dimensional image it is possible to visualise optical sections through the image. These properties of the confocal laser scanning microscope make it ideal for studies of microbial diversity in complex samples. When leaves of *S. uncinata* were studied, the morphology of several different cyanobacteria could be seen in great detail. A filamentous type with terminal heterocyst could be *Microchaete* sp. Thuret (Geitler, 1932) or the closely related *Fortiea* sp. that is known to be epiphytic on mosses (pers. com. J. Komárek, Faculty of Biological Sciences, University of South Bohemia, Ceske Budejovice, Czech Republic). The green fluorescence around a unicellular cyanobacterium is probably due to accumulation of carotenoids in the capsular material. This is typical of *Asterocapsa* sp. (Komárek and Anagnostidis, 1998). Epiphytic *Asterocapsa* spp. is known from maritime Antarctica, the Rocky Mountains in the USA and the Himalayas (pers. com. J. Komárek, see above) supporting the suggestion that it is the same type found epiphytic on Spitsbergen. On *B. pseudotriquetrum* epiphytic organisms with a different autofluorescence than cyanobacteria were found. Possibly this was a unicellular green alga, *Kentrosphaera* sp., in the stage of zoospore formation (Komárek and Fott, 1983).

5. Conclusions

The highest activity of nitrogen fixation by cyanobacteria growing epiphytically on mosses was associated with living moss cells. The specificity of the interaction between cyanobacteria and mosses may be due to a combination of growth pattern of the moss and EPS/protein matrixes produced by the cyanobacteria. A favourable growth pattern may be the shape of individual leaves (*S. uncinata*) or the relative position of the leaves in

relation to the stem and other leaves (*C. richardsonii*). The EPS/protein matrix seems to restrict and attach the cyanobacteria to the moss cells. Most of the cyanobacteria found growing as epiphytes on mosses were of the *Nostoc* type, but in addition a diverse epiphytic flora could be seen by different microscopy techniques. Especially useful was 2-photon confocal laser scanning microscopy for analyses of complex living samples of cyanobacteria/moss associations.

Acknowledgements

Discussions about systematic classification of the epiphytic organisms on mosses with Drs. Jiri Komárek and Richard Castenholz, and assistance with bryophyte taxonomy by Dr. Arve Elvebakk are gratefully acknowledged. We thank Gerda EM Lamers for her expert technical help with the confocal laser scanning microscopy and the staff at the Electron Microscopy facilities at University of Tromsø for help with the TEM and SEM. Gunvor Granås for her help with making the pictures and Tove Midtun for the drawings are gratefully acknowledged. The work has been partly supported by Norwegian Polar Institute and Roald Amundsen Centre for Polar Research, University of Tromsø, Norway.

REFERENCES

Alexander, V. 1974. A synthesis of the IBP tundra biome circumpolar study of nitrogen fixation. In: *Soil Organisms and Decomposition in Tundra*. A. Holding, J.O.W. Heal, S.F. MacLean, and P.W. Flanagan, eds. Tundra Biome Steering Committee, Stockholm, pp. 109–121.

Alexander, V., Billington, M., and Schell, D.M. 1978. Nitrogen fixation in arctic and alpine tundra. In: *Vegetation and Production Ecology of an Alaskan Arctic Tundra*, vol. 29. Ecological Studies. L.L. Tieszen, ed. Springer Verlag, New York, pp. 539–559.

Barsdate, K.J. and Alexander, V. 1975. The nitrogen balance of arctic tundra: Pathways, rates and environmental implications. *Journal of Environmental Quality* **4**: 111–117.

Basilier, K., Granhall, U., and Stenström, T. 1978. Nitrogen fixation in wet minerotrophic moss communities of a subarctic mire. *Oikos* **31**: 236–246.

Broady, P.A. 1979. The Signy Islands terrestrial reference sites: IX. The ecology of the algae of site 2, a moss carpet. *British Antarctic Survey Bulletin* **47**: 13–29.

Chapin, D.M. and Bledsoe, C.S. 1992. Nitrogen fixation in arctic plant communities. In: *Arctic Ecosystems in a Changing Climate. An Ecophysiological Perspective*. F.S. Chapin III, R.L. Jefferies, J.F. Reynolds, G.R. Shaver, and J. Svoboda, eds. Academic Press, Inc. San Diego, pp. 301–319.

Croome, R.L. 1973. Nitrogen fixation in the algal mats on Marion island. *South African Journal of Antarctic Research* **3**: 64–67.

Davey, A. 1983. Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. *Polar Biology* **2**: 95–100.

DeLuca, T.H., Zackrisson, O., Nilson, M.-C., and Sellstedt, A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature* **419**: 917–920.

Dodds, W.K., Gudder, D.A., and Mollenhauer, D. 1995. The ecology of *Nostoc*. *Journal of Phycology* **31**: 2–18.

Duckett, J.G., Prasad, A.K.S.K., Davies, D.A., and Walker, S. 1977. A cytological analysis of the *Nostoc*-bryophyte relationship. *New Phytologist* **79**: 349–362.

Fogg, G.E. and Stewart, W.D.P. 1968. *In situ* determinations of biological nitrogen fixation in Antarctica. *British Antarctic Survey Bulletin* **15**: 39–46.

Geitler, L. 1932. Cyanophyceae. In: *Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz*, 14 Band. L. Rabenhorst, ed. Akademische Verlagsgesellschaft mbH. Leipzig, pp. 1–1196.

Granhall, U. and van Hofsten, A. 1976. Nitrogenase activity in relation to intracellular organisms in *Sphagnum* mosses. *Physiologia Plantarum* **36**: 88–94.

Henry, G.H.R. and Svoboda, J. 1986. Dinitrogen fixation (acetylene reduction) in high Arctic sedge meadow communities. *Arctic and Alpine Research* **18**: 181–187.

Horne, A.J. 1972. The ecology of nitrogen fixation on Signy island, South Orkney islands. *British Antarctic Survey Bulletin* **27**: 1–18.

Jordan, D.C., McNicol, P.J., and Marshall, M.R. 1978. Biological nitrogen fixation in the terrestrial environment of a high Arctic ecosystem (Truelove Lowland, Devon Island, N.W.T.). *Canadian Journal of Microbiology* **24**: 643–649.

Kallio, P. 1974. Nitrogen fixation in subarctic lichens. *Oikos* **25**: 194–198.

Komárek, J. and Anagnostidis, K. 1998. Cyanoprokaryota 1. Teil: Chroococcales. In: *Süsswasserflora von Mitteleuropa*, 19/1. H. Ettl, G. Gärtner, H. Heynig, and D. Mollenhauer, eds. Gustav Fischer, Heidelberg, pp. 1–548.

Komárek, J. and Fott, B. 1983. Chlorophyceae (Grünalgen). Ordnung: Chlorococcales. In: *Das Phytoplankton des Süsswassers, Die Binnengewässer* 16, 7/1. G. Huber-Pestalozzi, ed. Schweizerbart Verlag, Stuttgart, pp. 1–1044.

Lennihan, R. and Dickson, L.G. 1989. Distribution, abundance and physiological aspects of *N. commune* in a high arctic ecosystem. *Journal of Phycology* **25**: 16.

Loonen, M.J.J.E. and Solheim, B. 1998. Does Arctic vegetation change when grazed by barnacle geese? A pilot study. *Norsk Polarinstitutt Skrifter* **200**: 99–103.

Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R., and Linkins, A.E. 1992. Microbial processes and plant nutrient availability in arctic soils. In: *Arctic Ecosystems in a Changing Climate. An Ecophysiological Perspective*. F.S. Chapin III, R.L. Jefferies, J.F. Reynolds, G.R. Shaver, and J. Svoboda, eds. Academic Press, Inc. San Diego, pp. 281–300.

Ohtani, S. and Kanda, H. 1987. Epiphytic algae on moss community of *Grimmia lawiana* around Syowa Station, Antarctica. *Proceeding NIPR Symposium. Polar Biology* **1**: 255–264.

Potts, M. 2000. *Nostoc*. In: *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. B.A. Witton and M. Potts, eds. Kluwer Academic Publisher, Dordrecht, pp. 465–504.

Potts, M. 1994. Desiccation tolerance of prokaryotes. *Microbiological Reviews* **58**: 755–805.

Rempfler, A. 1989. Wasser und nährstoffhaushaltliche Aspekte im Jahresgang hocharktischer Geosysteme (Raum Ny-Ålesund, Brøggerhalvøya, Nordwestspitzbergen). *Die Erde* **120**: 225–238.

Rodgers, G.A. and Stewart, W.D.P. 1977. The cyanophyte-hepatic symbiosis. I. Morphology and physiology. *New Phytologist* **78**: 441–458.

Scheirer, D.C. and Brasell, H.M. 1984. Epifluorescence microscopy for the study of nitrogen fixing blue-green algae associated with *Funaria hygrometrica* (Bryophyta). *American Journal of Botany* **71**: 461–465.

Scheirer, D.C. and Dolan, H.A. 1983. Bryophyte leaf epiflora: An SEM and TEM study of *Polytrichum commune* Hedw. *American Journal of Botany* **70**: 712–718.

Shaver, G.R. and Chapin, F.S. III. 1980. Responses to fertilization by various growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology* **61**: 662–675.

Smith, V.R. 1984. Effects of abiotic factors on acetylene reduction by Cyanobacteria epiphytic on moss at a subantarctic island. *Applied and Environmental Microbiology* **48**: 594–600.

Solheim, B., Endal, A., and Vigstad, H. 1996. Nitrogen fixation in arctic vegetation and soils from Svalbard, Norway. *Polar Biology* **16**: 35–40.

Solheim, B., Johanson, U., Callaghan, T.V., Lee, J.A., Gwynn-Jones, D., and Bjørn, L.O. 2002. The nitrogen fixation potential of arctic cryptogam species are influenced by enhanced UV-B radiation. *Oecologia* **133**: 90–93.

Solheim, B. and Zielke, M. 2002. Associations between cyanobacteria and mosses. In: *Cyanobacteria in Symbiosis*. B. Bergman, A.N. Rai, and U. Rasmussen, eds. B. Kluwer Academic Publishers, Dordrecht, pp. 137–152.

Stal, L.J. 2000. Cyanobacterial mats and stromatolites. In: *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. B.A. Witton, and M. Potts, eds. Kluwer Academic Publishers, Dordrecht, pp. 61–120.

Stewart, W.D.P. and Rodgers, G.A. 1977. The cyanophyte-hepatic symbiosis. II. Nitrogen fixation and the interchange of nitrogen and carbon. *New Phytologist* **78**: 459–471.

Vincent, W.F. 2000. Cyanobacterial dominance in the polar regions. In: *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. B.A. Witton, and M. Potts, eds. Kluwer Academic Publishers, Dordrecht, pp. 321–340.

Weakley, B.S. 1981. *A Beginner's Handbook in Biological Transmission Electron Microscopy*. Sec. ed. Churchill Livingston, New York.

Zielke, M., Ekker, A.S., Olsen, R.A., Spjelkavik, S., and Solheim, B. 2002. The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the High Arctic, Svalbard. *Arctic, Antarctic and Alpine Research* **34**: 293–299.