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# Effects of Abiotic Factors on Acetylene Reduction by Cyanobacteria Epiphytic on Moss at a Subantarctic Island

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**Acetylene reduction (AR) rates by cyanobacteria epiphytic on a moss at Marion Island (46°54′ S, 37°45′ E) increased from −5°C to a maximum at 25 to 27°C. $Q_{10}$ values between 0 and 25°C were between 2.3 and 2.9, depending on photosynthetic photon flux density. AR rates declined sharply at temperatures above the optimum and were lower at 35°C than at 0°C. Photosynthetic photon flux density at low levels markedly influenced AR, and half of the maximum rate occurred at 84 μmol $m^{-2}$ $s^{-1}$, saturation occurring at ca. 1,000 μmol $m^{-2}$ $s^{-1}$. Higher photosynthetic photon flux density levels decreased AR rates. AR increased up to the highest sample moisture content investigated (3,405%), and the pH optimum was between 5.9 and 6.2. The addition of P, Co, and Mo, individually or together, depressed AR.**

Investigations conducted under the auspices of the Tundra Biome group of the International Biological Programme have demonstrated that cyanobacteria associated (epiphytically or endophytically) with bryophytes in subarctic and Arctic bogs and mires are significant agents of nitrogen fixation (2, 15). Recently, similar findings have been reported for subantarctic (35, 36) and Antarctic (12) sites. Studies of the factors influencing the rates of N fixation by cyanobacteria have been carried out at many of the Northern hemisphere subpolar areas (1, 3, 6, 21, 22, 24, 31). Where substrate pH is sufficiently high to allow cyanobacterial growth, moisture and temperature appear to be important factors influencing nitrogenase activity. A complex relationship between fixation and light intensity, which involves temperature and oxygen tension, has also been noted (6, 31). To date, only one investigation on the influence of abiotic factors (temperature and moisture) on N fixation (acetylene reduction) by moss-cyanobacteria associations at southern subpolar sites has been reported (12).

In April 1983, laboratory studies were carried out at Marion Island (46°54′ S, 37°45′ E) to ascertain the effects of temperature, light, moisture content, pH, and selected nutrients on acetylene reduction by cyanobacteria epiphytic on the moss *Brachythecium subplicatum* (Hamp.) Jaeg. This paper presents the results of these studies.

## MATERIALS AND METHODS

**Site description.** *B. subplicatum* forms a dense mat of vertically oriented stems in the lower-altitude mires and bogs, especially where there is pronounced lateral flow of water through the peat or at the peat surface. These "drainage lines" are less oligotrophic than the surrounding ombrogenous areas (26). *B. subplicatum* samples from a minerotrophic drainage line receiving runoff from an adjacent slope supporting burrowing petrels (*Procellariidae*) were previously shown to possess an abundance of epiphytic cyanobacteria, a large proportion of which were heterocystous (36). High rates of acetylene reduction were exhibited by these samples. Most of the investigations reported here on the influence of abiotic factors on acetylene reduction were performed on samples from this drainage line. Additional samples for the investigation into the relative reduction rates along the lengths of the moss stems were collected from a burrow entrance on the adjacent slope and from the base of the slope.

**Sample collection and handling.** For each of these investigations, moss-cyanobacteria samples were collected a day earlier by cutting the moss stems ca. 3 cm below the mat surface. A 10-$cm^2$ section of excised mat provided ca. 120 stems, which were separated and mixed. Six stems were placed in preweighed glass test tubes (1.3 [inside diameter] by 6 cm) which served as incubation tubes for the acetylene reduction (AR) assay. The stems were positioned longitudinally along one side of the tube, adhering in position because of their wetness. The tubes were kept in humidified trays at 4 to 8°C until needed for the AR assay, which was usually performed within 12 h. Variations of this sample treatment procedure for assessing AR at different positions on the moss frond and the influence of moisture content, pH, or nutrients were as follows.

**AR on different segments of moss fronds.** *B. subplicatum* fronds were collected from a burrow entrance on the slope (site 1), the base of the slope (site 2), and the drainage line in the mire below the slope (site 3). Starting at the tip, the fronds were cut into 1-cm segments. The top three segments of fronds from all sites were green, and segment 4 was yellow-green. Segment 5 of site 2 fronds was also yellow-green, but the segments from the mire and burrow entrance were brown and contaminated with peat. Segment 6 of fronds from the base of the slope and the drainage line was also heavily contaminated with peat, whereas that of fronds from the burrow entrance was almost completely decomposed and was discarded. Ten segments of each length class were placed in each of four incubation tubes for the AR assay. Separate segments from each site were examined microscopically for cyanobacteria, which were rated on a relative abundance scale of 0 (absent) to 5 (very abundant).

**Influence of sample moisture content.** A manifold was constructed by inserting one point of 21 double-sided blood-sampling needles along a length of latex tubing closed at one end and connected via a silica gel drying tube to an air pump. The free end of each needle was inserted into unstoppered, preweighed incubation tubes, each containing six *B. subplicatum* stems. A further tube containing moss stems was weighed and sealed with a serum stopper. Dry air at 16 to 18°C was distributed by the manifold through the 21 tubes.

At approximately half-hour intervals, a tube was removed from the manifold, weighed, and stoppered. The stoppered tubes were kept next to the manifold under light and temperature conditions identical to those for the tubes on the manifold. In this manner, a range of moisture contents (expressed per unit [dry weight] of sample) was attained. After the last sample had been removed from the manifold, they were all assayed for AR.

**Influence of pH.** Eleven solutions ranging in pH from 3.4 to 10.0 were made up by adding dilute HCl or dilute NaOH to a solution of 0.2 mmol of $NaHCO_3$ per liter. The top 3 cm of ca. 50 *B. subplicatum* stems was soaked in 50 ml of each solution for 12 h in the dark and then was placed in incubation tubes (eight stems per tube, five replicates). The remaining stems were discarded, and the pHs of the solutions were remeasured. This pH value was taken to be that of the medium surrounding the cyanobacteria on the moss samples. The samples were assessed for AR as described below, except that the preincubation period was shortened to 5 min and the incubation was shortened to 1 h.

**Influence of phosphate P, cobalt, and molybdenum.** Approximately 50 *B. subplicatum* stem segments were placed in solutions (10 ml) containing either Co (10 ng $ml^{-1}$, as $CoCl_2$), Mo (150 ng $ml^{-1}$, as $Na_2MoO_4$), P (2.5 μg $ml^{-1}$, as $KH_2PO_4$), or a mixture of these three elements. A further 50 stem segments were placed in distilled water. The pH of this water and of all of the nutrient solutions had been adjusted to pH 6 before the samples were added. After 20 min, the stem segments for each solution were placed in preweighed incubation tubes (six fronds per tube, five replicates), which were then reweighed and placed in a humidified tray at 4°C (photosynthetic photon flux density [PPFD], 30 to 50 μmol $m^{-2}$ $s^{-1}$) for 3 h before incubating for AR. After the incubation, the samples were dried and weighed. Externally held water accounts for 43 to 54% (mean, 49%) of the total moisture content of saturated *B. subplicatum* fronds (S. Russell, personal communication), so that the amount of each nutrient added per dry weight of sample was assumed to be approximately equal to half of the estimated water content of the sample multiplied by the concentration of the nutrient in the solution in which the sample was soaked.

**$C_2H_2$ incubation procedure.** The incubation tubes were sealed with serum stoppers and positioned, moss side uppermost, in racks which were placed in water baths so that the moss fronds lay horizontally 3 to 4 mm below the coolant (water or ethylene glycol-water mixture) surface. After 30 min of preincubation at the temperature and radiation levels required for the particular incubation, 10% of the tube volume was replaced with $C_2H_2$ which had been scrubbed twice through concentrated $H_2SO_4$ and once through water. After 3 h, the tubes were removed and shaken vigorously for 15 s on a vortex mixer, and the headspace was subsampled into 3-ml blood-sampling tubes (Venoject; Terumo Corp., Tokyo, Japan, re-evacuated to a consistent vacuum). The samples were then dried at 105°C for 48 h and weighed.

Endogenous ethylene production (assessed for each treatment by incubating samples without $C_2H_2$) was never observed. $C_2H_4$ contamination of the scrubbed $C_2H_2$ was checked before each series of incubations. After 10 to 12 days of storage (in triple-walled 5-liter plastic bags used commercially as wine containers), low levels of $C_2H_4$ occurred in the $C_2H_2$, in which case a fresh batch was prepared.

**Ethylene detection.** $C_2H_4$ in the incubation atmospheres were determined within 6 h of subsampling by gas chromatography on Poropak N. $N_2$ was used as the carrier gas, and detection was by flame ionization. $C_2H_4$ concentrations were reported directly by an HP 3390A integrating reporter connected to the gas chromatograph. Acetylene reduction rates were expressed per gram [dry weight] of moss-cyanobacteria sample.

**Control and measurement of light and temperature during incubations.** A Philips 400-W SON high-pressure sodium vapor discharge lamp (S. A. Philips [Pty] Ltd., Newville, South Africa) illuminated the water baths from above, and one or more layers of grey shade-netting were placed over individual racks of tubes to provide a range of radiation levels. Finer control of light intensity was possible by moving the racks horizontally in the water baths or by moving the lamp vertically, or both.

PPFD was measured with a Li-Cor 190SB quantum sensor connected to an Li-188B integrating meter (both from Li-Cor, Inc., Lincoln, Nebr.). An incubation tube was cut longitudinally in half, and one side was sealed over the white central disc of the quantum sensor, which was then placed in the water baths to measure radiation. By using the shade netting and vertical positioning of the lamp, PPFD in the incubation tubes could be varied up to 2,000 μmol $m^{-2}$ $s^{-1}$.

The water baths were fitted with temperature controls and pumps capable of maintaining a ±10 cm $s^{-1}$ flow of coolant past the incubation tubes. Temperature in the tubes was measured by using NiCr-NiAl thermocouples connected to a KM3013 digital meter with built-in reference junction (Kane-May Ltd., Hertfordshire, England). Even under high radiation loads, the temperature in the tubes did not differ from that of the coolant liquid by more than 0.3°C.

**Statistical evaluation of the data.** Significances reported in the text for the differences in mean AR rates between sample treatments were established by student's *t* tests, and the significances of the various correlation coefficients were established by *t* tests of their Fischer-*Z* transformations. The significance of the differences in activation energies calculated from the Arrhenius plots of temperature versus AR at various PPFD levels was tested by covariance analysis in which the null hypothesis stated that the slopes of the Arrhenius curves did not differ across PPFD.

## RESULTS

**Influence of $CO_2$ and $HCO_3^-$ on AR rates.** $CO_2$ depletion in the incubation tubes under conditions of adequate light and warm temperatures might be expected to affect AR through its influence on cyanobacterial photosynthesis. Either 0.5 ml of water, 0.5 ml of $NaHCO_3$ (0.2 mmol liter $^{-1}$), or 0.5 ml of water plus 5% $CO_2$ was added to six replicated subsamples of moss, and these were assayed for AR at 20°C at a PPFD of 200 μmol $m^{-2}$ $s^{-1}$. Reduction rates for the water- and the $HCO_3$-treated fronds were similar, but enhanced $CO_2$ concentrations in the incubation tubes significantly ($P = 0.001$) depressed AR.

**AR on different segments of moss fronds.** AR rates at different positions on the moss fronds are shown in Fig. 1. The rates (nmol of $C_2H_2$ reduced g [dry weight]$^{-1}$ $h^{-1}$) are expressed as a percentage of the maximum rate recorded along the frond. There were no conspicuous differences in the pH or moisture content of the segments either within or across sites, except that the lowermost (segments 5 and 6) portions of the fronds were slightly (0.2 to 0.3 pH units) more acidic. The pattern of AR along the moss fronds varied according to site. Maximum rates were significantly ($P = 0.05$) different between sites and decreased in the order site 1 > site 2 > site 3. The highest AR rates in samples from the burrow entrance occurred in the top 1 cm, and rates de-

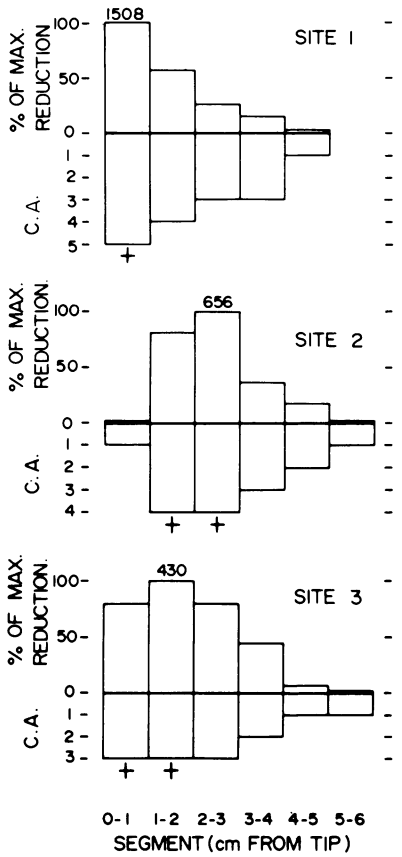


FIG. 1. AR for different segments of moss fronds from three sites: 1, entrance to bird burrow on slope; 2, base of slope; 3, minerotrophic drainage line in mire below slope. Values are expressed as a percentage of the maximum rate (nanomoles of $C_2H_2$ reduced per gram per hour) found for the frond; these are indicated above the 100% bar for each site. Cyanobacterial abundances (scale 0 to 5) are indicated by bars below the abscissa. Plus signs indicate that a large proportion of the cyanobacteria was heterocystous.

creased down the frond. For moss stems from the foot of the slope, maximum reduction rates occurred 2 to 3 cm from the apex. Samples collected from the mire drainage line showed the highest reduction rates 1 to 2 cm from the apex; however, the value for these segments was not significantly ($P \leq 0.05$) different from those found for the 0 to 1 and 2 to 3 cm classes.

Cyanobacterial abundance also varied along the length of the fronds in a pattern which depended on the collection site (Fig. 1) and correlated well with the observed AR values, accounting for >90% ($P = 0.05$) of the variation in activity within sites and 47% ($P = 0.01$) of the total variation between segments and across sites.

The possibility of AR by heterotrophic bacteria in the peat contaminating the lower stem segments cannot be discounted since darkened control incubations were not performed. However, heterotrophic AR in the mire peats has only been demonstrated for samples which have been fortified with a readily oxidizable, energy-rich substrate such as glucose (V. R. Smith, Proceedings of the Fourth Scientific Committee on Antarctic Research Symposium on Antarctic Biology, in press).

The estimation of cyanobacterial abundance was very tedious and could not be performed on the large number of incubation samples required for the investigations into the effects of abiotic parameters on $C_2H_2$ reduction. Because of the uniform reduction rates exhibited by the top 3 cm of fronds from the mire drainage line, samples from this site were used in these investigations.

**Influence of temperature on AR.** The effect of temperature on AR rates was investigated at three PPFD levels (Fig. 2). AR increased markedly between 0 and 25°C, the increases being accurately described by Arrhenius equations at all three PPFD levels. $Q_{10}$ values between 0 and 25°C varied from 2.33 to 2.56 (100 μmol $m^{-2}$ $s^{-1}$), 2.51 to 2.78 (200 μmol $m^{-2}$ $s^{-1}$), and 2.62 to 2.91 (400 μmol $m^{-2}$ $s^{-1}$); hence, the effect of temperature on $C_2H_2$ reduction was influenced by radiation levels. "Activation energies" calculated between 0 and 25°C from the Arrhenius equations increased with PPFD (60.3 kJ $mol^{-1}$ at 100 μmol $m^{-2}$ $s^{-1}$; 65.7 kJ $mol^{-1}$ at 200 μmol $m^{-2}$ $s^{-1}$; 68.6 kJ $mol^{-1}$ at 400 μmol $m^{-2}$ $s^{-1}$). Covariance analysis of the slopes of the Arrhenius plots indicated that these differences were highly significant ($F = 34.4$; $P < 0.001$).

The influence of subzero temperatures on AR was only investigated at a PPFD level of 200 μmol $m^{-2}$ $s^{-1}$. The rate at −1°C fitted the Arrhenius curve derived from the data for 0 to 25°C (inset to Fig. 2), but below this temperature the observed reduction rates declined much more rapidly than predicted by the curve. Low but significant AR rates (28 nmol $g^{-1}$ $h^{-1}$) occurred at −3°C, but at −5°C, only 3 of the 10 replicated incubations exhibited ethylene production.

The optimum temperature for AR appeared to be between 25 and 27°C. At all three PPFD levels, a single exponential equation described the decline in rates above the temperature optimum, and the observed (and calculated) rates at 35°C were significantly ($P = 0.001$) lower than at 0°C.

**Influence of radiation on AR.** Michaelis-Menten kinetics adequately described the relationship between AR and PPFD levels between 12 and 1,200 μmol $m^{-2}$ $s^{-1}$ (Fig. 3a). The equation $AR = (AR_{max} \times PPFD)/(K + PPFD)$ accounted for 98% of the variation in the AR data shown in Fig. 3a.

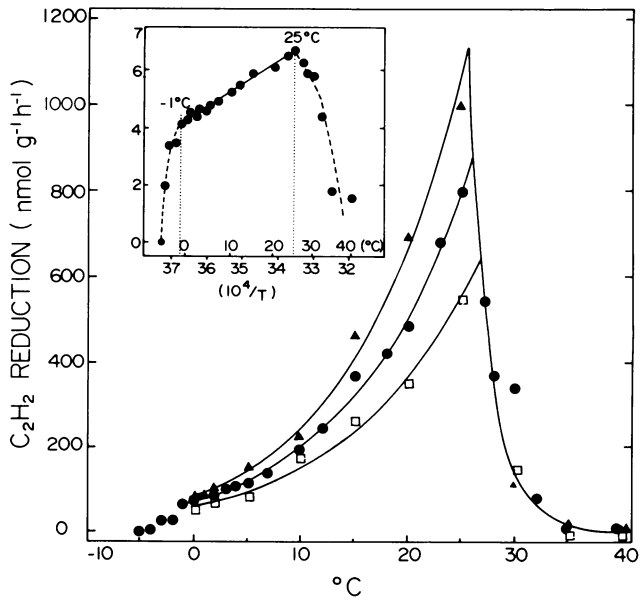


FIG. 2. Relationship between AR and temperature at 100 (□), 200 (●), and 400 (▲) μmol $m^{-2}$ $s^{-1}$ (PPFD). Each data point is the mean of 10 replicates. Inset: Arrhenius plot of log AR versus $10^4/T$ at 200 μmol $m^{-2}$ $s^{-1}$.

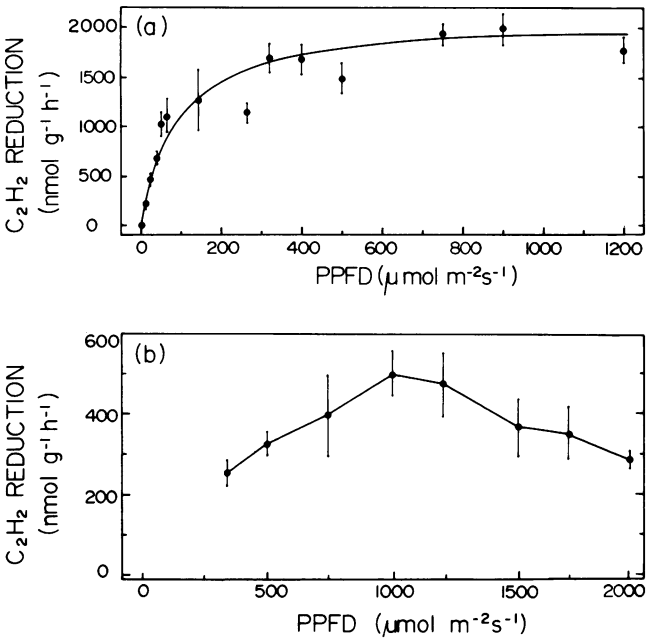


FIG. 3. Relationship between AR at 20°C and PPFD between (a) 0 and 1,200 μmol $m^{-2}$ $s^{-1}$ and (b) 350 and 1,950 μmol $m^{-2}$ $s^{-1}$. Vertical bars indicate ± one standard error of the mean ($n$ = 5).

The value of $K$ (the PPFD at which half of the maximum AR rate occurred) was 84 μmol $m^{-2}$ $s^{-1}$, indicating that AR by the moss-associated cyanobacteria responds markedly to light at low levels.

At 20°C, the lowest PPFD at which AR occurred was found empirically to be between 3 and 12 μmol $m^{-2}$ $s^{-1}$. At low PPFD levels (<150 μmol $m^{-2}$ $s^{-1}$), a logarithmic relationship fit the AR data well ($r$ = 0.970, $P$ = 0.01) and predicted zero AR at 7 μmol $m^{-2}$ $s^{-1}$ and less. The data in Fig. 3a suggest that saturation occurred at ca. 900 μmol $m^{-2}$ $s^{-1}$, and there was some indication that AR was inhibited at higher radiation levels, although the decrease in rates between 900 and 1,200 μmol $m^{-2}$ $s^{-1}$ was not significant at $P \leq 0.05$. Another series of incubations was therefore carried out (Fig. 3b), in which the radiation levels were varied between PPFD 350 and 1,950 μmol $m^{-2}$ $s^{-1}$. Depression of AR at PPFD > 1,000 μmol $m^{-2}$ $s^{-1}$ was clearly demonstrated, so that rates at 1,950 μmol $m^{-2}$ $s^{-1}$ were significantly ($P$ = 0.01) lower than at 1,000 μmol $m^{-2}$ $s^{-1}$.

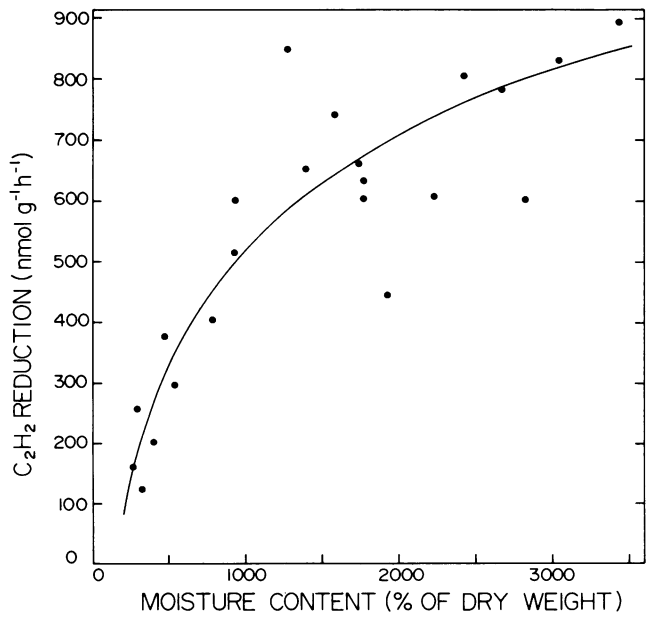


FIG. 4. Relationship between sample moisture content and AR reduction at 25°C and 200 μmol $m^{-2}$ $s^{-1}$.

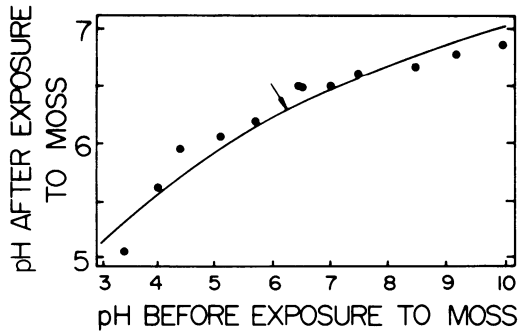


FIG. 5. Influence of *B. subplicatum* fronds on pH of solutions adjusted to a range of initial pH values.

**Influence of sample moisture content on AR.** AR increased markedly between the lowest sample moisture content tested (260%) and the field moisture value (3,405%) (Fig. 4), the relationship being AR = 257 log($H_2O$%) − 1249 ($n$ = 22, $r$ = 0.838, $P$ = 0.001) and predicting zero reduction at ≤130% moisture. This value was not tested empirically; however, moisture contents of moss samples from mire habitats rarely fall below 800%.

**Influence of pH on AR.** The moss fronds were capable of altering the pHs of the solutions to a substantial degree (Fig. 5) so that the final pH values were 5.0 to 6.9. The pH at the surface of the *B. subplicatum* fronds was apparently 6.3 (arrowed on the curve in Fig. 5), the value at which the moss samples had no effect on the original solution and which is in close agreement with those (6.2 to 6.3) measured directly on saturated mats of this species in the field.

AR increased markedly between pH 5 and 6 (Fig. 6), optimum fixation occurring between pH 5.9 and 6.2. Reduction rates then declined with increasing pH.

**Influence of P, Co, and Mo on AR.** The addition of P, Co, and Mo, either individually or together, depressed AR compared with frond samples which had been treated with water only (Table 1).

## DISCUSSION

The differences in AR rates for different parts of the moss stems could not be ascribed to variations in light, tempera-

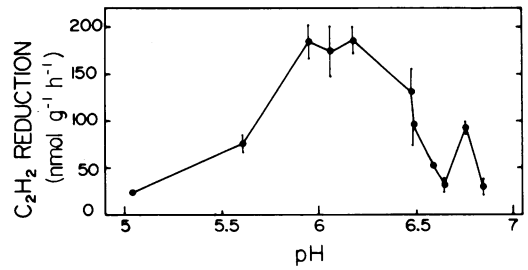


FIG. 6. Relationship between pH and AR at 25°C and 200 μmol $m^{-2}$ $s^{-1}$. Vertical bars indicate ± one standard error of the mean.

TABLE 1. Effects of P, Co, and Mo on $C_2H_2$ reduction by cyanobacteria epiphytic on *B. subplicatum* from a minerotrophic mire[a]

| Element(s) added | Approx amt added (ng mg of sample$^{-1}$) | | | $C_2H_2$ reduction (nmol g$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|
| | P | Co | Mo | |
| None (water only) | | | | 1,335 ± 302 |
| P | 59 | | | 680 ± 174 |
| Co | | 0.25 | | 902 ± 111 |
| Mo | | | 3.6 | 780 ± 17 |
| Co, Mo | | 0.25 | 3.7 | 813 ± 173 |
| P, Co, Mo | 60 | 0.25 | 3.6 | 946 ± 43 |

[a] Incubations were done at 25°C and 200 μmol m$^{-2}$ s$^{-1}$.

ture, pH, or moisture content, but they closely reflected the relative densities of the colonizing cyanobacteria. Field studies on a tundra mire at Stordalen (Sweden) also showed marked variations in AR along *Sphagnum riparium* and *Drepanocladus exannulatus* thalli which were caused by uneven colonization by cyanobacteria rather than abiotic factors such as light and moisture content (6, 22).

Marion Island experiences a typical subantarctic oceanic climate characterized by moderately low temperate throughout the year; the mean air temperatures in the warmest and coldest months are 7.3 and 3.2°C, respectively, and diurnal temperature variations are also small. Rain falls at the island on more than 300 days per year (34), and periods of drought lasting more than 3 or 4 days are uncommon. Even during these dry periods, the bryophyte layer in the mires remains wet due to its high water-holding capacity and upward capillary movement of water from the waterlogged peat substratum. At many subpolar and cold temperate sites moisture is an important factor determining the distribution of cyanobacteria (14, 28, 32, 33), and N fixation activity in free-living and lichenized forms depends on thallus moisture content, often up to high saturation values (3, 4, 11, 36). AR by moss-*Nostoc* associations at the Vestfold Hills in Antarctica was appreciably inhibited by partial desiccation over a range of temperatures (−3.5 to 25°C) (12). Field moisture contents of *B. subplicatum* at Marion Island are generally above 1,000%, the lowest recorded value being 570% (S. Russell, personal communication). It is therefore unlikely that desiccation ever completely inhibits N fixation by the moss-cyanobacteria associations, but since AR responded to moisture content up to high values (ca. 3,400%) (Fig. 4), fluctuations in moisture content possibly influence N fixation rates in the field.

N-fixing cyanobacteria are rarely found at substratum pH values below 5 (2, 14, 19, 20), and nitrogenase activity in these organisms is also strongly pH dependent (21). *Nostoc commune* at Signy Island, although able to fix N at pH 6.6, showed maximum rates at sites with pH values of 8.7 to 9 (18, 28). Laboratory studies of cyanobacteria associated with *D. exannulatus* at Stordalen demonstrated a very pronounced AR optimum at pH 6.8 (22). However, some investigations have shown little correlation between in situ N fixation and field pH (6). In the present study, *B. subplicatum* fronds demonstrated a marked ability to buffer the external medium to a narrow pH range, probably due to a high cation exchange capacity at the moss surface, but alkaline leaf exudates may also contribute to an elevated pH in the phyllosphere (9). The optimum pH range for AR was similar to the pH of the moss fronds in the field.

Light and temperature profiles in the *B. subplicatum* stratum and the uppermost peat layer are shown for a sunny day in May in Fig. 7. PPFD and irradiance were rapidly attenuated with depth by the moss fronds. The air temperature at the time of measurement was 9.1°C, and the temperature decreased markedly within the first 2 cm of the moss layer, at which depth the water table occurred. Temperature then decreased only slowly with depth in the waterlogged layer. Freezing of the moss layer is infrequent and of short duration, occurring only in the upper 2 to 3 cm. The temperature in this layer is generally between 4 and 10°C, the maximum recorded being 12°C (S. Russell, personal communication).

Nitrogen fixation is usually considered to be more sensitive to variations and extremes of temperature than are other physiological processes such as photosynthesis, respiration, or overall growth (10, 30, 37, 39). N-fixing organisms are considered to be mesophilic (21) and generally not able to fix N effectively below 0°C (2). However, many studies at subpolar sites have demonstrated appreciable fixation at low temperatures down to 0°C (4, 5, 12, 16, 23, 28) and even lower (17, 31). Active $CO_2$ fixation by *N. commune* in Antarctica occurs at −5°C (8), and it has been suggested (12) that this low-temperature photosynthesis enables nitrogenase activity to proceed until it is inhibited by complete cellular freezing. Recently, N fixation by this species has been recorded at −7°C (13). The lack of liquid water below 0°C probably limits N fixation, rather than low temperature inactivation of nitrogenase (28). In the study reported here, freezing possibly accounted for a greater decline in AR rates below −1°C than predicted from the curve in Fig. 2. At −1°C, moisture may not have been frozen on the moss fronds due to capillarity; in addition, osmotic and matric potentials would depress the freezing point of the cyanobacterial cytoplasm.

The optimum temperature for AR in this study was 25 to 27°C, higher than those (15 to 20°C) generally reported for moss- or lichen-associated cyanobacteria from Northern

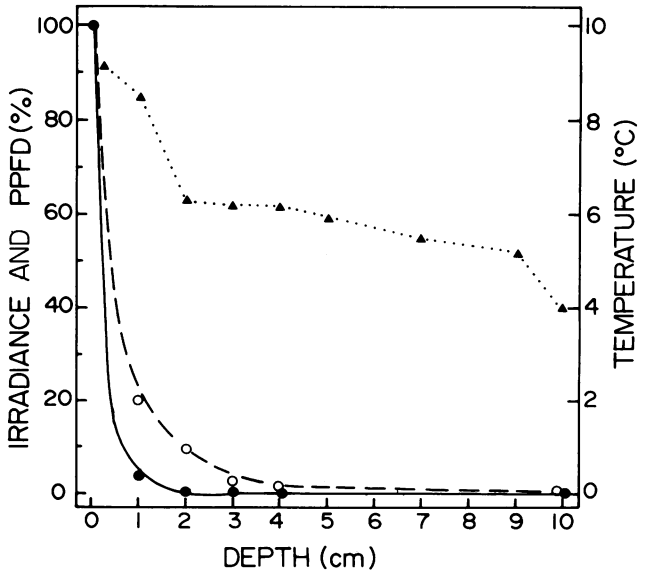


FIG. 7. Temperature (▲), PPFD (●), and irradiance (○) profiles through a mat of *B. subplicatum* in the field. PPFD and irradiance levels are expressed as a percentage of the levels incident on the top of the moss layer (1,700 μmol m$^{-2}$ s$^{-1}$ and 1,065 W m$^{-2}$, respectively).

hemisphere tundra mires and wet meadows (3, 17, 20) but similar to the value (25°C) found for *Nostoc* isolated from moss leaves at Devon Island, Northwest Territories, Canada (29). $Q_{10}$ values (2.3 to 2.9) reported here for AR were lower than those (4 to 6) found for N-fixing organisms from cold subpolar sites and were more similar to those (ca. 3) for organisms from more temperate areas (21). The relatively high temperature optima and low $Q_{10}$ values found here for cyanobacterial AR agree with similar observations on the specific growth rates of algae and bacteria at Marion Island (27; H. J. Lindeboom, unpublished Ph.D. thesis).

Light is generally considered to be an important factor limiting N fixation in moss-algae communities in cold temperate and subarctic regions (2, 22, 25). In the studies reported here, PPFDs incident on the moss-cyanobacterial samples as a whole were measured. The actual values experienced by the cyanobacterial cells were probably only a fraction of the measured values due to shading by the moss tissue and surrounding mucilage. A single horizontal layer of *B. subplicatum* fronds attenuated the PPFD by 60 to 70%, and only 3.5% of the PPFD (and 20% of the irradiance) penetrated the moss layer in situ to a depth of 1 cm (Fig. 7). It is therefore unlikely that saturating PPFD values for AR (e.g., 1,000 μmol $m^{-2}$ $s^{-1}$) (Fig. 3b) are experienced in situ, especially since cloudy weather occurs at the island more than 90% of the time and PPFD levels are generally 200 to 400 μmol $m^{-2}$ $s^{-1}$ (7). The moss-associated cyanobacteria will, therefore, normally experience illumination at levels at which N fixation is strongly dependent on light intensity (lower part of the curve in Fig. 3a).

The observation that the addition of solutions containing P depressed AR levels compared with the addition of an equal amount of distilled water agrees with results from similar studies on *N. commune* at Barrow, Alaska (3). However, at Stordalen, in situ AR by *S. riparium*-associated cyanobacteria correlated positively with added $PO_4$ levels (6). Phosphorus deficiency generally affects N fixation before it influences general cell growth (21) and, although the peat solution in the study mire did not contain detectable levels of $PO_4$ P (>0.002 μg $ml^{-1}$), nutrients leached from the adjacent slope contribute to the minerotrophic status of the mire. Cyanobacteria are able to accumulate and store P in the form of polyphosphate (21, 38). It is therefore unlikely that the samples collected from this site were nutrient-limited (especially regarding P), which might explain the lack of an AR response to supplied nutrients. The influence of phosphate P on AR by cyanobacteria epiphytic on *Brachythecium rutabulum* (Hedw.) B.S.G. from an oligotrophic mire was examined by soaking fronds in either water or a 10-μg/ml P solution (pH 6) for 12 h, rinsing, and incubating with $C_2H_2$. Very low reduction values were recorded for these samples, and although P-treated replicates showed higher rates than the water-treated replicates (23 ± 12 and 13 ± 3 nmol $g^{-1}$ $h^{-1}$, respectively; $n = 4$), the difference was not significant ($P \leq 0.05$). Very few cyanobacteria occurred in these *B. rutabulum* samples. It is likely that nutrient enrichment allows a denser colonization of cyanobacteria on mosses at minerotrophic sites and that this is associated with higher N fixation rates.

From the results reported here, and considering the general climatic regime at Marion Island and light attenuation within the moss layer, light appears to be the main factor influencing N fixation by moss-associated cyanobacteria. Although marked AR responses to temperature, water content, and pH occurred under laboratory conditions, several properties of the bryophyte layer allow for only small fluctuations of these parameters in the field. The high water-holding capacity of the mosses reduces desiccation, and the cation exchange capacity at the moss surface results in pH buffering and concentration of nutrients in the phyllosphere. The magnitude of temperature fluctuations is also smaller in the moss layer than on bare rock or peat. Reduced $pO_2$ levels have also generally been shown to stimulate nitrogenase activity (2, 3, 21). AR response to lower $O_2$ concentrations could not be examined in this investigation since a $O_2$ absorber was not available. Photosynthetically produced $O_2$ would quickly alter the composition of anaerobic gas mixtures added at the start of the incubation. However, microsites with reduced $pO_2$ tensions may exist in the moss layer in the field and would be of considerable importance in allowing higher rates of N fixation.

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