CHAPTER 8-3
NUTRIENT RELATIONS: NITROGEN

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Figure 1. ***Physcomitrella patens*** growing on previously flooded soil. Note the nitrogen-fixing blue-green bacterium, ***Nostoc***, at the **arrow**. Photo by Michael Lüth, with permission.

N Forms

Nitrogen is available in many forms. The most abundant of these, N_2 gas, cannot be used by plants or animals and must be converted by **Cyanobacteria** or bacteria before plants can use it. Animals can only obtain it by eating other organisms that have already placed the N into amino acids. Other forms of N that plants can absorb include **ammonium** (NH_4^+), **nitrite** (NO_2^-), **nitrate** (NO_3^-), and organic forms such as **amino acids** and **urea**. As we shall soon see, not all bryophytes have the same ability to use these forms and some are toxic to most taxa.

Nitrate and Ammonium

Plants, including bryophytes, can take in and use both NO_3^- (nitrate) and NH_4^+ (ammonium). The form of nitrogen needed by bryophytes varies with species and habitat. Aquatic higher plants use nitrogen in three inorganic forms: NO_2^- (nitrite) (Schwoerbel & Tillmanns 1964, 1977), NO_3^-, NH_4^+ (Schwoerbel & Tillmanns 1972; Rudolph & Voigt 1986). Bryophytes usually absorb NH_4^+ more easily than they absorb NO_3^- (Schwoerbel & Tillmanns 1974; Simola 1975; Miyazaki & Satake 1985; Schuurkes *et al*. 1986). Cation vs anion exchange sites may determine the use of nitrate (anion) vs ammonium (cation), causing ***Sphagnum*** to have a strong preference for ammonium because of its extensive cation exchange sites (Wanek & Pörtl 2008).

Vanderpoorten (2000) reported that NH_4^+ N is one of the best factors to explain differences in aquatic ***Amblystegium*** (Figure 2) distributions in river systems. Frahm (1975) found that the brook moss ***Fontinalis antipyretica*** var. ***gigantea*** (Figure 3) had a low tolerance for NH_4^+, but Schwoerbel and Tillmanns (1974, 1977) found conflicting evidence showing that this species uses NO_3^- and NH_4^+, with NH_4^+ being taken up first if provided together with NO_3^-. In fact, it is unable to uptake NO_3^- in the dark (Schwoerbel & Tillmanns 1974). To show the complexity of the N relationships, growth on a nitrate medium requires the bryophytes to convert it to ammonium ions before they can assimilate it (Brown 1982). It is possible that various strains have developed within species that have different tolerance levels for some of their nutrients.

Figure 2. ***Amblystegium fluviatile***, an aquatic moss sensitive to ammonium levels. Photo by Michael Lüth, with permission.

Figure 3. ***Fontinalis antipyretica*** var. ***gigantea*** dry, a moss with mixed responses to ammonium as its nitrogen source. Photo by Janice Glime.

Physiology of Nitrate and Ammonium

Assuming that bryophytes operate as do **tracheophytes** (lignified vascular plants), NO_3^-, once in the plant, is converted to NH_4^+. In leaves, the intermediate product, NO_2^-, is reduced by **nitrite reductase** (enzyme that facilitates addition of hydrogen and loss of oxygen from NO_2^- during photosynthetic electron transport process). No intermediate product is released and the final product is NH_4^+. Since photosynthesis provides the **NADH** (nicotinamide adenine dinucleotide + H, the active coenzyme form of vitamin B_3) and **ferredoxin** needed for conversion of nitrogen oxides to NH_4^+, the conversion process is enhanced by the same things that enhance photosynthesis – high light and warm temperatures (Salisbury & Ross 1978). Thus, more ammonium is produced.

		nitrite reductase		
NO_3^-	→	**NO_2^-**	→	**NH_4^+**
nitrate		nitrite		ammonium

Morphological Anomalies

Brown (1982) suggested that the *p*H or alkalinity affects availability of N for plants, with NO_3^- being more available in neutral or alkaline soils and NH_4^+ in acidic soils and water. But NH_4^+ is usually toxic to plants in any appreciable quantity. Sironval (1947) found that NH_4^+ ions caused degeneration of the **caulonema** (part of protonema from which buds arise) of ***Funaria hygrometrica*** (Figure 4) and Southorn (1977) found they caused morphological abnormalities in the same species. Killian (1923) likewise found morphological abnormalities in the leafy liverwort ***Scapania*** (Figure 5). On the other hand, Burkholder (1959) found that cultured bryophytes did equally well on both NO_3^- and NH_4^+ salts.

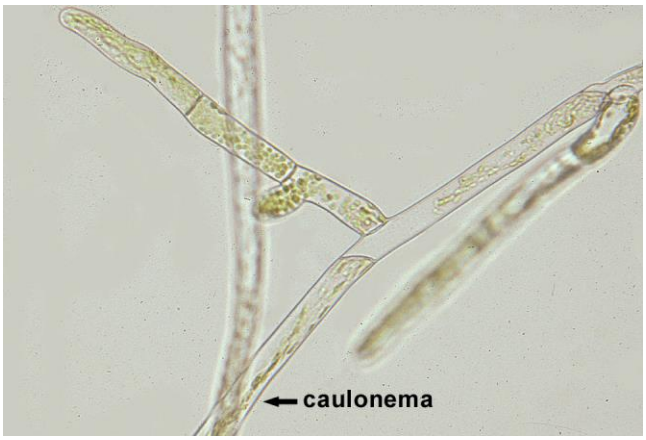

Figure 4. ***Funaria hygrometrica*** protonema showing caulonema, a stage that seems to degenerate when ammonium ions are added. Photo by Janice Glime.

Figure 5. ***Scapania undulata***, an aquatic leafy liverwort that exhibits morphological abnormalities when ammonium concentrations are too high. Photo by David T. Holyoak, with permission.

An interesting consequence of *p*H differences was suggested by Machlis (1962). In ***Sphaerocarpos texanus*** (Figure 6), male plants are smaller than females in the field. Machlis attributed this to the ability of male plants to absorb NH_4^+ ions more readily than females, causing them to have a lower *p*H, which could suppress growth. He supported this suggestion by growing the plants on potassium, which caused no *p*H change, and likewise no reduction in the size of male plants.

Figure 6. ***Sphaerocarpos texanus***, a thallose liverwort in which male plants absorb NH_4^+ ions more readily than do females, causing males to have a lower *p*H, possibly accounting for growth suppression and smaller males. Photo by Martin Hutten, with permission.

Benefit or Detriment?

In a study designed to determine the effects of various forms of N on bryophyte function, Alghamdi (2003) studied the popular, fast-growing aquarium moss ***Taxiphyllum barbieri*** (Java moss, Figure 7). He found that the benefit to the moss depends on what parameter you measure (Figure 8). For example, dry biomass increase was greatest in high NO_3^- concentrations (30 mg L^{-1} N), whereas the greatest increase in length occurred in high NH_4^+ concentrations (30 mg L^{-1} N). This difference resulted in the least biomass increase per stem length in high NH_4^+ concentrations, despite the relatively high increase in length in that treatment. The overall appearance of the mosses in high NH_4^+, then, was to appear long and thin compared to those in other treatments, but not dissimilar to the plants in the control (standard nutrient solution but with no N source). Based on the lower growth in the NH_4NO_3 media, Alghamdi reasoned that in the presence of NH_4^+, the NO_3^- became unusable because of the inhibition of nitrate reductase by NH_4^+ (see Syrett & Morris 1963; Orebamjo & Stewart 1975). At the same time, the lower concentration of NH_4^+ (15 mg L^{-1} N) in combination compared to NH_4^+ alone (30 mg L^{-1} N) reduced the growth. This relationship was consistent with much greater growth at 30 mg L^{-1} N than at 10 mg L^{-1} N as NH_4^+ (Figure 8).

Figure 7. ***Taxiphyllum barbieri***, an aquarium moss subjected to high ammonia concentrations from fish waste products. Photo by Tan Sze Wei, Aquamoss website <www.aquamoss.net>, with permission.

NO_2^- caused only modest improvements in biomass and length over N-free controls (Figure 8), but caused considerable increase in chlorophyll *a* (Alghamdi 2003; Figure 10). The chlorophyll *a*:*b* ratio was highest in the high NO_3^- treatment, due to mosses in that treatment having the least chlorophyll *b* per biomass of moss, a concentration even lower than that of controls (Figure 10). In fact, effects of inorganic N form on chlorophyll *b* resulted in either no improvement over N-free controls, or depressed levels of chlorophyll *b*. However, chlorophyll *a* was higher in nearly all nitrogen treatments than in controls. Baxter *et al*. (1992) found a similar but slight decrease in total chlorophyll concentration in ***Sphagnum cuspidatum*** (Figure 9), typically a submersed species, with increasing levels of NH_4^+, but in Alghamdi's experiments, ***Taxiphyllum barbieri*** (Figure 7) actually had total chlorophyll increase, although not statistically significant, with increase from 1 to 30 mg L^{-1} N as NH_4^+ (Figure 10).

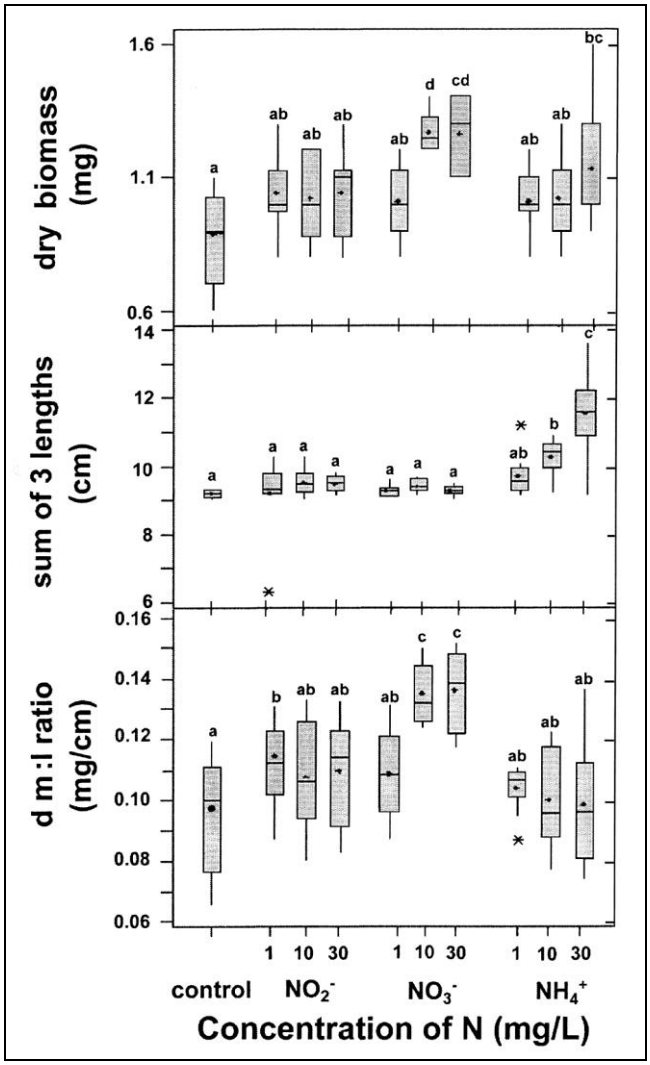

Figure 8. Effects of various forms of inorganic N (control = no N) on growth in length (l) and biomass (d m) of ***Taxiphyllum barbieri***. Box mean (dot) and median (horizontal line); bottom of box is first quartile and top is third quartile. Whiskers represent lowest and highest observations still inside region defined by lower limit Q1-1.5 (Q3-Q1) and upper limit Q3+1.5 (Q3-Q1); *represents outliers that extend beyond whiskers; n=15 sets of 3 stems. Means with same letters are not significantly different from each other (DNMRT, $\alpha = 0.05$). Based on Alghamdi 2003.

Figure 9. ***Sphagnum cuspidatum***, an aquatic species that has a decrease in chlorophyll with an increase in ammonium ions. Photo by Jonathan Sleath, with permission.

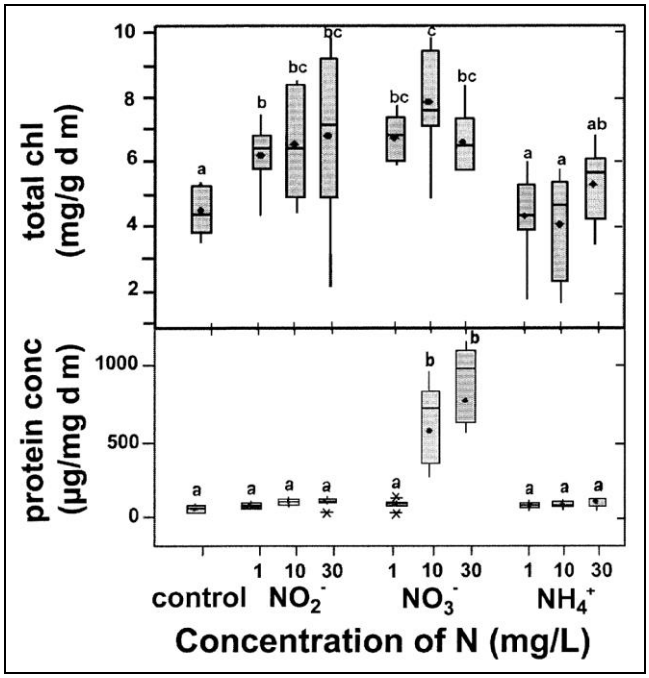

Figure 10. Effects of various forms of inorganic N (control = no N, NO_2^- = nitrite, NO_3^- = nitrate, NH_4^+ = ammonium) on chlorophyll *a* and protein concentrations of ***Taxiphyllum barbieri***. Notation as in Figure 8; n = 15 sets of 3 stems. Based on Alghamdi 2003.

Protein concentrations in ***Taxiphyllum barbieri*** (Figure 7) showed a very different picture from other measurements, with little difference among treatments except at 10 and 30 mg L^{-1} NO_3^- (Figure 10; Alghamdi 2003). In ***Sphagnum cuspidatum*** (Figure 9) Baxter *et al*. (1992) found the addition of NH_4^+ (as NH_4Cl) generally caused an increase in amino acids, at least within the first 15 days, in both locations studied, with arginine increasing the most at the unpolluted site and actually decreasing at the NH_4^+-polluted site. The latter study suggests that ***Sphagnum cuspidatum*** may acclimate to a higher level of NH_4^+ in a way that it eventually requires higher levels than populations not continuously exposed to such high levels. Clearly the uses of the various forms of N in bryophytes are complex and one cannot give a simple answer as to which form is best.

Species Differences

In ***Sphagnum*** (Figure 18, Figure 33-Figure 23), differences exist among the species. ***S. flexuosum*** (Figure 11) is apparently unable to utilize NO_3^- (Schuurkes *et al*. 1986), and Touffet (1971) found that NO_3^- actually reduced the growth of ***Sphagnum*** and was less effectively utilized than NH_4^+ when it was the only N resource. Nevertheless, in many ***Sphagnum*** species nitrate reductase, an inducible enzyme (Deising 1987), permits use of NO_3^-. High levels of NH_4^+ inhibit nitrate reductase, and hence reduce growth, by inhibiting NO_3^- uptake (Rudolph *et al*. 1987). Rudolph and Voigt (1986) demonstrated that 322 µM was a favorable concentration of NO_3^- in ***S. magellanicum*** (Figure 12), whereas at 225 µM NH_4^+ the chlorophyll content decreased. At 600 µM NH_4^+, nitrate reductase activity was reduced by as much as 20%. These factors most likely limit mosses in particular habitats.

Figure 11. ***Sphagnum flexuosum***, a species that is unable to use nitrate. Photo by Michael Lüth, with permission.

Figure 12. ***Sphagnum magellanicum***, a species that benefits from added nitrate. Photo by Janice Glime.

Growth is promoted by added nitrate and ammonium in ***Sphagnum fallax*** (Figure 13), a species of hollows and lawns (Twenhöven 1992). ***Sphagnum magellanicum*** (Figure 12), typically a hummock species, exhibits no benefit with the same treatments. In fact, both species on hummocks exhibit reduced growth in added nitrate and

ammonium. On the other hand, growth is reduced in levels that are lower than the levels resulting from the present atmospheric inputs, suggesting that these bogs were originally N limited. This changes the competitive status of these two species. ***Sphagnum fallax*** is typically competitive, whereas ***S. magellanicum*** is stress tolerant. When N deposition is elevated in previously N-limited conditions, ***S. fallax*** is able to outcompete ***S. magellanicum***.

In the sub-Antarctic on Marion Island, increased NH_4^+ caused an increase in CO_2 assimilation for four moss species, but NO_3^- had a greater effect. Cl^- added with the NH_4^+ may have caused the lesser increase with NH_4^+ additions (Smith 1993). These increases are significant in this habitat with such low soil nutrient levels.

Figure 13. ***Sphagnum fallax***, a competitive species, with capsules. Photo by David T. Holyoak, with permission.

Long Term Effects

The negative effects of elevated nitrate and ammonia in fens are demonstrated in Dutch fens (Paulissen *et al*. 2004). These researchers found that ***Scorpidium scorpioides*** (Figure 14) and other brown mosses declined, whereas ***Sphagnum squarrosum*** (Figure 15) and ***Polytrichum commune*** (Figure 16-Figure 17) increased, lowering the *p*H. ***Scorpidium scorpioides*** did best on nitrate; ammonium nitrate decreased its growth somewhat, and ammonium itself was very toxic. ***Sphagnum squarrosum*** and ***Polytrichum commune*** experienced little affect from the N treatment.

Figure 14. ***Scorpidium scorpioides***, a species that declines when nitrates and ammonia are supplemented. Photo by Jan-Peter Frahm, with permission.

Figure 15. ***Sphagnum squarrosum***, a species that declines when nitrates and ammonia are supplemented. Photo by Janice Glime.

Figure 16. ***Polytrichum commune***, a species that increases when fertilized with nitrate and ammonium. Photo by Michael Lüth, with permission.

Figure 17. ***Polytrichum commune***, with capsules. This is a bog competitor that benefits from added nitrate and ammonium. Photo by Michael Lüth, with permission.

Organic Nitrogen

Most agricultural plants seem to absorb their nitrogen in the form of NH_4^+ or NO_3^-, but it seems that bryophytes have more options. ***Sphagnum*** (Figure 18, Figure 23, Figure 33) is able to use urea (along with phosphate) in the

Alaskan wetlands, resulting in an increase in biomass compared to controls (Sanville 1988). In nature, amino acids likewise can be abundant, present as breakdown products of plant and animal wastes, litter, and corpses. Yet few culture studies or field tracer studies have included these organic forms until recently. Is it possible that bryophytes can use this organic N as their primary source? If so, they may benefit from organic leachates in early stages of litter decomposition of a soil environment.

In bogs and poor fens, NH_4^+ seems to be the predominant form of available N (Rosswall & Granhall 1980). NO_3^- is often lost through denitrification (Hemond 1983). Not surprisingly, some studies show that ***Sphagnum*** seems to require most of its inorganic N as NH_4^+ (Schuurkes *et al*. 1986). But Simola (1975, 1979) showed that ***Sphagnum nemoreum*** (=***S. capillifolium***; Figure 18) and ***S. fimbriatum*** (Figure 19-Figure 20) both could use amino acids. Simola (1975) examined the effects of common peat amino acids – those most likely to be available to the ***Sphagnum***. For ***Sphagnum nemoreum*** NH_4NO_3 proved to be the best N source, with the ammonium ion being used more effectively than nitrate. The amino acids arginine and alanine as the only N source proved to provide satisfactory growth. On the other hand, this species made no use of the amino acids leucine, lysine, isoleucine, or methionine. Lysine actually inhibited growth. This species is more tolerant to organic nitrogen than are tracheophytes, especially of the non-proteinogenic amino acid hydroxyproline. More recently, McKane (1993), using tracer studies, found that for ***Sphagnum***, ***Aulacomnium palustre*** (Figure 21), and ***Hylocomium splendens*** (Figure 22), the amino acid **glycine** was actually the preferred form of nitrogen over NH_4^+ and NO_3^-.

Figure 18. ***Sphagnum nemoreum***, a species that can use amino acids as a nitrogen source. Photo by Michael Lüth, with permission.

It appears that in Arctic ecosystems, organic nitrogen (amino acids, especially glycine) may actually be the preferred source of N for some bryophytes, including ***Sphagnum rubellum*** (Figure 23) (Kielland 1997). Even amino acids with higher molecular weights, such as **aspartate** and **glutamate**, can be absorbed at higher rates than inorganic N. Kielland suggested that the high capacity for absorbing amino acids might be an adaptation to the low inorganic N availability in the Arctic.

Figure 19. ***Sphagnum fimbriatum*** habitat, a species that can use amino acids, most likely available from decomposing leaf litter in its habitat. Photo by Dick Haaksma, with permission.

Figure 20. ***Sphagnum fimbriatum***, a species that can use amino acids as a nitrogen source. Photo by Michael Lüth, with permission.

Figure 21. ***Aulacomnium palustre***, a species that "prefers" glycine over ammonium and nitrate. Photo by Janice Glime.

Figure 22. ***Hylocomium splendens***, a species that "prefers" glycine over ammonium and nitrate. Photo by Michael Lüth, with permission.

Figure 23. ***Sphagnum rubellum***, a species that exhibited decreased growth when receiving elevated ammonium nitrate. Photo by Michael Lüth, with permission.

The Arctic is not the only place where amino acids can provide N for bryophytes. ***Hylocomium splendens*** (Figure 22) in the boreal forest can utilize glycine (Forsum *et al*. 2006). When ammonium, nitrate, and glycine were applied in spray solutions similar to the concentrations in precipitation, this moss took up the greatest labelled N compared to other concentrations. This included a 17% contribution from amino acid N.

Even floodplain bryophytes can use amino acids. Schuler *et al*. (1955) found that in culture the thallose liverwort ***Sphaerocarpos texanus*** (Figure 6) grew more typically on a mix of amino acids than it did on NH_4NO_3 alone.

Burkholder (1959) examined the effects of 20 amino acids (0.0001 M AA to 0.0016 M AA) with and without the addition of NH_4NO_3 on the color and growth of ***Atrichum undulatum*** (Figure 24). **Glycine**, **L-cystine**, **L-cysteine**, and **L-tyrosine** were the only treatments with amino acids alone in which the moss retained its green color. Others were yellow-green, brown-green, or brown (in DL-serine and DL-tryptophan). When grown in combination of each of these 20 amino acids with NH_4NO_3, plants in all treatments grew more than in any of the amino acids alone except in the highest concentration (0.0016 M) of **DL-tryptophan**. Growth was generally greatest in the lower concentration of amino acid (0.0001 M) plus NH_4NO_3.

Figure 24. ***Atrichum undulatum*** with capsules, a species that is able to use some amino acids, but not others. Photo by Andrew Hodgson, with permission.

The report of amino acid utilization by the aquatic Java moss (***Taxiphyllum barbieri***, Figure 7) (Alghamdi 2003), seems unusual among the aquatic mosses and may somehow relate to its ability to live in aquaria and tropical streams where most other bryophytes seem unable to survive. Could this in some way relate to the higher annual temperatures of its tropical habitat? Or is the lack of evidence for amino acid usage in many other species simply a lack of testing?

Alghamdi (2003) chose common soil water-soluble amino acids (**glycine**, **methionine**, **serine**, **arginine**, and **alanine**) to compare their effects on growth, branching, chlorophyll, and protein on the aquatic moss ***Taxiphyllum barbieri*** (Figure 7). He found that four of these amino acids induced branching, relative to the controls, but no branching appeared in any of the **methionine** treatments (Figure 25).

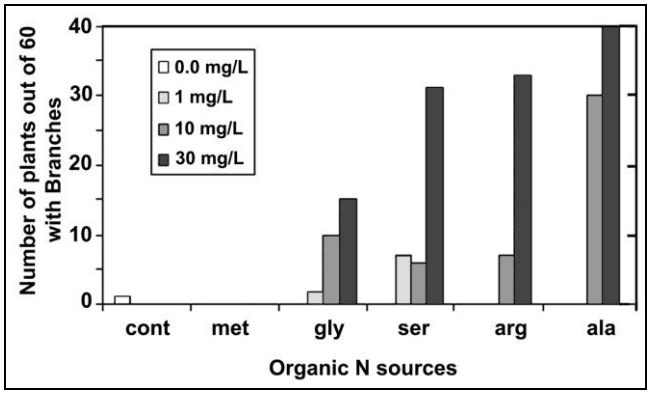

Figure 25. Effects of water soluble amino acids on number of branches in the Java moss, ***Taxiphyllum barbieri***. **cont** = control, **gly** = glycine, **meth** = methionine, **ser** = serine, **arg** = arginine, **ala** = alanine. From Alghamdi 2003.

Methionine proved to be inhibitory to growth in length whereas **serine** caused an increase in both dry biomass and length relative to controls (Figure 26;

Alghamdi 2003). **Arginine** as the only N source at 1, 10, and 30 mg L^{-1} caused a striking increase in the biomass and ratio of dry biomass to length, but maintained a length somewhat less than that of the N-free controls (Figure 26). This resulted in unusually short, wide plants, combined with high protein concentrations but below normal chlorophyll concentrations at the lowest level applied (1 mg L^{-1}; Figure 27).

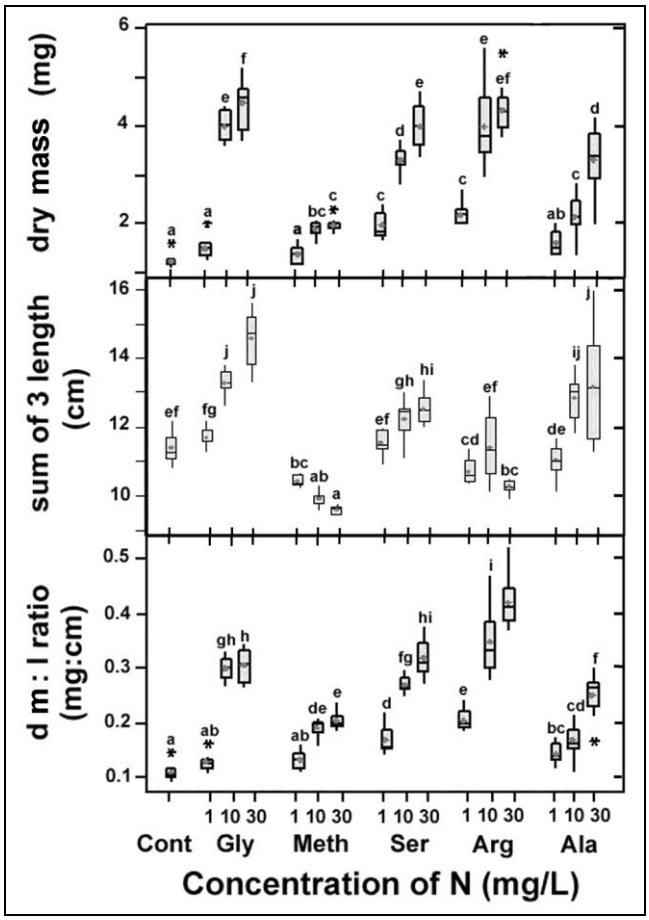

Figure 26. Effect of water soluble amino acids on the biomass, length, and robustness (wt:length) of the Java moss, ***Taxiphyllum barbieri***. Cont = control, Gly = glycine, Meth = methionine, Ser = serine, Arg = arginine, Ala = alanine. Length and biomass represent sum of 3 stems; n = 10 sets of 3 stems. Notation as in Figure 8. Based on Alghamdi 2003.

Methionine likewise caused an increase in biomass and decrease in length growth with concentration increase (1, 10, 30 mg L^{-1}). **Alanine** caused an increase in both length and biomass with concentration, with the overall effect being one of a more robust plant at higher concentrations, having a higher biomass to length ratio than that of the controls. The mosses responded to 1 mg L^{-1} **glycine** much as they did to the N-free medium, but at higher concentrations (20 and 30 mg L^{-1}) their length and biomass both increased considerably over that of controls.

Alghamdi (2003) then compared the effects of glycine, which seemed to produce the "healthiest" plants, to those of the inorganic forms of N. This aquatic moss did less well on the inorganic forms NH_4NO_3 or NO_3^- than on NH_4^+ alone or NH_4^+ + the amino acid **glycine** and did best on glycine alone, producing more biomass, longer stems, and more branches (Figure 28, Figure 29). In fact, glycine seemed to induce branching (Table 1).

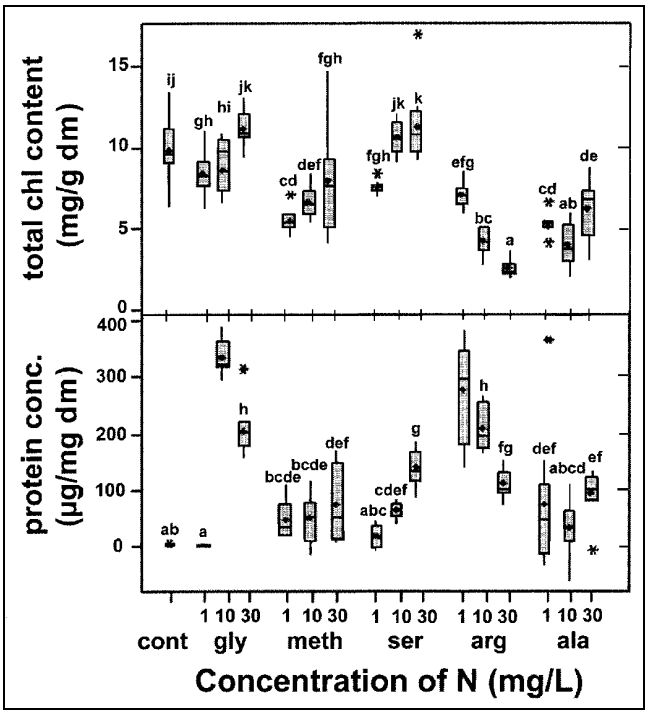

Figure 27. Effect of water soluble amino acids on the protein content and total chlorophyll concentration of the Java moss, ***Taxiphyllum barbieri***. cont = control, gly = glycine, meth = methionine, ser = serine, arg = arginine, ala = alanine. n = 10 sets of 3 stems. Notation as in Figure 8. From Alghamdi 2003.

In the same series of experiments, Alghamdi (2003) examined the effects of inorganic N and glycine on the chlorophyll and protein content of ***Taxiphyllum barbieri***. Glycine, both alone and in combination with NH_4^+, resulted in the highest protein concentrations (Figure 30). The effects on chlorophyll were less clear, but the highest total chlorophyll occurred in the highest glycine concentration (Figure 30). NH_4^+ at 20 mg L^{-1}, however, produced similar chlorophyll concentrations, but at 30 mg L^{-1} the chlorophyll content decreased.

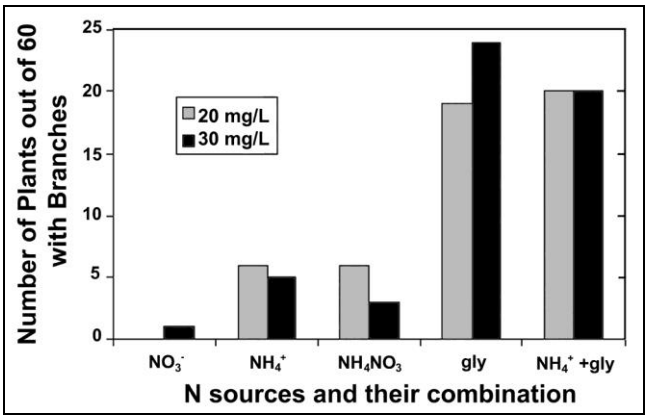

Figure 28. Effect of N source as nitrate (NO_3^+), ammonium (NH_4^+), glycine (gly), and combinations at two concentrations on number of branches in ***Taxiphyllum barbieri***. The combinations have half the total N from each source. From Alghamdi 2003.

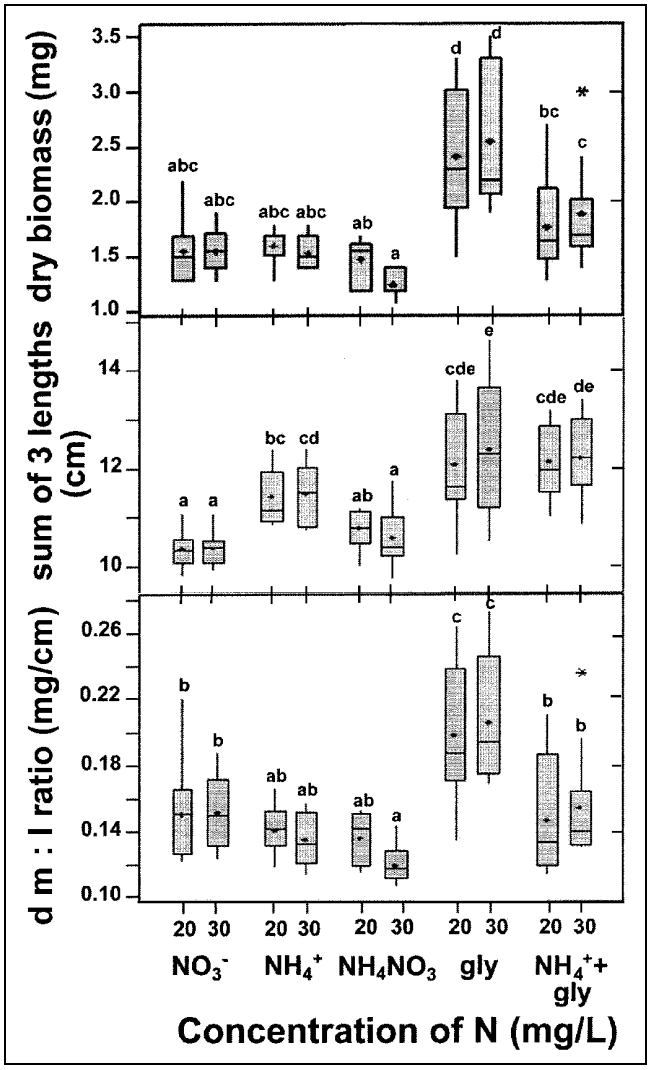

Figure 29. Effect of nitrate (NO_3^+), ammonium (NH_4^+), glycine (gly), and combinations on the increase in biomass (d m) and length (l) and robustness (wt:length) of the Java moss, ***Taxiphyllum barbieri***. Notation as in Figure 8; n = 10 sets of 3 stems. From Alghamdi 2003.

Table 1. Effect of various N forms on moss branching in ***Taxiphyllum barbieri***. From Alghamdi 2003.

Treatment	Moss Branching
glycine	long with many short branches
NO_3^-	short and no branches
NH_4^+	long and few short branches
glycine + NH_4^+	long with many short branches and slightly thin
NH_4NO_3	short, thin and few short branches

Other organic compounds, such as nucleic acids, are also released from organism tissues as they decay. Based on his data showing that ***Atrichum undulatum*** (Figure 24) had good growth in a medium with yeast nucleic acids as its N source, Burkholder (1959) tested growth of this species on the nucleic acid bases. Growth of leafy shoots was good in **adenine** and **guanine**, but there was no growth in uracil or thymine. Growth in xanthine, uric acid, and cytosine was less than that in NH_4NO_3.

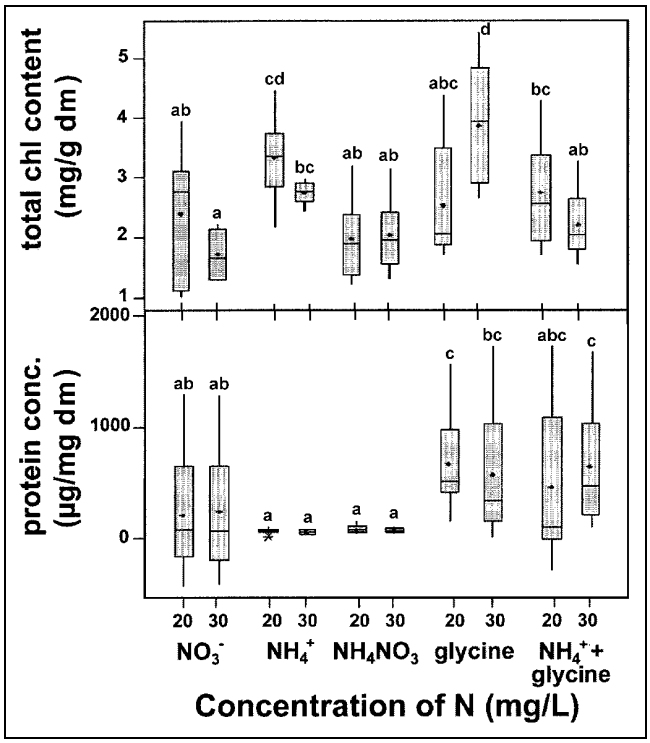

Figure 30. Effects of inorganic N compared to glycine on the protein and chlorophyll content of Java moss (***Taxiphyllum barbieri***). Notation as in Figure 8; n = 10 sets of 3 stems. From Alghamdi 2003.

Both uracil (in the presence of NH_4NO_3) and aspartic acid caused ***Sphagnum squarrosum*** (Figure 15) to become thalloid (resembling its protonema), as did hydroxyproline + glycine, occasionally (Burkholder 1959). Not all mosses responded in the same way. Growth of ***Leptobryum pyriforme*** (Figure 31) and ***Splachnum sphaericum*** (Figure 32) and others was "excellent" on a medium with **NH_4NO_3** plus **uracil**, but was poor in ***Sphagnum squarrosum***. On the other hand, while growth of ***Leptobryum pyriforme*** was good with **uric acid** and **cytosine**, ***Splachnum sphaericum*** had poor growth. The ability to use nucleic acids, amino acids, and other organic N compounds could permit bryophytes to take advantage of partially decomposed litter in which these nitrogen sources leak from the dead tissues.

Figure 31. ***Leptobryum pyriforme***, a species that grows well with uric acid and cytosine. Photo by Michael Lüth, with permission.

Figure 32. ***Splachnum sphaericum*** with capsules, a species with good growth on NH_4NO_3 plus uracil, but poor growth with uric acid and cytosine. Photo by Michael Lüth, with permission.

When N (as NH_4NO_3) was added to a mire in central Sweden, ***Sphagnum fuscum*** (Figure 33), ***S. magellanicum*** (Figure 12), and ***S. rubellum*** (Figure 23) exhibited increased concentrations of amino acids in the capitulum (Nordin & Gunnarsson 2000). But the growth in length decreased at the same time. The researchers demonstrated that when the amino acid N concentrations exceeded 2.0 mg N g^{-1} dry mass, growth was negatively affected. The amino acid N concentrations did not serve as a good measure of N deposition rates when the deposition rates were less than 1.0 g m^{-2} yr^{-1}.

Brown (1982) suggested that in low N environments the mosses may be able to move organic molecules containing N from dying and dead cells to the growing apex. It is very likely that these molecules would be amino acids, as well as dipeptides and other organic compounds.

Some amino acids, leaking into the environment from decaying vegetation, could cause developmental anomalies leading to abnormal growth forms in bryophytes. For example, amino acids, such as **hydroxyproline**, can cause desuppression in the development of underleaves in liverworts (Basile & Basile 1980; Basile *et al*. 1988), causing them to look like normal leaves. In the moss ***Atrichum*** (Figure 24), amino acids inhibited leafy shoot development (Burkholder 1959). This might be another example of the **Gaia hypothesis** (Lovelock, 1988), wherein the ecosystem behaves like a superorganism and species depend on other species for their biochemical needs during development. The N relationships of bryophytes are proving to be more complex than we previously thought.

Figure 33. ***Sphagnum fuscum***, a species that exhibited decreased growth when receiving elevated ammonium nitrate. Photo by Jutta Kapfer, with permission.

Nitrogen Uptake

With the variety in forms of N used by various species, we might expect sites and mechanisms of uptake to vary as well. Atmospheric deposition of N serves as the major source of N for many bryophytes (Soares & Pearson 1997). These researchers raised concerns about the ability of increased levels of these N sources in pollution to inhibit nitrate reductase and affect cation, total N, and organic acid concentrations.

Using ***Racomitrium lanuginosum*** (Figure 34), ***Rhytidiadelphus loreus*** (Figure 35), and ***Philonotis fontana*** (Figure 36) and a single field misting with 3 mol m^{-3} NH_4^+ and NO_3^- Soares and Pearson (1997) found a 20% increase in tissue N after 48 hours. Labelled N experiments on ***R. lanuginosum*** revealed N partitioning, with the highest N uptake in the upper stem and leaves. High concentrations of N resulted in reduced N uptake efficiency. The ammonium decreased nitrogen reductase activity and caused organic acids and cations to decline. However, nitrate treatments cause the opposite response.

Figure 34. ***Racomitrium lanuginosum***, a species has elevated N in the upper stems and leaves following added ammonium and nitrate. Photo by Michael Lüth, with permission.

Figure 35. ***Rhytidiadelphus loreus***, a species that rapidly takes up added N in the first 48 hours. Photo by Michael Lüth, with permission.

Figure 36. ***Philonotis fontana***, a species that rapidly takes up added N in the first 48 hours. Photo by Michael Lüth, with permission.

What controls the rate of uptake in various species? Jauhiainen *et al*. (1998) found that among seven ***Sphagnum*** species, the greatest uptake rate was by individuals (not species) that had the largest capitula and a high number of ion exchange sites. These species were the lawn species ***S. pulchrum*** (Figure 37), ***S. fallax*** (Figure 13), ***S. papillosum*** (Figure 38), and ***S. magellanicum*** (Figure 12). However, when compared on the basis of dry mass, the most effective species were the hummock species ***S. fuscum*** (Figure 33) and ***S. rubellum*** (Figure 23). These species were also the most effective ones in retaining available nitrogen.

Figure 37. ***Sphagnum pulchrum***, a lawn species with a large capitulum and high nitrogen uptake. Photo by Michael Lüth, with permission.

Kopáček and Blažzka (1994) examined ammonium uptake in alpine streams of the High Tatra Mountains, Slovakia. Maximum uptake rates of ammonium N by bryophytes ranged 6-11 mg m^{-2} h^{-1}. The uptake rate did not seem to relate to *p*H during 3- to 5-hour testing periods. Nevertheless, nitrification of ~50% of the NH_4^+-N added occurred in non-acidified streams, but was negligible in acidified streams.

In the aquatic liverworts ***Jungermannia vulcanicola*** (Figure 39-Figure 40) and ***Scapania undulata*** (Figure 5, Figure 41), uptake activities were similar (Miyazaki & Satake 1985). Uptake was greatest at the plant tips and decreased toward the base. Uptake of ammonium at the tip was between 1.9 X 10^{-5} and 5.8 X 10^{-5} g N g dry wt^{-1} h^{-1}. Nitrate uptake was less than that of ammonium.

Figure 38. ***Sphagnum papillosum***, a lawn species with a large capitulum and high nitrogen uptake. Photo by Michael Lüth, with permission.

Figure 39. ***Jungermannia vulcanicola***, a Japanese species with a high tolerance for acid. Photo by Angela Ares, with permission.

Figure 40. ***Jungermannia vulcanicola***, a species in which N uptake is greatest at the plant tips and decreases toward the base. Photo by Angela Ares, with permission.

Figure 41. ***Scapania undulata***, showing a typical habitat. Photo by Michael Lüth, with permission.

Bryophytes have a variety of options for obtaining N. In the Antarctic, ***Bryum pseudotriquetrum*** (Figure 42-Figure 43) and ***Sarconeurum glaciale*** (Figure 44) are able to retain more of the N from precipitation than does the dry soil of the fellfields where they live (Greenfield 1992). The N forms are retained by ion exchange and chelation, enabling them to supplement the low nutrient levels in the rocks and poor-nutrient soils.

Figure 42. ***Bryum pseudotriquetrum*** in the Antarctic, a species that retains N from precipitation. Photo courtesy of Catherine Beard.

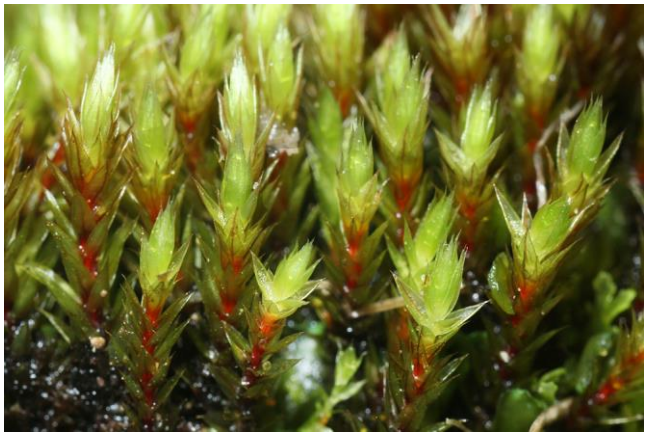

Figure 43. ***Bryum pseudotriquetrum***, a moss that retains N from precipitation. Photo by Barry Stewart, with permission.

Figure 44. ***Sarconeurum glaciale*** with the lichen ***Xanthoria mawsonii*** on it. ***Sarconeurum glaciale*** retains N from precipitation. Photo from Australian Antarctic Data Centre, through Creative Commons.

Bryophytes are diverse in their abilities and physiologies. We have often assumed that they are unable to take N from the soil. However, Ayres *et al*. (2006) demonstrated clearly that mosses are able to derive N from the soil. In fact, they suggested that uptake from soil might be common among mosses, but this prediction needs to be tested.

Nitrogen Fixation

With 78% of our atmosphere being composed of nitrogen and only about 5% of biomass being nitrogen, one would expect this element to be no problem for living systems to obtain. But unlike phosphorus, it cannot normally be obtained from bedrock. And just as you and I can make no use of the free, gaseous nitrogen we breathe, most plants can't either. Instead, plants require their nitrogen fixed into ammonium (NH_4^+) or nitrate (NO_3^-) salts (or converted to amino acids) before they can obtain and convert it to specific amino acids and proteins they need.

Nitrogen fixation is the process of trapping atmospheric nitrogen and converting it to NH_4^+ and in some cases, converting it to NO_3^-. Elbert *et al*. (2012) estimated that cryptogamic covers, including **Cyanobacteria**, algae, fungi, lichens, and bryophytes, account for nearly half of biological N fixation in terrestrial communities. Bryophytes play a crucial contributor in many communities by providing suitable habitat for the N-fixers.

N fixation by **Cyanobacteria** associations with bryophytes may be important in many ecosystems where it has hardly been recognized (Cullimore & McCann 1972; Madhusoodanan & Dominic 1996). Nitrogen fixation is a major source of usable nitrogen for bryophytes, particularly in bogs and fens. Like many tracheophytes, bryophytes can use N released by N fixation from associated bacteria and **Cyanobacteria**. The **heterocysts** (large, transparent, thick-walled cell in filaments of some **Cyanobacteria**; site of nitrogen fixation; Figure 45) of **Cyanobacteria** make them a rich source of amino acids as a result of their **nitrogen-fixing** activity. That is, they are able to convert atmospheric N to a form usable by other living organisms.

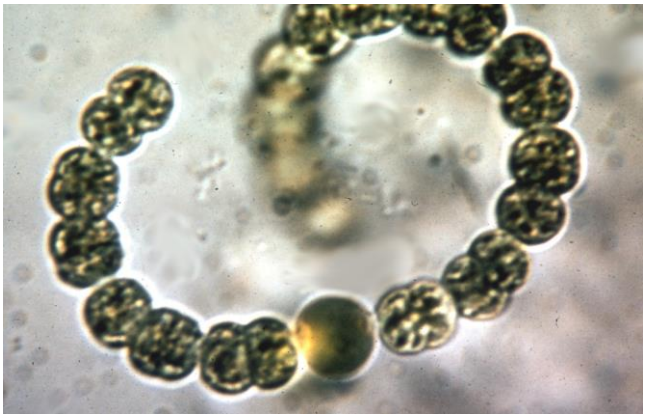

Figure 45. ***Anabaena*** (**Cyanobacteria**) showing heterocyst in middle lower part of picture. Photo by Janice Glime.

In the process of nitrogen fixation in **Cyanobacteria**, the simple CH_2O group from sugars, fixed by cells adjacent to the heterocyst, is moved into the heterocyst (Figure 46). Atmospheric nitrogen (N_2) enters adjacent cells and is passed to the heterocyst. In the heterocyst **nitrogen reductase** (enzyme that catalyzes addition of H^+ to N to form NH_4^+) catalyzes the transformation of N_2 to the reduced NH_4^+ with H^+ obtained from the CH_2O group.

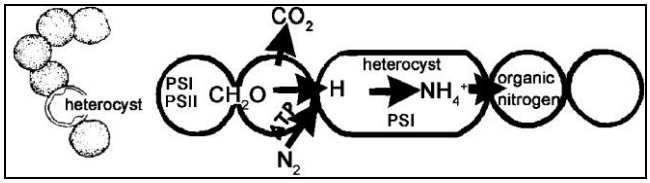

Figure 46. Nitrogen fixation in **Cyanobacteria**, with atmospheric nitrogen entering an adjacent cell and being transferred to the **heterocyst**, where it is converted to ammonium (NH_4^+). The ammonium is then moved to the adjacent cell where it is converted into organic compounds, typically amino acids. Diagram by Janice Glime.

Many studies have shown that some bryophytes, especially peatland bryophytes, obtain N through N fixation processes of surface-dwelling **Cyanobacteria** (Figure 47) as well as other bacteria (Cullimore & McCann 1972; Granhall & Selander 1973; Alexander *et al*. 1974; Basilier *et al*. 1978; Smith & Ashton 1981; Smith 1984; Nakatsubo & Ino 1986, 1987; Bentley 1987; Given 1987; Bergman *et al.* 1993; Madhusoodanan & Dominic 1996). In the **Cyanobacteria**, the most significant contributions come from taxa such as ***Nostoc*** (Figure 1, Figure 48), ***Anabaena*** (Figure 49), and ***Calothrix*** (Figure 50) that have the special cells called **heterocysts**. These cells provide a "safe" environment for nitrogen fixation because they lack the oxygen-generating reactions of photosystem II. The enzyme **nitrogen reductase** is unable to make the conversion in an aerobic environment, hence requiring a location where photosynthetic oxygen is not available. Since only the **Cyanobacteria** and some true bacteria are able to use the abundant atmospheric nitrogen, this conversion makes a significant contribution to usable nitrogen in the ecosystem.

Figure 47. **Cyanobacteria** on ***Campylopus*** at geothermal vent in New Zealand. Photo by Janice Glime.

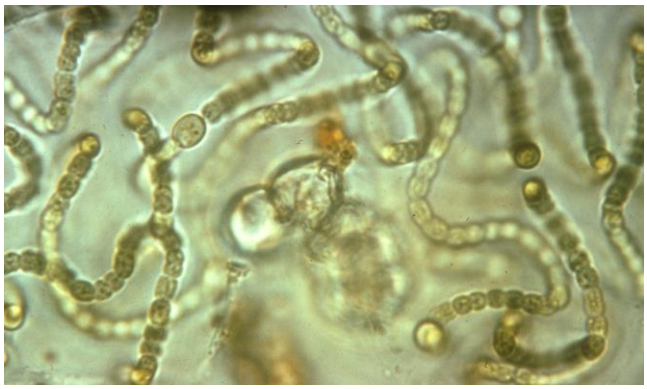

Figure 48. ***Nostoc***, a typical N-fixing **Cyanobacterium** that can be found associated with bryophytes. Note the enlarged heterocysts. Photo by Janice Glime.

Figure 49. ***Anabaena***, a common N-fixing symbiont that lives among bryophyte leaves. Photo by Yuuji Tsukii, with permission.

The **Cyanobacteria** fix more nitrogen than is essential for their own needs and release the excess to their environment. Significant contributions of N through N fixation by **Cyanobacteria** occur in grasslands (Vlassak *et al*. 1973), boulder communities (Snyder & Wullstein 1973a, Jones & Wilson 1978), tropical forests, especially in

epiphyllous communities (those growing on a leaf) (Bentley 1987), poor ***Sphagnum*** (Figure 18, Figure 23, Figure 33) mires (Basilier 1979), boreal forests (DeLuca *et al*. 2002; Gundale *et al*. 2011), and polar turfs (Alexander 1975; Alexander *et al*. 1978).

Figure 50. ***Calothrix***, a nitrogen-fixing **Cyanobacterium** that can live in association with ***Phaeoceros***. Note the heterocyst at the base of each filament. Photo by Yuuji Tsukii, with permission.

In the terrestrial moss ***Hymenostylium recurvirostre*** (Figure 51), association with ***Nostoc*** (Figure 48) is common. Labelled ^{15}N from N gas, converted by ***Nostoc***, resulted in the highest concentrations in the new rhizoids, then new shoots, then old shoots and old rhizoids (Jones & Wilson 1978). Jones and Wilson suggest that these locations indicate the nitrogen is being translocated from old to young tissues. Not only is free NH_4^+ available, but also large quantities of extracellular amino acid leakage is associated with this ***Nostoc***. In view of the discussion above on bryophyte use of amino acids, it is likely that the moss and its neighbors might be using these amino acids as part of their N source.

Figure 51. ***Hymenostylium recurvirostrum*** with capsules, a species that commonly has ***Nostoc*** associates. Photo by Michael Lüth, with permission.

In some of the liverworts and hornworts, **Cyanobacteria** seem to behave symbiotically (Saxena 1981), but more frequently it seems to be only a matter of suitable habitat. For example, in the moist Pacific northwest, approximately 85% of the sampled epiphytic leafy liverwort ***Porella navicularis*** (Figure 52-Figure 53) harbors ***Nostoc*** (**Cyanobacteria;** Figure 48) in distinct colonies under the leaf curled margins and in other plant crevices (Dalton & Chatfield 1985). Nitrogen fixation is measured by the acetylene reduction method, and the product C_2H_2 is used as the measure of fixation. The production of fixed N on ***P. navicularis*** resulted in a mean of 53.5 nmol C_2H_2 g^{-1} d m h^{-1} and reached up to 316 nmol C_2H_2 g^{-1} d m h^{-1}. Dalton and Chatfield (1985) at first thought the ***Porella*** association was symbiotic, but the low number of heterocysts (3-7%) is typical of free-living ***Nostoc***; symbiotic ones typically have a frequency of 30-40%. In either case, the effect is the same; by providing a suitable habitat for **Cyanobacteria**, the mosses facilitate an increase of available N in the system.

Figure 52. ***Porella navicularis*** on tree. Photo from Botany website, UBC, with permission.

Figure 53. ***Porella navicularis***, a suitable substrate for ***Nostoc*** and N fixation. Photo from Botany website, UBC, with permission.

Temperate bryophytes often have associated **Cyanobacteria**, especially ***Nostoc***. Soil associations with bryophytes can benefit the ecosystem in several ways. Not only do they provide additional usable N to the ecosystem, as in the ***Hymenostylium recurvirostre*** (Figure 51) association in Upper Teesdale (Wilson 1975), but they also provide a buffer against erosion and leaching of nutrients already in the upper soil layers.

Few studies have quantitatively addressed the role of micro-organisms in bryophyte communities, particularly in peatlands where their role is significant (Gilbert *et al*. 1999). Nevertheless, these micro-organisms are undoubtedly key players in nutrient cycling through the microbial loop.

Table 2. Comparison of N fixation rates by **Cyanobacteria** associated with bryophytes in various habitats. Rates converted to nmol N using the 3:1 ratio of reduced acetylene to fixed N given by Nakatsubo and Ino (1987) and Vlassak *et al*. (1973). gfm = grams fresh mass; gdm = grams dry mass. Table compiled by Medora Burke-Scoll.

Location	Habitat	Bryophyte and Cyanobacteria partner	Rate	Reference
Tropical	Lava and on volcanic island	*Funaria hygrometrica* + *Nostoc* & *Anabaena*	0.42 nmol N cm^{-2} hr^{-1}	Rodgers & Henriksson 1976
Tropical	Undisturbed forest floor	*Chiloscyphus coalitus* + *Anabaena* &/or *Nostoc*	1.87 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Chiloscyphus fissistipus* + *Anabaena* &/or *Nostoc*	8.2 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Bazzania adnexa* + *Anabaena* &/or *Nostoc*	1.23 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Hypnum chrysogaster* + *Anabaena* &/or *Nostoc*	3.1 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Pohlia nutans* + *Anabaena* &/or *Nostoc*	3.27 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Tortella calycina* + *Anabaena* &/or *Nostoc*	2.57 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Tropical	Undisturbed forest floor	*Pohlia nutans* + *Anabaena* &/or *Nostoc*	3.27 nmol N gdm^{-1} hr^{-1}	Brasell *et al.* 1986
Temperate	Grassland	*Ceratodon purpureus* + *Nostoc*	10.4 nmol N gdm^{-1} hr^{-1}	Vlassak *et al.* 1973
Temperate Japan	Aquatic	*Sphagnum capillaceum* + *Stigonema, Hapalosiphon, Scytonema,* & *Nodularia*	0.13 nmol N gfm^{-1} hr^{-1}	Morimoto & Maruyama 1982
Temperate	Peatland	*Sphagnum* + *Stigonema, Hapalosiphon, Scytonema,* & *Nodularia*	0.13 nmol N gfm^{-1} hr^{-1}	Morimoto & Maruyama 1982
Temperate	Coniferous forest floor (Bilberry-spruce forest)	*Sphagnum girgensohnii* + *Anabaenopsis*	None detected *included only plant apex.	Basilier 1979
Temperate	Forest margin	*Sphagnum papillosum* + endophytic *Nostoc*	0.033 nmol N gdm^{-1} hr^{-1} (only plant apex)	Basilier 1979
Temperate	Fen	*Sphagnum angustifolium* + endophytic *Nostoc*	43.3 nmol N gdm^{-1} hr^{-1} (only plant apex)	Basilier 1979
Temperate	Fen	*Drepanocladus aduncus* + unidentified epiphytic Cyanobacteria	25.67 nmol N gdm^{-1} hr^{-1} (only plant apex)	Basilier 1979
Temperate	Fen	*Sphagnum riparium* + epiphytic *Hapalosiphon*	26.67 nmol N gdm^{-1} hr^{-1} (only plant apex)	Basilier 1979
Temperate	Lakeside	*Sphagnum annulatum* + *Nostoc*	15.3 nmol N gdm^{-1} hr^{-1} (only plant apex)	Basilier 1979
Temperate	Desert	*Grimmia* + *Azotobacter*	0.065 nmol N gdm^{-1} hr^{-1}	Snyder & Wullstein 1973b
Temperate	Desert	*Syntrichia ruralis* + *Azotobacter*	0.061 nmol N gdm^{-1} hr^{-1}	Snyder & Wullstein 1973b
Boreal Iceland	Iceland Lava field	*Grimmia* + *Anabaena* & *Nostoc*	0.13 nmol N/20 cm plant · hr^{-1}	Englund 1976
Boreal	Iceland Lava field	*Racomitrium* + *Anabaena* & *Nostoc*	0.1 nmol N/20 cm plant · hr^{-1}	Englund 1976
Subalpine	Forest floor	*Sphagnum* + Cyanobacteria	0.743 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Peatland	*Sphagnum lindbergii* + *Nostoc* & *Scytonema*	1.3 nmol N gdm^{-1} hr^{-1}	Granhall & Selander 1973
Subalpine	Peatland	*Sphagnum* + Cyanobacteria	0.29 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Aquatic	*Sphagnum* + Cyanobacteria	0.13 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Forest floor	*Atrichum* + Cyanobacteria	0.053 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Forest floor	*Dicranum* + Cyanobacteria	0.023 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Forest floor	*Pleurozium schreberi* + Cyanobacteria	0.026 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Forest floor	*Plagiomnium cuspidatum* + Cyanobacteria	0.15 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979

Subalpine	Forest floor	*Polytrichum* + Cyanobacteria	0.011 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Forest floor	*Bazzania trilobata* + Cyanobacteria	0.033 nmol N gdm^{-1} hr^{-1}	Lambert & Reiners 1979
Subalpine	Coniferous forest floor	Feather mosses	0.23 nmol N gdm^{-1} hr^{-1}	Granhall & Lindberg 1978
Subalpine	Coniferous forest floor	*Sphagnum*	7.47 nmol N gdm^{-1} hr^{-1}	Granhall & Lindberg 1978
Alpine zone of Mt. Fuji	Mountain summit	*Aongstroemia fuji-alpina, Ceratodon purpureus,* & *Bryum + Nostoc*	3.4 nmol N cm^{-2} hr^{-1}	Nakatsubo & Ohtani 1991
Antarctic	East Ongul Island, Antarctica. Sand near a rocky peak.	*Ceratodon purpureus* & *Bryum pseudotriquetrum + Nostoc*	2.37 nmol N cm^{-2} hr^{-1}	Nakatsubo & Ino 1987
Antarctic	Marion Island (highly minerotrophic receiving nutrient-rich mire runoff)	*Brachythecium subplicatum + Anabaena, Calothrix, Hapalosiphon, Nostoc, Sphaerocystis, Stigonema,* & *Tolypothrix*	103.5 nmol N gdm^{-1} hr^{-1}	Smith & Russell 1982
Antarctic	Marion Island (exposed wind-swept rocky ridges)	*Ditrichum strictum* (balls) + *Anabaena, Calothrix, Hapalosiphon, Nostoc, Sphaerocystis, Stigonema,* & *Tolypothrix*	0.12 nmol N gdm^{-1} hr^{-1}	Smith & Russell 1982
Antarctic	Marion Island (submerged)	*Grimmia falcate + Anabaena, Calothrix, Hapalosiphon, Nostoc, Sphaerocystis, Stigonema,* & *Tolypothrix*	5.15 nmol N gdm^{-1} hr^{-1}	Smith & Russell 1982
Antarctic	Fumaroles near summit of Mt. Melbourne	*Campylopus pyriformis* & *Cephaloziella exiliflora + Mastigocladus laminosus*	11 nmol N gdm^{-1} d^{-1}	Broady *et al*. 1987

Arctic, Antarctic, and Alpine

In bryophyte-**Cyanobacteria** associations in the Antarctic (Smith & Russell 1982; Smith 1984; Nakatsubo & Ino 1987; Line 1992; Pandey *et al*. 1992), Arctic (Alexander *et al*. 1978), and alpine/subalpine zones (Lambert & Reiners 1979), N fixation may be a very important contribution of this limiting nutrient to the nutrient-poor ecosystems (Smith & Ashton 1981). Although Smith and Ashton failed to show much acetylene reduction to indicate fixation activity in the field on sub-Antarctic Marion Island at ~0°C, they considered that during the warm summer, fixation by **Cyanobacteria**l flora of bryophytes could approach that exhibited in the lab at ~20°C, thus contributing significantly to the available N in the ecosystem. In a 48-hour field incubation with an air temperature of -1.7°C and moss moisture of 300-1500%, only the moss ***Ditrichum strictum*** associations had any positive acetylene reduction (1.17 & 1.21 µg g^{-1} $48h^{-1}$). The more protected, but nevertheless very cold, ***Clasmatocolea humilis*** and ***Cryptochila grandiflora*** (=***Jamesoniella grandiflora***; Figure 54) associations failed to demonstrate any fixation.

Figure 54. ***Cryptochila grandiflora***, an Arctic species that apparently has no cyanbacterial N fixation. Photo by Juan Larrain, through Creative Commons.

Arctic and Subarctic

In the Arctic soils of Svalbard, Norway, N fixation by both Free-living and bryophyte associations of **Cyanobacteria** is the only significant source of N input to the soil ecosystem (Solheim *et al*. 1996). The most important bryophytes for harboring such associations were ***Calliergon richardsonii*** (Figure 55) and ***Sanionia uncinata*** (Figure 56). An interesting factor in the fixation was grazing by geese (Figure 57). Grazed areas had a 10-fold maximum fixation (693.6±1.5 nmol C_2H_4 h^{-1} gdm^{-1}) compared to ungrazed areas (65.3±16.6 nmol C_2H_4 h^{-1} gdm^{-1}), perhaps because in these areas the **Cyanobacteria** also occurred on the grass. The transfer of fixed N to the plants supported high plant productivity. On the other hand, where birds harbored under cliffs, the concentration of bird droppings inhibited N fixation.

Figure 55. ***Calliergon richardsonii***, an important substrate for **Cyanobacteria** in the Arctic. Photo by Michael Lüth, with permission.

Figure 56. ***Sanionia uncinata***, an important substrate for **Cyanobacteria** in the Arctic. Photo by Hermann Schachner, through Creative Commons.

Figure 57. Barnacle Goose foraging, creating conditions for a higher N fixation rate. Photo by Arthur Chapman, through Creative Commons.

Increased levels of UV-B radiation in the sub-Arctic could have an effect on the rate of nitrogen fixation in bryophyte-**Cyanobacteria** associations (Solheim *et al*. 2002). These researchers found that it causes a 50% decrease in N-fixation potential in the dominant lichen ***Peltigera aphthosa*** (Figure 58), a species with ***Nostoc*** as its N-fixing symbiont. Furthermore, the moss ***Sanionia uncinata*** (Figure 56) in vegetation exposed to experimentally enhanced levels of UV-B for 3 and 4 years in the high Arctic in Svalbard exhibited a 50% reduction in N-fixation potential compared to controls after 3 years. ***Hylocomium splendens*** (Figure 22) failed to show a reduction in N fixation potential after seven years of exposure to increased UV-B. In that same experiment, a 50% increase in precipitation caused a 6-fold increase in N fixation potential.

Nitrogen fixation by **Cyanobacteria** seems to have been important in the colonization of Surtsey, a subArctic island south of Iceland, formed by volcanic eruptions from 1963-1967 (Henriksson *et al*. 1987). By 1987 it had extensive colonies of mosses [***Bryum argenteum*** (Figure 59-Figure 60), ***Ceratodon*** (Figure 61), ***Racomitrium*** **spp.** (Figure 62)] that had **Cyanobacteria** associates capable of N-fixation, primarily ***Nostoc calcicola***. ***Racomitrium canescens*** (Figure 62) exhibited an unidentified N-fixing ***Nostoc*** species living **inside** its cells.

Figure 58. ***Peltigera aphthosa***, a species with Cyanobacteria symbionts. This lichen declines in the presence of elevated UV-B radiation. Photo by Steven K. Sullivan, through Creative Commons.

Figure 59. ***Bryum argenteum***, a pioneer on Surtsey. Photo by Paul Davison, with permission.

Figure 60. ***Bryum argenteum*** capsules – a species that reproduces mostly by fragments. Photo by Dick Haaksma, with permission.

Figure 61. ***Ceratodon purpureus*** with capsules, a colonizer on Surtsey. Photo by Michael Lüth, with permission.

Figure 62. ***Racomitrium canescens***, a species known to sometimes have ***Nostoc*** inside its cells. Photo by Marko Vainu, through Creative Commons.

Antarctic and SubAntarctic

Like Surtsey, the Antarctic lacks litter, so bryophytes have little litter source for N. Without litter, making soil is a slow process. Hence, having an N-fixing partner is often an essential part of life (Smith & Ashton 1981; Smith & Russell 1982).

In support of the suggestion that contributions to N in the summer may be significant, Nakatsubo and Ino (1987) found that approximately 330 mg N m^{-2} was fixed per growing season in some areas of the Antarctic. Fogg and Stewart (1968) found that most N fixation occurs at temperatures above 10°C, thus explaining the lack of activity in the Smith and Ashton (1981) study. Temperatures in the moss-**Cyanobacteria**l associations in summer in the maritime Antarctic typically are in excess of 10°C, often reaching 20°C during midday (Huntley 1971). Smith (1984) found that the fixation rate increased at temperatures from -5°C to a maximum at 25-27°C, decreasing sharply after that. Saturation occurred at ~1000 µmol m^{-2} s^{-1} photon flux density, decreasing at higher levels. Once suitable temperatures were available, moisture seemed to be the most important criterion, causing an increase in fixation up to the highest water content measured: 3,405%! The chemical conditions suitable for fixation seem to be restrictive, with an optimum *p*H in this system of 5.9-6.2 and a negative response to the addition of P, Co, or Mo (Smith 1984). Hence, under warmer conditions, fourteen out of nineteen bryophyte associations did indeed exhibit fixation, with values increasing as moisture content increased (Smith & Russell 1982). Rates ranged from 0.36 to 310.57 nmol C_2H_2 g^{-1} dw h^{-1} (acetylene reduction as indirect measurement of N fixation) among the fourteen with measurable fixation. Surprisingly, in their study, temperature and radiation seemed to have no effect on the rate.

Alpine and Subalpine

The alpine zone likewise is nitrogen limited due to the slow decay rate and limited organic layer. **Cyanobacteria** are important in binding the soil and in providing reduced N. In the subalpine zone of the White Mountains of New Hampshire, USA, the moss ***Plagiomnium cuspidatum*** (Figure 63) provides a suitable habitat for **Cyanobacteria** (Lambert & Reiners 1979). Nevertheless, in an association under the subalpine forest, the ***Sphagnum*** (Figure 15) association was the only one with significant N fixation activity. Lambert and Reiners attributed the activity, in the capitulum, to bacteria, although they considered **Cyanobacteria** to be a possibility.

Figure 63. ***Plagiomnium cuspidatum***, a species that hosts **Cyanobacteria** in the White Mountains, northeastern USA. Photo by Hermann Schachner, through Creative Commons.

On Mt. Fuji, the moss communities of the dry SW slope are nearly devoid of N-fixing activity, but on the moist NE-facing cliffs they exhibit high activity, especially with ***Nostoc*** colonies (Nakatsubo & Ohtani 1991), again demonstrating the importance of moisture. In the somewhat less severe climate of the Alaskan blue spruce taiga system, feather mosses such as ***Pleurozium schreberi*** (Figure 64) and ***Hylocomium splendens*** (Figure 22) are important substrates for N-fixing aerobic and facultative anaerobic bacteria (Billington & Alexander 1983). Here the mosses were quite important, exhibiting daily June and July rates of 74, 119 and 109 µg C_2H_4 m^{-2} d^{-1} of N fixation, respectively, for 3 years of study.

Figure 64. ***Pleurozium schreberi***, a common substrate for N-fixing **Cyanobacteria** in the boreal forest. Photo by Janice Glime.

Peatland Associations

Sphagnum (Figure 18, Figure 23, Figure 33) is highly colonized by a variety of **Cyanobacteria**, both on its surface (Hooper 1982), and in its hyaline cells (Figure 65-Figure 66; Granhall & Hofsten 1976; Granhall & Lindberg 1978), especially by ***Nostoc*** (Figure 48) and ***Hapalosiphon*** (Figure 67) (Sheridan 1991). In bogs and fens, **Cyanobacteria** on bryophyte surfaces can contribute considerable usable N to the ecosystem (Alexander *et al*. 1974; Basilier *et al*. 1978, Basilier 1979; Lambert & Reiners 1979; Rosswall & Granhall 1980, Hooper 1982). Chapman and Hemond (1982) determined that the contribution was greater than that from the only other known input, bulk precipitation (as NO_3^-). Three types of ***Sphagnum*** (Figure 18, Figure 23, Figure 33) N-fixing associations fix N: epiphytic **Cyanobacteria**, intracellular **Cyanobacteria**, and N-fixing bacteria (Granhall & Selander 1973, Granhall & Hofsten 1976).

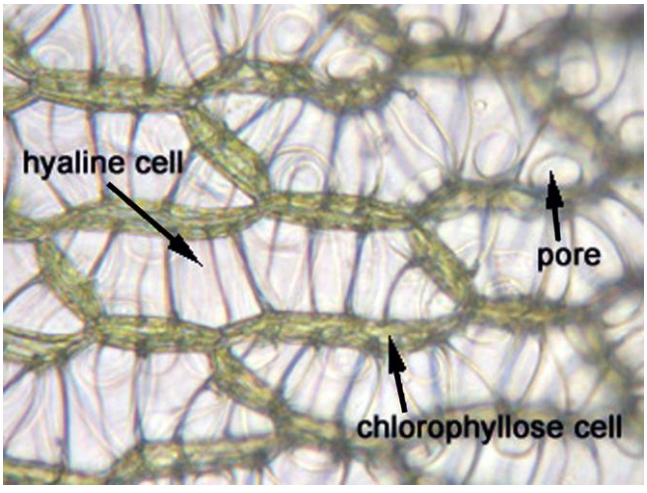

Figure 65. ***Sphagnum*** cells showing the hyaline cell. Photo from Botany website, UBC, with permission.

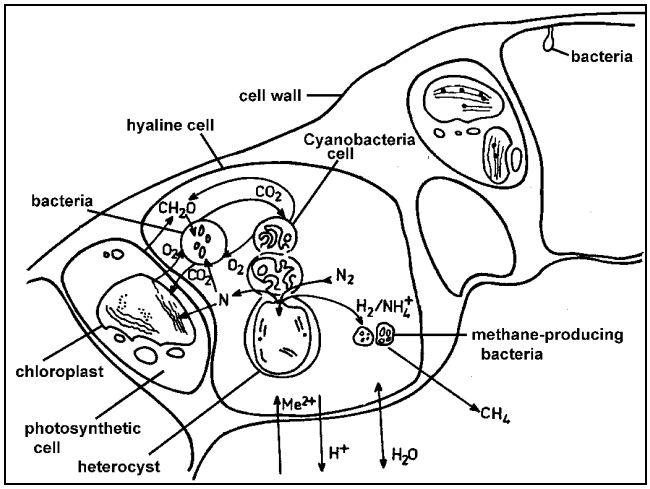

Figure 66. Potential interactions of micro-organisms within the hyaline cell of ***Sphagnum***. Redrawn from Granhall & Hofsten 1976.

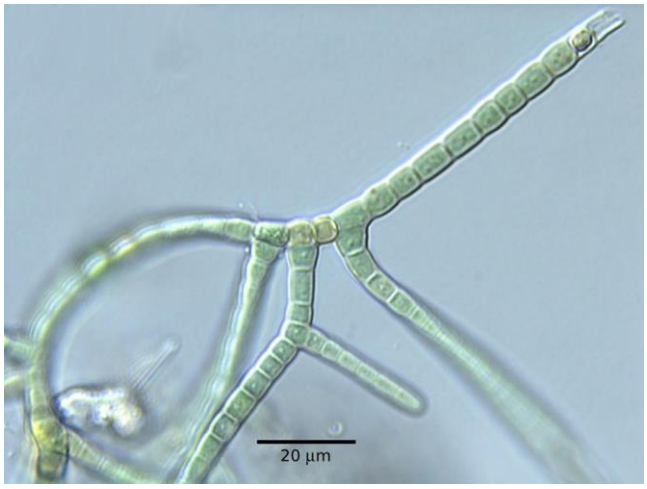

Figure 67. ***Hapalosiphon***, a common member of **Cyanobacteria** that occurs on bryophytes. Photo by Jason Oyadomari, with permission.

Basilier (1979) reported N-fixation activity by **Cyanobacteria** on ***Sphagnum*** (Figure 18, Figure 23, Figure 33), ***Drepanocladus*** (Figure 68), and ***Calliergon*** (Figure 55) in phosphorus-rich environments. Basilier and coworkers (1978), as well as Granhall and Selander (1973), found that the highest N fixation rates in their studies occurred on species of the mosses ***Sphagnum*** and ***Drepanocladus*** (*s.l.*), with a mean value of 9.4 g m^{-2} yr^{-1}. In fact, **Cyanobacteria** associated with ***Sphagnum*** can have higher N fixation per heterocyst than do free-living **Cyanobacteria** in the same condition (Basilier 1980). Granhall and Lindberg (1978) reported a total rate of 0.8-3.8 g fixed N m^{-2} yr^{-1} in wet ***Sphagnum*** communities in a mixed pine and spruce forest in central Sweden. Zimicki (1976) and Basilier *et al*. (1978) have estimated N fixation in various sites for ***Sphagnum riparium*** (Figure 69) to be 0.5-6.4 g m^{-2} yr^{-1}.

Basilier *et al*. (1978) found that the fixation rate in the ***Sphagnum riparium*** (Figure 69) association was strongly light dependent, but that *p*H in the range of 4.3 to 6.8 had little effect. Maximum fixation occurred around noon with the middle of the growing season exhibiting the highest rates. Interestingly, they found that rates on the apical

portions and non-green portions of the ***Sphagnum*** were lower than other green parts, and that the highest rates occurred on the periphery of the moss community. On the other hand, using ^{15}N as a tracer, Basilier (1980) later found that enrichment of N from **Cyanobacteria** fixation appeared within two hours in the apex of ***Sphagnum***. It appears that habitat comparisons need to be made to determine where the highest rates might occur – and why.

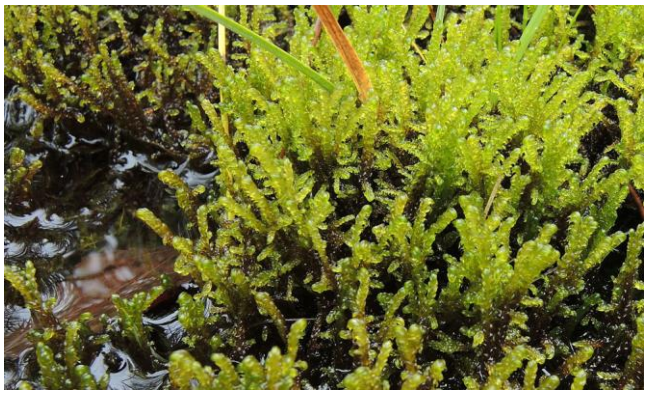

Figure 68. ***Drepanocladus cossonii***, a species that houses N-fixing **Cyanobacteria** in P-rich environments. Photo by Michael Lüth, with permission.

Figure 69. ***Sphagnum riparium***, a substrate for N-fixing **Cyanobacteria**. Photo by Michael Lüth, with permission.

Once the **Cyanobacteria** convert the N to NH_4^+ and amino acids, these are available not only for the bryophytes they occupy, but also for the tracheophytes rooted among them. In Thoreau's Bog in Massachusetts, N fixation exceeded atmospheric N deposition (Hemond 1983), and Hemond concluded that microbial N fixation provides sufficient quantity of N that N may never be limiting to primary productivity in a bog (or poor fen) ecosystem.

Boreal Forests

The boreal forest productivity is limited primarily by available soil N. Bryophytes on the forest floor serve as C and N pools. Recently, researchers have realized the role of N-fixation by **Cyanobacteria** in association with bryophytes in the boreal forest. DeLuca *et al*. (2002) reported that N-fixation reached only 0.5 kg N ha^{-1} yr^{-1}. On the other hand, they found that ***Nostoc*** (Figure 48) living in association with ***Pleurozium schreberi*** (Figure 64) fixes 1.5-2.0 kg N ha^{-1} yr^{-1} in Scandinavian and Finnish boreal forests. They suggest that previous estimates of N-fixation in boreal forests may be too low.

Pleurozium schreberi (Figure 64) is able to modulate its N content based on the amount of N input. With N addition, the N-fixation per unit moss mass and per unit area decreases sharply (Gundale *et al*. 2011). This causes the N pool in the moss to remain stable except at very high additions. This effect on the bryophytes provides at least part of the explanation for the constancy of N acquisition by woody plants up to 12 kg ha^{-1} yr^{-1} additional N. The researchers suggested that bryophytes limit the acquisition of anthropogenic N by woody plants in the boreal forest.

Egorov (2007) found that the nitrogen regime of most of the moss species in the Khibiny Mountains of Eurasia was self-supporting. He attributed this to nitrogen fixation by the epiphytic **Cyanobacteria** on the mosses, accounting for 28% of the total N in the mosses during the growing period.

Hylocomium splendens (Figure 22) is another important feather moss in the boreal forest. And like ***Pleurozium schreberi*** (Figure 64) it is a major contributor to the conversion of N to a usable form by providing a suitable substrate for **Cyanobacteria** (Zackrisson 2009). It is interesting that both of these feather mosses contribute greater N-fixation rates at northern latitudes (64-69° N) than at the more southern latitudes. This is mostly accomplished by species of ***Nostoc*** (Figure 48) and ***Stigonema*** (Figure 70) as the **Cyanobacteria** N fixers. Of further interest is the greater tolerance to N pollution in ***Hylocomium splendens*** when compared to ***P. schreberi***. Consistent with its tolerance to N pollution, ***H. splendens*** exhibited a somewhat higher N-fixation rate at high fertility sites. But ***Hylocomium splendens*** contributed about 50% less to the total N than did ***P. schreberi***. Together, these two species contribute 1.6 kg fixed N ha^{-1} yr^{-1}.

Figure 70. ***Stigonema turfacea***, member of a genus that is common on bryophytes as a nitrogen fixer. Photo by Jason Oyadomari, with permission.

Temperate Forests

Lindo and Whitely (2011) pointed out that we know about the symbiotic Cyanobacteria-bryophyte associations that contribute significantly to the nitrogen levels on the florest floor through nitrogen fixation. But contributions of this process in the canopy are poorly understood. Older trees can contribute bio-available nitrogen to the ecosystem through the **Cyanobacteria**-bryophyte associations where

atmospheric nirogen is fixed in the canopy, potentially making a major contribution to the nitrogen dynamics of the forest. This seems to be especially true in the temperate rainforest. Lindo and Whitely (2011) found that **Cyanobacteria** density was significantly greater in epiphytic bryophytes compared to mosses on the forest floor, with the highest rates (0.76 kg N ha^{-1} yr^{-1}) at 30 m in the canopy compared to the forest floor (0.26 kg N ha^{-1} yr^{-1}). Thus this relationship is important in the canopy of large, old trees in a coastal temperate rain forest with high epiphytic bryophyte biomass.

Tropics

Although associated **Cyanobacteria** are best known from bryophytes in northern habitats, they also exist in the tropics. In the cloud forest on a volcano in the French West Indies, ***Sphagnum erythrocalyx*** is substrate for the N-fixing **Cyanobacterium *Hapalosiphon flexuosus*** (see Figure 67) (Sheridan 1991). The mean rate of methane production caused by N reduction was 19.1 nmol C_2H_4 gdw^{-1} h^{-1} with an annual contribution of N by N fixation of 4.02 kg N ha^{-1} yr^{-1}. The production in the uppermost green capitula was 4.5X that in the basal portions.

Epiphylls

Epiphylls are those organisms that live on leaves. These are common in warmer climates where the leaves remain on the plant for more than one year. In rainforests, epiphyllous liverworts provide the moist microhabitat needed for high rates of nitrogen fixation by associated bacteria and **Cyanobacteria** (Bentley & Carpenter 1980; Bentley 1987; Carpenter 1992), which may be transferred to the host leaves (Bentley & Carpenter 1984).

At least some micro-organisms living in association with epiphyllous liverworts are able to transfer this fixed nitrogen directly to their host plants (Figure 71; Bentley & Carpenter 1984), thus constituting a loose arrangement that benefits the tracheophyte as well as the bryophyte. In the palm ***Welfia georgii***, 10-25% of the N in the leaf was derived from the micro-organisms harbored there among the leafy liverwort cover.

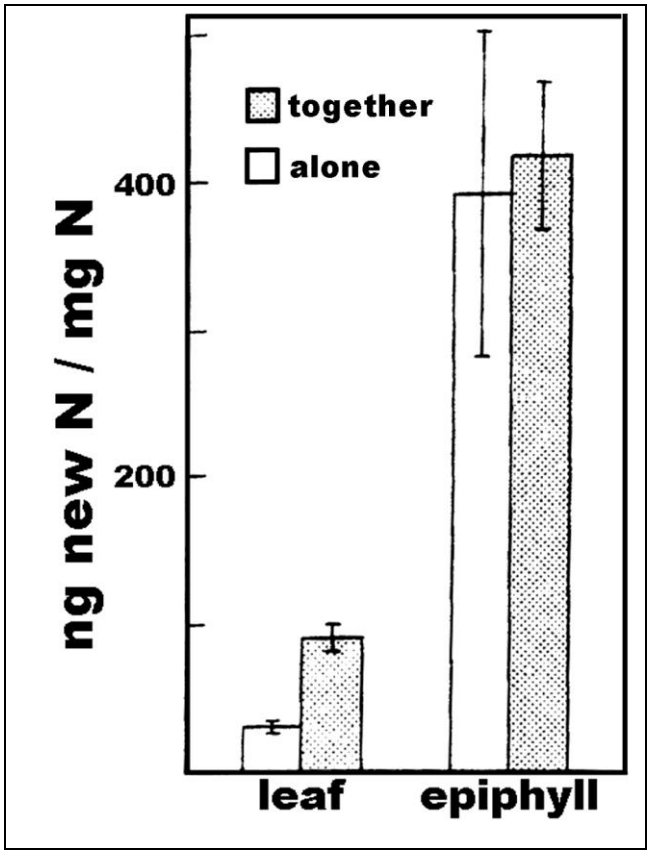

Figure 71. Means and standard errors of 5 hrs of production of fixed nitrogen in leaves of the palm *Welfia georgii* incubated alone (with epiphylls removed) and leaves with intact epiphylls, indicating a much greater transfer of new N to the leaf when epiphylls are present. Redrawn from Bentley & Carpenter 1984.

Liverwort Symbiosis

Several attempts have been made to explain the high degree of N fixation in liverwort associations. In an early attempt, Griggs (1937) grew liverworts from Katmai volcanic ash on N-free sand for three years to determine their success compared to that of liverworts on the same medium, but with the addition of 4 mg L^{-1} NH_4NO_3. During that three-year period, the ones with the additive grew no better, but toward the end of the three years, the N-free cultures became pale and unhealthy. When 4 mg L^{-1} NH_4NO_3 was added to the N-free cultures, they promptly revived. Griggs took this as evidence that no N fixation had occurred.

Nevertheless, at least the thallus of the liverwort ***Blasia pusilla*** (Figure 72) has symbiotic **Cyanobacteria** that do perform N fixation (Rodgers 1978; Peters 1991). In fact, there are many genetic strains of ***Nostoc*** (Figure 48) associated with ***Blasia*** (West & Adams 1997; Costa *et al*. 2001). The presence of ***Nostoc*** induces both structural and metabolic changes within the ***Blasia*** thallus (Kimura & Nakano 1990; Meeks 1990).

Figure 72. ***Blasia pusilla***. **Arrow** indicates ***Nostoc*** colony. Photo by Walter Obermayer, with permission.

Nostoc (Figure 48) is only capable of invading the liverwort when the ***Nostoc*** is in its mobile stage (Kimura & Nakano 1990). That is, when the segments (called **hormogonia**) of a filament separate, they are mobile by a gelatinous sol-gel transformation that permits them to

slither and glide. In this stage they are able to invade the thallus of ***Blasia pusilla*** (Figure 72) and induce the morphological changes that permit the partnership to work. At the same time, the ***B. pusilla*** signals the ***Nostoc*** by producing two **auricles** (earlike lobes), each with an enclosed chamber housing a slime papilla that fills the chamber with mucilage (Renzaglia 1982a). The mucilage attracts the ***Nostoc***, which then takes up residence in the chamber (Figure 73). Once the ***Nostoc*** arrives, the auricle increases in size and closes its opening. Following the invasion, the surrounding cells of the ***Blasia*** thallus have attenuated growth and produce branched filaments from hyaline cells that penetrate the ***Nostoc*** colonies (Kimura & Nakano 1990). These filaments form a labyrinth of wall ingrowths into the ***Nostoc*** cells, suggesting that they may have the role of transfer cells for exchanging metabolites (Ridgway 1967; Duckett *et al*. 1977). Once it has settled into its thallus home, the ***Nostoc*** produces numerous heterocysts, which are essential for the N fixation.

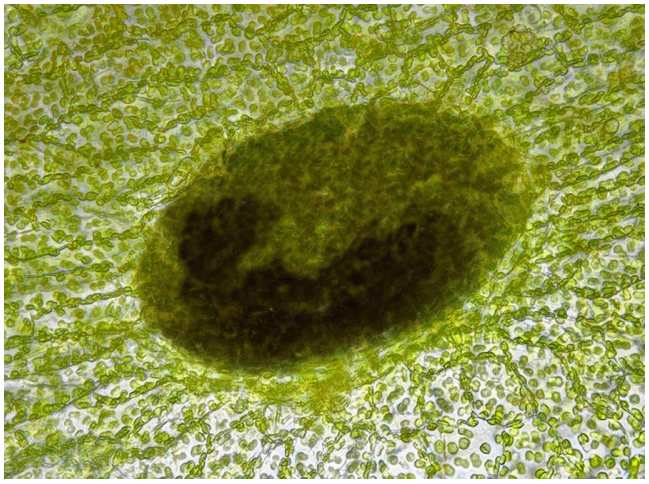

Figure 73. ***Blasia pusilla Nostoc*** colony, the site of N fixation. Photo by Dick Haaksma, with permission.

When the ***Nostoc*** (Figure 48) grows deeply embedded within the liverwort thallus, it no longer has access to dissolved CO_2. Stewart and Rodgers (1977; 1978) determined that the ***Nostoc*** obtains its carbon through transfer from the ***Blasia*** (Figure 72) thallus to ***Nostoc***, suggesting that this is really a **mutualistic** relationship (one in which both partners benefit). Within the thallus the ***Nostoc*** requires a higher light intensity and higher temperature (above 17ºC) for maximal activity compared to those living alone (max activity above 12ºC) (Rodgers 1978). Hence, the liverwort provides a safe compartment that will remain moist much longer than the external environment, and even provides the needed carbon source for its symbiont.

The ability to colonize rapidly, symbiont intact, is facilitated in ***Blasia pusilla*** (Figure 72) by the production of two types of gemmae (Figure 74-Figure 75). These gemmae permit the symbiont to travel with the gemma and easily renew the partnership arrangement upon germination (Renzaglia 1982b; Duckett & Renzaglia 1993). Taxa that depend on spores for their dispersal would not benefit from this convenience.

Figure 74. ***Blasia pusilla*** showing gemmae on stalk. Photo by Des Callaghan, with permission.

Figure 75. ***Blasia pusilla*** gemmae "star" gemmae. Photo by Dick Haaksma, with permission.

As already noted, the epiphytic leafy liverwort ***Porella navicularis*** (Figure 52-Figure 53) provides a suitable substrate for ***Nostoc*** (Figure 48) in western North America (Dalton & Chatfield 1985). This association is present in a broad geographic range. The presence of these ***Nostoc*** (Figure 48) symbionts in liverworts seems to be restricted to taxa that are pioneers (Schuster 1992a, b), living in temporary or poorly colonized habitats that are likely to be low in usable N.

Hornwort Associations

Hornworts (**Anthocerotophyta**) are well known for their symbiotic associations with **Cyanobacteria**, especially ***Nostoc*** (Figure 48) in association with ***Phaeoceros*** (Figure 76) and ***Anthoceros*** (Figure 77) (Peirce 1906; Ridgway 1967; Enderlin & Meeks 1983; Steinberg & Meeks 1987). A wide diversity of ***Nostoc*** strains infect these hornworts (West & Adams 1997), and it appears that ***Anthoceros*** harbors a ***Nostoc*** that is unique from that of ***Blasia*** (Figure 72) (Leizerovich *et al.* 1990). But ***Phaeoceros*** also hosts the filamentous ***Calothrix*** (**Cyanobacteria**; Figure 50) (West & Adams 1997). This multiplicity of symbiotic genera is apparently unusual; Rai

et al. (2000) indicate that typically only one genus will infect a particular taxonomic group of plants.

Figure 76. ***Phaeoceros carolinianus*** showing bluish green color typical of plants with *Nostoc* inhabitants. Photo by Michael Lüth, with permission.

For the association to begin, the ***Nostoc*** (Figure 48) must form **hormogonia** (portions of filament in **Cyanobacteria** that become detached and reproduce by cell division) that can break away and move through the environment to reach the hornwort (Wong & Meeks 2002), just as in ***Blasia*** (Figure 72). But it seems that the hornwort makes certain that this occurs, if there is ***Nostoc*** in the vicinity. Free-living ***Nostoc*** rapidly forms hormogonia when in the presence of ***Anthoceros punctatus*** (Figure 77), or even in the presence of agar preconditioned with ***A. punctatus*** (Campbell & Meeks 1989), indicating a diffusable substance from ***A. punctatus*** that stimulates this response.

Figure 77. ***Anthoceros punctatus***, a species that stimulates formation of hormogonia in ***Nostoc***. Photo by Jonathan Sleath, with permission.

Both ***Nostoc*** (Figure 48) and the hornwort seem to be modified physiologically once joining in symbiosis (Joseph & Meeks 1987; Campbell & Meeks 1992). Before the partnership can work, the ***Nostoc*** must form heterocysts (large, transparent, thick-walled cells found in filaments of certain **Cyanobacteria**; sites of N fixation) (Wong & Meeks 2002). This is where the enzyme **nitrogenase**, needed for the N fixation, is located in both free-living and symbiotic strains (Rai *et al*. 1989). When mutants of ***Nostoc punctiforme*** (Figure 78), unable to form **heterocysts**, were introduced to ***Anthoceros punctatus*** (Figure 77), the partnership formed, but no N fixation occurred; the mutants did not produce any nitrogenase.

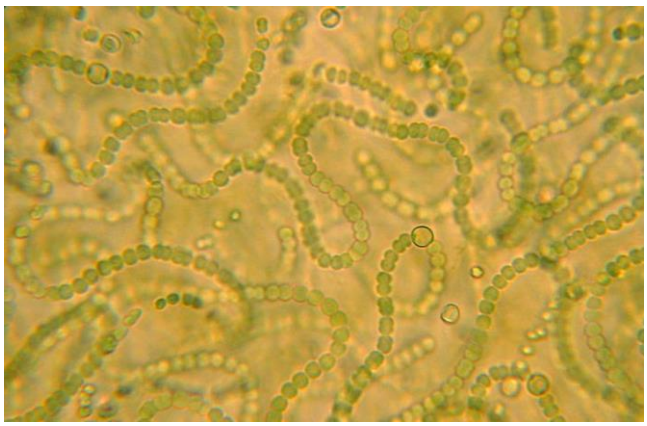

Figure 78. ***Nostoc punctiforme***, a species that does not fix N when it cannot form heterocysts. Photo by Thibul, through Creative Commons.

As in the ***Blasia*** (Figure 72) symbionts, the nitrogenase of the ***Nostoc*** (Figure 48) must have an anaerobic environment in which to fix nitrogen. Campbell and Meeks (1992) demonstrated this by showing that the symbiont could produce fixed N only under anaerobic conditions when grown outside its host. However, when it grew in its ***Anthoceros punctatus*** (Figure 77) host, it could be grown anaerobically; the special cavities where it grew on the host provided the anaerobic conditions needed.

Perhaps one explanation for the success of N fixation within the host lies in the structure of the symbiont heterocyst, contrasting with that of the free-living ***Nostoc*** (Figure 48) strains. When growing inside the host, the ***Nostoc*** heterocyst lacks the outer polysaccharide layer typical that in of free-living ***Nostoc*** (Campbell & Meeks 1992). Rather, it appears that when the ***Nostoc*** grows in the cavities of ***Anthoceros punctatus*** (Figure 77), the cavities replace that wall function. ***Anthoceros*** also mediates the nitrogenase activity, supressing it in the presence of NO_3^- (Campbell & Meeks 1992) and NH_4^+ (Steinberg & Meeks 1991). The end product of the ***Nostoc*** fixation is NH_4^+, accounting for 75% of the introduced radioactive N after 0.5 min, but only 14% after 10 minutes of incubation (Meeks *et al*. 1985), indicating a rapid transformation to something else. Glutamine and glutamate are quickly synthesized via the glutamine synthetase-glutamate synthase pathway, preventing the toxic buildup of NH_4^+. Thus one end result of the symbiosis is that the intracellular levels of NH_4^+ are low compared to those of symbiont-free ***Anthoceros***.

Only 10% of the NH_4^+ is assimilated into the ***Nostoc*** (Figure 48); 1% is lost to the medium; ***Anthoceros*** (Figure 77) incorporates the remainder (Meeks *et al*. 1985). Prakasham and Rai (1991) demonstrated that there is a specific methylammonium transport system in the symbiotic ***Nostoc***, which may account for the reduced NH_4^+ levels and rapid transfer to the host. In symbiont-free ***Anthoceros*** supplied with high levels of NH_4^+, the glutamate dehydrogenase system is functional, permitting

an NH_4^+ buildup (Meeks *et al*. 1983). Therefore, it appears that the ***Nostoc*** partner provides a very effective and safe source of NH_4^+ for the ***Anthoceros*** host (Meeks *et al*. 1985).

As in the ***Blasia*** (Figure 72) partnership, ***Nostoc*** (Figure 48) living within the hornwort gets its carbon primarily from its host plant (Stewart & Rodgers 1977). In fact, ***Nostoc*** isolated from ***Anthoceros punctatus*** (Figure 77) had only 12% of the Rubisco activity of free-living strains, with an equal reduction in CO_2 fixation (Steinberg & Meeks 1989; Rai *et al*. 1989). However, the distribution and levels of Rubisco were similar in the two strains (Rai *et al*. 1989), with 4.3% and 5.2% of the protein as Rubisco in symbionts and free-living ***Nostoc***, respectively (Steinberg & Meeks 1989), suggesting that there is regulation of the Rubisco activity and not an alteration at the gene transcription level. This could be related to the fact that the structure of the chlorophyll complex differs somewhat; the ***Nostoc*** contains the typical cyanophycean granules, but it lacks **phycobilisomes**, the cellular organelle located on the surface of the thylakoids of the chlorophyll complex and in which the biliprotein pigments (**phycocyanin**, **phycoerythrin**) are present (Honegger 1980).

Because the ***Nostoc*** (Figure 48) has reduced ability to fix its own carbon, this transfer of fixed carbon from ***Anthoceros punctatus*** (Figure 77) to ***Nostoc*** is necessary for the fixation of N_2. When the ***Nostoc***-hornwort association was deprived of light for 28 hours, the rate of acetylene reduction (as a measure of N fixation) declined by 99%, but resumed up to 64% of its illuminated activity when supplied with glucose in the dark (Steinberg & Meeks 1991), indicating the need for light and photosynthetic activity for the partnership to work. These researchers found that photosynthates produced immediately by the **Cyanobacterium** can supply at least one-third of the reductant needed for nitrogenase activity in the short-term for the symbiosis to work. When gametophytes were deprived of light, but sporophytes were provided with light, nitrogenase activity continued (Stewart & Rodgers 1977), suggesting a transfer of sugar from the sporophyte to the gametophyte, then to the ***Nostoc***. These factors suggest that the ***Nostoc***, living in the reduced light of the interior of the hornwort thallus, may be dependent upon the hornwort for glucose or similar carbohydrate as an energy source in order to continue its N fixation, thus completing a true mutualistic relationship with its host.

The local sites of the host plants act as islands that effectively keep the ***Nostoc*** (Figure 48) strains in isolation. Even within a single host plant there may be a great diversity of cyanobacterial strains, and these strains seem to be restricted to one site (Costa *et al*. 2001). Nevertheless, some host plants shared strains of ***Nostoc*** that could be found growing 2000 m away. Furthermore, strains found in ***Blasia*** (Figure 72) could also be found in the lichen *Peltigera neopolydactyla* (Figure 79). Although different cavities can easily host different strains in both ***Blasia*** and the **Anthocerotophyta**, a single cavity seems only to host one strain.

Lunar Rocks

Liverworts were among the few organisms to grow successfully on lunar rocks. But why? The thallose liverwort ***Marchantia polymorpha*** (Figure 80) exhibited a tremendous increase in growth following being sprinkled with Apollo 11 or 12 lunar rock material. Hoffman (1974) followed up on this observation by testing the effects of basalt from Minnesota and C-horizon substrate from the Valley of Ten Thousand Smokes, Alaska. In both cases, the growth of ***M. polymorpha*** was significantly increased. But what caused this surge of growth? Nitrogen was absent in any form in both the lunar material and the basalt, and neither P nor K was abundant, so the three typical fertilizer nutrients seem not to be the cause. The macronutrients Ca, Mg, and S were all more abundant in basalt than in the C-horizon soil, but the C-horizon soil caused the greater stimulation. Iron remains a possibility, being abundant in all three substrata. We already know that it stimulates the growth of ***Funaria hygrometrica*** (Figure 4) (Hoffman 1966). And it is also known to stimulate N fixation in Cyanobacteria (Mills *et al*. 2004; Moore *et al*. 2009). On the other hand, no data were gathered on the *p*H, which could affect the solubility, and therefore availability, of all the nutrients. Some have speculated that survival of the liverwort was possible due to partnering **Cyanobacteria** that could trap and convert the atmospheric nitrogen. Perhaps we need to look for soil and rock components that foster the N fixation reaction.

Figure 79. ***Peltigera neopolydactyla***, a lichen with the same strains of ***Nostoc*** as those found in ***Blasia***. Photo by Jason Hollinger, through Creative Commons.

Figure 80. ***Marchantia polymorpha*** with **gemmae**, a species that can grow on lunar rocks. Here it shows red-violet coloration similar to that which develops on the ventral part of the thallus in response to N and P deficiency. Photo by Dick Haaksma, with permission.

Other Stressful Habitats

In **cryptogamic crusts** (*i.e.* soil crusts of algae, lichens, bryophytes, fungi, and micro-organisms; Figure 81) of prairies, deserts, and grasslands, **Cyanobacteria** are able to maintain an active state longer when water is held by the bryophytes. This increases their contribution to the usable N in the soil (Vlassak *et al*. 1973; Giddens 1982; Belknap *et al*. 2001). The crust itself is vital to maintaining both water and nutrients in the soil during and following heavy storms.

In geothermal fields and following fires, bryophytes again provide the moist environment needed to maintain N-fixing micro-organisms (Brasell *et al*. 1986). Hence, we must ask if the bryophytes are net users of nitrogen, or do they facilitate a net gain to the system. At least in some habitats they definitely facilitate a gain by providing the right habitat for fixation to occur, accompanied by leakage of the new N products.

Figure 81. Cryptogamic crust with the moss ***Syntrichia inermis***. Photocourtesy of Lloyd Stark.

Likewise, bryophyte-**Cyanobacteria** associations are important in the colonization of volcanic lava. **Cyanobacteria** are common on bryophytes of dry lava fields (Englund 1976) as well as on the moist, warm bryophyte surfaces near steam vents (Broady *et al* 1987). Both ***Anabaena variabilis*** (=***Tricormus variabilis***; Figure 82) and ***Nostoc muscorum*** were associated with ***Funaria hygrometrica*** (Figure 4) on the newly formed volcano Surtsey off the Icelandic coast (Rodgers & Henriksson 1976). Although the ***Funaria*** did not directly affect the fixation rate, growth of both the ***Funaria*** and the **Cyanobacteria** benefitted by the association, and the N content of ***Funaria*** also increased as a result of the cyanobacterial N fixation.

Although moss associates are responsible for most N fixation in Arctic and subarctic ecosystems, legume associations are considered the predominant N fixers in temperate ecosystems (Stewart 1967). Nevertheless, in some temperate habitats bryophytes are the only plants able to occupy the habitat. For example, on granite outcrops, bryophytes, especially ***Grimmia*/*Schistidium*** (Figure 83), are well known for their role in accumulating soil and nutrients and holding the moisture needed for tracheophyte establishment. Microbial nitrogen fixation on these bryophytes is part of this successional story (Snyder & Wullstein 1973a; Jones & Wilson 1978).

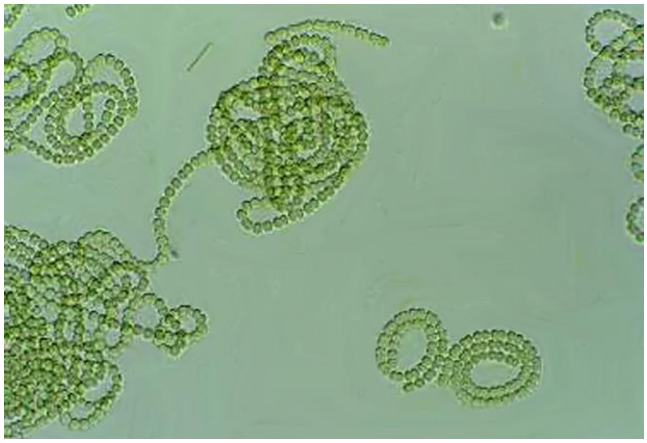

Figure 82. ***Anabaena variabilis***, a species associated with ***Funaria hygrometrica***. Photo from Cyanosite, through public domain.

Figure 83. ***Schistidium apocarpum*** with capsules on granite rock where they accumulate nutrients and prepare the substrate for tracheophytes. Photo by Michael Lüth, with permission.

Nitrogen Translocation

We know that N is needed in amino acids, proteins, nucleic acids, and ribonucleic acids. But where do they go in the plants? Eckstein and Karlsson (1999) compared their locations in the boreal forest moss ***Hylocomium splendens*** (Figure 22) and the wet habitat moss ***Polytrichum commune*** (Figure 16-Figure 17). They demonstrated that both **endohydric** (having internal conduction) and **ectohydric** (using external conduction) species were able to move N compounds from one **ramet** (attached branch serving like a separate organism) to another. Current-year segments of both species appeared to be strong **sinks** for nitrogen, as demonstrated by their considerable increase in the labelled N pool during the season. **Sinks** are locations where something, such as plant nutrients, organic pollutants, or metal ions, is stored and immobilized through natural processes.

In the period of June to September, ***Polytrichum commune*** (Figure 16-Figure 17) lost labelled N from all segments (Eckstein & Karlsson 1999). The researchers attributed this to transfer of N to underground structures (sinks). However, in ***Hylocomium splendens*** (Figure 22), the one-year-old segments had increased labelled N, whereas the older segments lost 50% of the labelled N they had absorbed. This ability to transfer nutrients from one

part to another is especially beneficial in nutrient-poor environments.

N Sequestering

Sinks can be seasonal, with actively used nutrients moving from locations such as leaves to storage locations as winter approaches or simply be storage of excess. Once incorporated into the bryophytes, nutrients, including N compounds, can either be sequestered or recycled. In some cases they are moved to young, growing tissues. In the tropics, epiphytic bryophytes can sequester inorganic nitrogen from atmospheric deposition. Clark *et al*. (2005) estimated that the epiphytic bryophytes and epiphytic assemblages retained 33-67% of the inorganic N deposition from cloud water and precipitation, retaining 3.4 kg N ha^{-1} yr^{-1}, accounting for 50% of the inorganic N in atmospheric deposition. This effectively removes 50% of the suitable N sources and sequesters them in the bryophyte tissues.

In the boreal species ***Hylocomium splendens*** (Figure 22) in a subarctic birch woodland, retention of labelled N varied from three to ten years, depending on the method used (Eckstein 2000). The ability to transport the N compounds to other locations in the plant and a relatively long life span for the growth segments could explain the long residence time of the labelled N. This species uses **acropetal** (from base upward) transport, thus minimizing losses from by the environment by storage in older segments.

Some nutrients are lost to grazing, and in the Arctic, Snow Geese (*Chen caerulescens*; Figure 84) contribute to this herbivory (Kotanen 2002). But mosses can play a role in this goose scenario. Tissues of grasses and sedges that are eaten by the geese are not compensated for their losses, with tissue N responding poorly to N additions. Kotanen suggests that the abundant mosses in these freshwater wetlands sequester the added N, preventing it from reaching forage plants and returning to the ecosystem through feces. But in tracer studies, Kotanen found that mosses did not prevent the grasses and sedges from likewise taking up ammonium and nitrate at or below the moss surface. Nevertheless, most of the added N was absorbed by the mosses before it reached the soil, diverting N away from the forage plants and sequestering it in the moss peat.

Figure 84. ***Chen caerulescens*** grazing on grass that competes with mosses. Mosses, however, take up added N. Photo by Walter Siegmund, through Creative Commons.

N Deficiency Effects

For agricultural plants we know all the symptoms of deficiency. Even the house plant owners are often aware of deficiency symptoms. But for bryophytes, we know little.

One of the symptoms of nutrient deficiency in crop plants is presence of red coloration in the leaves. When the thallose liverwort ***Marchantia polymorpha*** (Figure 80) was grown without nitrate and phosphate, the ventral cell layers developed a red-violet color in the cell walls (Voth & Hamner 1940).

We know that some algae use diminishing N availability in their medium as a signal to go into a sexual phase and produce resting zygotes (Trainor 1959; Singh & Chaudhary 1990; Matsuda *et al*. 1992). Do any bryophytes also use any nutrient signal to become sexual?

In the thallose liverwort ***Marchantia*** (Figure 80), a low ratio of N to C stimulates production of sexual branches (Lockwood 1975). In seeming contrast, the liverwort ***Fossombronia brasiliensis*** produces more gametangia when N is supplied as nitrate than when it is supplied as ammonium (Chin *et al*. 1987). In ***Bryum argenteum*** (Figure 59-Figure 60), reduced nutrient levels stimulate the production of sex organs (Joenje & During 1977), but it wasn't clear which nutrient(s) deficiency might be critical for the reproduction.

Several species of the thallose liverwort ***Riccia*** (Figure 85) produce archegonia and antheridia in response to limiting nitrates (Selkirk 1979). On the other hand, urea not only increased archegonial production significantly in ***Riccia crystallina*** (Figure 85) but also increased growth (Sood 1974). It is more interesting that in this species the amino acids hydroxyproline, serine, threonine, asparagine, glutamic acid, alanine, and leucine increased archegonia production, whereas glycine, tryptophan, aspartic acid, and valine increased production of antheridia.

Figure 85. ***Riccia crystallina***, a species that produces more archegonia and grows more when given urea. Photo by David T. Holyoak, with permission.

In other cases, organic N compounds alter the photoperiodic induction of gametangia. In the leafy liverwort ***Cephalozia lunulifolia*** (=***C. media***; Figure 86), the amino acids arginine, cysteine, and tryptophan plus kinetin can override photoperiodic control (Lockwood 1975). And these amino acids had similar negating effects over the photoperiodic short-day initiation of gemmae.

Furthermore, adding inorganic N as nitrate or ammonium did not override the effects of the amino acids.

Low levels of N can also reduce gemma production in the thallose liverwort ***Marchantia polymorpha*** (Figure 80) (Wann 1925; Duckett & Pressel 2009). This seems also to explain the loss of gemma production in this species two years following a fire (Duckett & Pressel 2009). On the other hand, ***Ceratodon purpureus*** (Figure 61) and ***Funaria hygrometrica*** (Figure 4) on bonfire sites have early gemma production, as do ***Bryum*** (Figure 59-Figure 60) species in arable fields (Duckett *et al*. 2004; Pressel *et al*. 2007). Ball (2010) reported that nitrate levels go up following a fire, and that these results are persistent. The charcoal resulting from the fire stimulates the conversion of ammonia to nitrates through the action of bacteria. This suggests that some of these bryophytes may benefit differently from different forms of nitrogen.

Figure 86. ***Cephalozia lunulifolia***, a liverwort in which the amino acids arginine, cysteine, and tryptophan plus kinetin can override photoperiodic control of gametangia and gemmae initiation. Photo by Hermann Schachner, through Creative Commons.

N Enrichment

The unusual way in which bryophytes respond to nitrogen addition has interesting effects in the ecosystem. As already noted, increases in nitrogen often result in a reduction of bryophyte cover and diversity or replacement of one species by another. But even though the bryophyte productivity decreases as N deposition increases, the stored N can increase within the bryophyte (Gundale *et al*. 2011).

This has interesting implications for the ecosystem, because it buffers the N reaching the tree roots, at least in boreal forests (Gundale *et al*. 2011). Predictably, N fixation by associated **Cyanobacteria** decreases as N fertilization increases. In the boreal feather moss ***Pleurozium schreberi*** (Figure 64), the tissue concentrations of nitrogen increased but the biomass decreased with increasing nitrogen addition. Because feather mosses provide considerable biomass on the boreal forest floor, they can have considerable impact on the nitrogen that is able to reach the trees, trapping nitrogen from precipitation, providing niches for **Cyanobacteria**, and sequestering nitrogen from airborne dust.

Many studies in peatlands have included enrichment of N to determine effects on bryophyte productivity. In an Arctic heath community, where N and P are colimiting, Gordon *et al*. (2001) found that applications of N (0, 10, & 50 kg ha^{-1} yr^{-1}) and P (0 & 5 kg ha^{-1} yr^{-1}) caused a decrease in lichen cover; applications of 10 kg ha^{-1} yr^{-1} resulted in a higher proportion of physiologically active bryophyte shoots. Nevertheless, individual bryophyte species responded differently, suggesting that we cannot draw generalizations from limited fertilization experiments.

Added N can affect different life stages differently. In Wales populations of ***Racomitrium lanuginosum*** (Figure 34), growth was stimulated initially with the highest N addition level (60 kg N ha^{-1} yr^{-1}) (Jones *et al*. 2002). However, after 6 months, all concentrations (20, 40, & 60 kg N ha^{-1} yr^{-1}) caused decreased growth compared to the control with no N addition. By contrast, optimum regeneration from fragments occurred at 20-40 kg on bare soil, but under a canopy of the grass *Festuca ovina* (Figure 87) it was best at 0-20 kg N.

Figure 87. *Festuca ovina*, a grass that benefits the growth of ***Racomitrium lanuginosum*** fragments on the soil at its base. Photo by J. C. Schou (BioPix), with permission.

Thus we have seen that N enrichment, including that from atmospheric pollution, can be detrimental to bryophytes, especially in some conditions. This has resulted in the disappearance of some species (Strengbom *et al*. 2001). Strengbom and coworkers found that in a boreal forest after fertilization had been stopped for nine years, there were no signs of bryophyte recovery. Mycorrhizal fungi produced more sporocarps on the formerly fertilized plots than on those still receiving N, but the species composition was very different from that of never-fertilized controls. After 47 years of no fertilization, the mosses ***Brachythecium reflexum*** (Figure 88) and ***Plagiothecium denticulatum*** (Figure 89) showed enhancement from the previous N fertilization. On the other hand, the common moss ***Hylocomium splendens*** (Figure 22) was still less abundant than in the controls that were never treated with N. These changes were in contrast to the constancy of tracheophyte composition during and after cessation of N treatments.

Figure 88. ***Brachythecium reflexum***, a species that showed enhancement of coverage 47 years after N fertilization ceased. Photo by Michael Lüth, with permission.

Figure 89. ***Plagiothecium denticulatum***, a species that showed enhancement of coverage 47 years after N fertilization ceased. Photo by Hermann Schachner, through Creative Commons.

In a boreal forest of northern Sweden, simulated N deposition had no short-term effects on the above- or below-ground biomass of the understory (Nordin *et al*. 1998). The recovery increased with the N dose. In the plots with 0.5 kg N ha^{-1} the highest concentrations of labelled N occurred in the bryophytes [***Dicranum majus*** (Figure 90) and ***Pleurozium schreberi*** (Figure 64)], whereas in higher applications the grass *Deschampsia flexuosa* (Figure 91) exhibited the highest levels. The elevated N also resulted in greater herbivory on the blueberry *Vaccinium myrtillus* (Figure 92). This raises the as-yet unanswered question of how increased N affects herbivory on bryophytes.

The reduction of bryophyte productivity with increased N deposition is a recurring theme (Koranda *et al*. 2007). Koranda and coworkers sought an explanation for this reduced productivity. Using fragments of ***Thuidium tamariscinum*** (Figure 93) and ***Hylocomium splendens*** (Figure 22), they assessed the effects of ammonium nitrate (30 kg ha^{-1} yr^{-1}) for 80 days. In this experiment, there was no growth change in ***T. tamariscinum***, whereas ***H. splendens*** showed growth reduction. The latter also exhibited a significant increase in N concentration, whereas only ***T. tamariscinum*** had a significant increase in amino acid N. Both species exhibited a reduction in lipid concentration, accompanied by strikingly enhanced turnover rates of carbon storage pools in the fertilized plants. Koranda and coworkers interpreted these results to indicate that the depressed growth of ***H. splendens*** may be caused by enhanced synthesis of N-containing organic compounds, most probably of cell wall proteins. Disturbance of the cellular carbon metabolism may also contribute.

Figure 90. ***Dicranum majus*** with capsules. This species exhibits among the highest concentrations of labelled N when given 0.5 kg N ha-1. Photo by Michael Lüth, with permission.

Figure 91. *Deschampsia cespitosa*, a species that exhibits the highest concentrations of labelled N at N applications higher than 0.5 kg N ha^{-1}. Photo by Rasbak, through Creative Commons.

Figure 92. *Vaccinium myrtillus*, a species that experiences greater herbivory when treated with elevated N. Photo by Anneli Salo, through Creative Commons.

Figure 93. ***Thuidium tamariscinum***, a species that exhibited no change in growth rate under elevated ammonium nitrate. Photo by Hermann Schachner, through Creative Commons.

In a nutrient-deficiency condition, with 10 weeks of watering with distilled water daily, ***Pseudoscleropodium purum*** (Figure 94) grew faster than did ***Brachythecium rutabulum*** (Figure 95) (Bates 1994). When those populations were subjected to a nutrient pulse of 8 daily additions of KH_2PO_4 and NH_4NO_3, followed by 10 weeks of no nutrient additions, growth of ***P. purum*** was significantly stimulated, whereas that of ***B. rutabulum*** was not. ***Pseudoscleropodium purum*** increased its uptake of P, less so of N, and conserved these more effectively in nutrient-deficient conditions than did ***B. rutabulum***. Cation exchange appears to be important in sequestering nutrient cations. These results can explain differences in habitat – ***P. purum*** lives where nutrient inputs are unpredictable, coming as wet deposition; ***B. rutabulum*** lives in a more continuous nutrient supply, apparently coming from the soil.

Figure 94. ***Pseudoscleropodium purum***, a species that conserves N and P in nutrient-deficient conditions. Photo by Phil Bendle, with permission.

Sphagnum magellanicum (Figure 12) has a different set of habitat conditions and illustrates differences in ammonium and nitrate enrichment effects. As noted earlier, this species was favored by nitrate concentrations up to 322 µM, whereas ammonium concentrations ≧ 255 µM caused decreases in chlorophyll content and growth (Rudolph & Voigt 1986). At 600 µM of added ammonium there was a 20% reduction in nitrate reductase activity and net photosynthesis.

Figure 95. ***Brachythecium rutabulum*** with water droplets, a species that is not stimulated by N and P additions. Photo by Christophe Quintin, through Creative Commons.

Calliergonella cuspidata (Figure 96) in a calcareous fen in the mountains of Switzerland showed no observable morphological changes due to increased N levels, whereas the same species showed a number of morphological changes in higher light intensities created by cutting of the tracheophyte vegetation (Bergamini & Peintinger 2002).

Figure 96. ***Calliergonella cuspidata***, a species that does not change morphology in response to increased N levels. Photo by Michael Lüth, with permission.

In a different set of experiments, Heijmans *et al*. (2001) elevated the nitrogen levels (5 g N m^{-2} $year^{-1}$ as ammonium nitrate) in a bog in The Netherlands for three years, added at 3-week intervals during the growing seasons. As one might expect, the tracheophyte biomass increased. But for the ***Sphagnum*** (Figure 18, Figure 23, Figure 33), growth was significantly reduced in the third growing season. It is likely that this was the result of encroaching tracheophyte cover.

Can we expect a different response from a submersed species of ***Sphagnum***, such as ***S. cuspidatum*** (Figure 9)? In a culture experiment lasting 12 weeks, this species was grown at various levels of ammonium (Paffen & Roelefs 1991). In highly enhanced CO_2, this species had increased growth in length and biomass, both with and without ammonium enrichment, but with only ammonium enrichment there was no increase in biomass.

Bryophytes have often been used as monitors. In terrestrial habitats, the moss bag became popular. In aquatic habitats, bryophytes can be used *in situ* or as transplants. The aquatic moss ***Fontinalis antipyretica*** (Figure 97) has been used to assess a variety of pollutants. Mosses such as this have the advantage of accumulating pollutants rather than representing the momentary levels found in chemical assays. For understanding its indications as a biomonitor for NH_4^+, it was necessary to understand the pattern of uptake and the way in which high concentrations could alter physiological performance (Vieira *et al*. 2009). These researchers learned that the concentrations that had significant impact on membrane permeability were the same as those that caused a significant lowering of photosynthetic capacity. As time passes in those higher concentrations, the damage threshold is lowered.

Figure 97. ***Fontinalis antipyretica***, a species that sequesters a variety of pollutants. Photo by Štěpán Koval, with permission.

Habitat Relations

Surprisingly, some of our best studies on canopy bryophytes are from the tropics. Clark *et al*. (1998) estimated the N accumulation of epiphytic bryophytes in a tropical montane forest in Costa Rica to be 1.8-3.0 g N m^{-2} yr^{-1}. N release from bryophyte litter in the canopy and on the ground was initially rapid, with ~30% released. Release from green shoots on the forest floor was greater, with ~47% of the initial N released in the first 3 months. The researchers found no evidence for net N immobilization by either litter or green shoots, but the remaining N in the litter, as already seen above in other species, was **recalcitrant** (substance that degrades at extremely slow rate if at all when released into environment). The epiphytic bryophytes retained 0.8-1.3 g N m^{-2} yr^{-1}. The ability of these epiphytes to retain inorganic N from atmospheric deposition gives them a major role in converting mobile forms of N to highly recalcitrant forms.

Previous research at this site indicated that epiphytic bryophytes retain inorganic N from atmospheric deposition to the canopy (Clark *et al*. 1998). Therefore, they play a major role in transforming N from mobile to highly recalcitrant forms in this ecosystem.

In a study of layer differences in a wooded meadow, Kull *et al*. (1995) found that upper layers have the best access to light and the lower layers have higher N-use efficiency and/or better ability to acquire N. The herbaceous layer has the highest level of foliar nitrogen compared to the tree and moss layers. However, the herbaceous layer is co-limited by light and nitrogen, whereas the moss layer is limited only by light.

On Signy Island in the Antarctic, the dry turf had lower total N concentrations per dry weight (0.79%) than did the wet carpet (2.17%) (Christie 1987). In December, the meltwater and pools of the dry turf had 230 μg N L^{-1} while 165 μg N L^{-1} was present in the wet carpet. ***Nostoc muscorum*** was present at both sites and exhibited high levels of nitrogen fixation. Biological N fixation accounted for 45.9 mg m^{-2} yr^{-1} in the dry turf and 192.4 mg m^{-2} yr^{-1} in the wet carpet. Christie attributed additional inputs to penguin activity.

Throughout this chapter we have seen differences both among species and among habitats. We have barely scratched the surface in understanding these differences and why they occur.

Nitrogen Cycling

In those habitats where bryophytes form a major component of the ecosystem, their role in N cycling can be important. This is particularly true in cold biomes and tropical rainforests (Cornelissen *et al*. 2007). As we have seen, bryophytes host N-fixing bacteria and **Cyanobacteria** that contribute significant usable N to the soil. They furthermore modify the soil climate through control of hydrology and temperatures. They provide safe sites to soil organisms that contribute to litter breakdown.

Temperature plays an important control on the rate of breakdown in the Alaskan tundra. Warming from 4° to 10° significantly increases the rates of nitrogen mineralization, causing a significant effect on the rate of N cycling in litter and tundra soils (Hobbie 1996). Among the growth forms, graminoid litter had the fast rate, whereas moss and deciduous shrub litter had the slowest decomposition rates. This is largely due to the placement of bryophyte nutrients into recalcitrant forms (Hobbie 1996; Cornelissen *et al*. 2007). Decomposition will be discussed further in a separate chapter of this volume.

Summary

Nitrogen is available to bryophytes as **ammonium** (NH_4^+), **nitrite** (NO_2^-), **nitrate** (NO_3^-), and organic forms such as **amino acids** and **urea**. Nitrite, however, is generally toxic. Ammonium can lower internal *p*H and suppress growth. Nitrite can cause an increase in chlorophyll *a*, whereas nitrate can cause a decrease in chlorophyll *b*, both causing an increase in the *a*/*b* ratio. But effects on amino acid and protein concentration vary among species and among habitats. In the Arctic, amino acids and urea are utilized by both bryophytes and tracheophytes. ***Sphagnum*** species often seem to benefit more from amino acids than from ammonium.

Much of the nitrogen uptake is from precipitation; some is from the soil. But our knowledge of nitrogen uptake mechanisms is meager, and the mechanisms

differ among species. These include ion exchange sites and chelation and can be affected by *p*H, iron and phosphorus concentrations, and temperature.

Some, perhaps many, bryophytes solve the nitrogen problem through symbiotic partners, especially **Cyanobacteria**, that carry out **nitrogen fixation**. This process seems to be especially important in the polar and alpine regions under warmer summer conditions up to ~25ºC. But more xeric conditions such as among **epiphyllous** tropical bryophytes and associated with prairie and grassland cryptogamic crusts also benefit from N fixation. In all of these habitats, bryophytes have an **important role** in maintaining the moisture necessary for the fixation to occur.

Peatlands have a high N fixation rate, and **Cyanobacteria** are common in association with ***Sphagnum***. They have a wider *p*H tolerance range (4.3-6.8) than the **Cyanobacteria** in the cold habitats (5.9-6.2).

The liverwort ***Blasia pusilla*** provides a special chamber in each **auricle** where it is moist with mucilage and the **Cyanobacteria** enter and grow. It then seals the chamber and produces filaments that penetrate the ***Nostoc*** colonies. Finally the ***Nostoc*** produces numerous heterocysts. The ***Nostoc*** even travels with the gemmae.

Anthoceros punctatus forms a similar partnership, as do most of the hornworts, but it even stimulates the *Nostoc* to form hormogonia, permitting it to slither toward the hornwort. In both liverwort and hornwort partnerships, the ammonium produced by the cyanobacterial heterocyst is quickly converted to glutamine and glutamate to avoid the buildup of toxic ammonium. The ***Anthoceros*** gets almost 90% of the fixed N and provides fixed C to its **Cyanobacteria** partner.

Moon rock, and rock taken from volcanic areas on Earth, stimulate the growth of bryophytes, but we don't know why. One possibility is the high concentration of iron; another is that symbionts thrived on these rocks, providing N fixation.

It appears that bryophytes play a major role as a substrate for N fixation in many nutrient-poor habitats, making than essential component of those ecosystems.

Nitrogen content varies with species, habitat, season, type of N available, and concentration of N in the ecosystem. It can be sequestered in slowly decaying tissues or translocated to growing regions.

N deficiency, or the wrong form of N (*e.g.* NH_4^+), can cause bryophytes to become long and thin, appearing etiolated. **Glycine**, **serine**, **arginine**, and **alanine** can induce branching. **Methionine** not only did not induce branching, but it also inhibited growth. Glycine caused the greatest weight and length gain of these amino acids in Java moss. Even nucleic acids are usable N sources, with good leafy shoot growth in **adenine** and **guanine**, but no growth in **uracil** or **thymine** in some species and good growth in others. In ***Sphagnum squarrosum*** **uric acid** and **cytosine** caused the plant to be come thalloid.

N enrichment can have initial stimulating effects followed by long-term negative effects, in some case because of competition from other kinds of plants. These differences vary by species and habitat.

Nitrogen cycling among bryophytes is not well understood. We do know that they can release it when dry tissues are rehydrated, but they can also sequester it, serving as sinks, or expose it in recalcitrant forms as tissues decay.

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Literature Cited

Alexander, V. 1975. Nitrogen fixation by blue-green algae in polar and subpolar regions. In: Stewart, W. D. P. (ed.). Nittrogen Fixation by Free-Living Micro-organisms. Cambridge University Press, Cambridge, pp. 175-188.

Alexander, V., Billington, M., and Schell, D. 1974. The influence of abiotic factors on nitrogen fixation rates in the Barrow Alaska Arctic tundra. Rept. Kevo Subarct. Res. Stat. 11: 3-11.

Alexander, V., Billington, M., and Schell, D. M. 1978. Nitrogen fixation in Arctic and alpine tundra. In: Tieszen, L. L. (ed.). Vegetation and Production Ecology of an Alaskan Arctic Tundra. Springer-Verlag, New York, pp. 539-558.

Alghamdi, A. A. 2003. The Effect of Inorganic and Organic Nitrogen Sources and Their Combination on Growth and Metabolism of *Vesicularia dubyana*. Ph. D. Dissertation, Michigan Technological University, Houghton, MI, 150 pp.

Ayres, E., Wal, R. van der, Sommerkorn, M., and Bardgett, R. D. 2006. Direct uptake of soil nitrogen by mosses. Biol. Lett. 2: 2862-2888.

Basile, D. V. and Basile, M. R. 1980. Ammonium ion-induced changes in form and hydroxy-proline content of wall protein in the liverwort *Gymnocolea inflata*. Amer. J. Bot. 67: 500-507.

Basile, D. V., Lin, J.-J., and Varner, J. E. 1988. The metabolism of exogenous hydroxyproline by gametophytes of *Plagiochila arctica* Bryhn et Kaal. (Hepaticae). Planta 175: 539-545.

Basilier, K. 1979. Moss-associated nitrogen fixation in some mire and coniferous forest environments around Uppsala, Sweden. Lindbergia 5: 84-88.

Basilier, K. 1980. Fixation and uptake of nitrogen in *Sphagnum* blue-green algal associations. Oikos 34: 239-242.

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

Bates, J. W. 1994. Responses of the mosses *Brachythecium rutabulum* and *Pseudoscleropodium purum* to a mineral nutrient pulse. Funct. Ecol. 8: 686-692.

Baxter, R., Emes, M. J., and Lee, J. A. 1992. Effects of an experimentally applied increase in ammonium on growth and amino-acid metabolism of *Sphagnum cuspidatum* Ehrh. ex. Hoffm. from differently polluted areas. New Phytol. 120: 265-274.

Belnap, J., Rosentreter, R., Leonard, S., Kaltenecker, J. H., Williams, J., and Eldridge, D. 2001. Biological Soil Crusts:

Ecology and Management. U.S. Dept. of the Interior, Bureau of Land Management, 110 pp.

Bentley, B. L. 1987. Nitrogen fixation by epiphylls in a tropical rainforest. Ann. Missouri Bot. Gard. 74: 234-241.

Bentley, B. L. and Carpenter, E. J. 1980. Effects of desiccation and rehydration on nitrogen fixation by epiphylls in a tropical rainforest. Microbial Ecol. 6: 109-113.

Bentley, B. L. and Carpenter, E. J. 1984. Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. Oecologia 63: 52-56.

Bergamini, A. and Peintinger, M. 2002. Effects of light and nitrogen on morphological plasticity of the moss *Calliergonella cuspidata*. Oikos 96: 355-363.

Bergman, B., Rai, A. N., Johansson, C., and Söderbäck, E. 1993. Cyanobacterial-plant symbioses. In: Galun, M. (ed.). Selected Papers from the International Symbiosis Congress, Balaban Publishers, Rehovot, Israel, 17-22 Nov 1991. Symbiosis 14: 61-81.

Billington, M. M. and Alexander, V. 1983. Site to site variations in nitrogenase activity in a subarctic black spruce (*Picea mariana*) forest. Can. J. Forest Res. 13: 782-788.

Brasell, H. M., Davies, S. K., and Mattay, J. P. 1986. Nitrogen fixation associated with bryophytes colonising burnt sites in Southern Tasmania, Australia. J. Bryol. 14: 139-149.

Broady, P., Given, D., Greenfield, L., and Thompson, K. 1987. The biota and environment of fumaroles on Mt. Melbourne, northern Victoria Land. Polar Biol. 7: 97-113.

Brown, D. H. 1982. Mineral nutrition. In: Smith, A. J. E. (ed.). Bryophyte Ecology. Chapman & Hall, London, pp. 383-444.

Burkholder, P. R. 1959. Organic nutrition of some mosses growing in pure culture. Bryologist 62: 6-15.

Campbell, E. L. and Meeks, J. C. 1989. Characteristics of hormogonia formation by symbiotic *Nostoc* spp. in response to the presence of *Anthoceros punctatus* or its extracellular products. Appl. Environ. Microbiol. 55: 125-131.

Campbell, E. L. and Meeks, J. C. 1992. Evidence for plant-mediated regulation of nitrogenase expression in the *Anthoceros-Nostoc* symbiotic association. J. Gen. Microbiol. 138: 473-480.

Carpenter, E. J. 1992. Nitrogen fixation in the epiphyllae and root nodules of trees in the lowland tropical rainforest of Costa Rica. Acta Oecol. 13: 153-160.

Chapman, R. R. and Hemond, H. F. 1982. Dinitrogen fixation by surface peat and *Sphagnum* in an ombrotrophic bog. Can. J. Bot. 60: 538-543.

Chin, C. M., Maclellan, A. J., and Renzaglia, K. S. 1987. Vegetative growth and reproduction of *Fossombronia brasiliensis* Steph.: The influence of photoperiod, temperature and inorganic nitrogen source. J. Bryol: 14: 581-591.

Christie, P. 1987. Nitrogen in two contrasting Antarctic bryophyte communities. J. Ecol. 75: 73-94.

Clark, D. L., Nadkarni, N. M., and Gholz, H. L. 1998. Growth, net production, litter decomposition, and net nitrogen accumulation by epiphytic bryophytes in a tropical montane forest. Biotropica 30: 12-23.

Clark, K. L., Nadkarni, N. M., and Gholz, H. L. 2005. Retention of inorganic nitrogen by epiphytic bryophytes in a tropical montane forest. Biotropica 37: 328-336.

Cornelissen, J. H. C., Lang, S. I., Soudzilovskaia, N.A., and During, H. J. 2007. Comparative cryptogam ecology: A review of bryophyte and lichen traits that drive biogeochemistry. Ann. Bot. 99: 987-1001.

Costa, J., Paulsrud, P., Rikkinen, J., and Lindblad, P. 2001. Genetic diversity of *Nostoc* symbionts endophytically associated with two bryophyte species. Appl. Environ. Microbiol. 67: 4393-4396.

Cullimore, D. R. and McCann, A. E. 1972. Epiphytic algae isolated from moss. Blue Jay 30: 167-168.

Dalton, D. A. and Chatfield, J. M. 1985. A new nitrogen-fixing cyanophyte-hepatic association: *Nostoc* and *Porella*. Amer. J. Bot. 72: 781-784.

Deising, H. 1987. In vivo studies on the regulation of nitrate reductase in *Sphagnum* species. Symp. Biol. Hung. 35: 59-69.

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

Duckett, J. G. and Pressel, S. 2009. Extraordinary features of the reproductive biology of *Marchantia* at Thursley Common. Field Bryol. 97: 3-11.

Duckett, J. G. and Renzaglia, K. S. 1993. The reproductive biology of the liverwort *Blasia pusilla* L. J. Bryol. 17: 541-552.

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

Duckett, J. G., Fletcher, P., Francis, R., Matcham, H. W., Russell, A. J., and Pressel, S. 2004. *In vitro* cultivation of bryophytes; practicalities, progress, problems and promise. J. Bryol. 26: 3-20.

Eckstein, R. L. 2000. Nitrogen retention by *Hylocomium splendens* in a subarctic birch woodland. J. Ecol. 88: 506-515.

Eckstein, R. L. and Karlsson, P. S. 1999. Recycling of nitrogen among segments of *Hylocomium splendens* as compared with *Polytrichum commune*: Implications for clonal integration in an ectohydric bryophyte. Oikos 86: 87-96.

Egorov, V. I. 2007. The nitrogen regime and biological fixation of nitrogen in moss communities (the Khibiny Mountains). Eurasian Soil Sci. 40: 463-467.

Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Büdel, B., Andreae, M. O., and Pöschl, U. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nat. Geosci. 5: 459-462.

Enderlin, C. S. and Meeks, J. C. 1983. Pure culture and reconstitution of the *Anthoceros-Nostoc* symbiotic association. Planta 158: 157-165.

Englund, B. 1976. Nitrogen fixation by free-living microorganisms on the lava field of Heimaey, Iceland. Oikos 27: 428-432.

Fogg, E. G. and Stewart, W. D. P. 1968. *In situ* determinations of biological nitrogen fixation in Antarctica. Brit. Antarct. Surv. Bull. 15: 39-46.

Forsum, Å., Dahlman, L., Näsholm, T., and Nordin, A. 2006. Nitrogen utilization by *Hylocomium splendens* in a boreal forest fertilization experiment. Funct. Ecol. 20: 421-426.

Frahm, J.-P. 1975. Toxitoleranzversuche an Wassermoosen. Gewass. Abwass. 57/58: 59-66.

Giddens, J. E. 1982. Nitrogen fixation in soil crusts of tall fescue (*Festuca arundinacea*) sods. Soil Sci. 133(5): 295-297.

Gilbert, D., Francez, A.-J., Amblard, C., and Bourdier, G. 1999. The microbial communities at the surface of the *Sphagnum*. Ecologie Brunoy 30(1): 45-52.

Given, D. R. 1987. Plants in the Antarctic. Newslett. Spec. Surv. Commiss. 8: 25.

Gordon, C., Wynn, J. M., and Woodin, S. J. 2001. Impacts of increased nitrogen supply on high Arctic heath: The importance of bryophytes and phosphorus availability. New Phytol. 149: 461-471.

Granhall, U. and Hofsten, A. 1976. Nitrogenase activity in relation to intracellular organisms in *Sphagnum* mosses. Physiol. Plant. 36: 88-94.

Granhall, U. and Lindberg, T. 1978. Nitrogen fixation in some coniferous forest ecosystems. In: Granhall, U. (ed.). Environmental Role of Nitrogen-fixing Blue-green Algae and Asymbiotic Bacteria, Ecol. Bull., Stockholm, pp. 178-192.

Granhall, U. and Selander, H. 1973. Nitrogen fixation in a subarctic mire. Oikos 24: 8-15.

Greenfield, L. G. 1992. Retention of precipitation nitrogen by Antarctic mosses, lichens and fellfield soils. Antarct. Sci. 4: 205-206.

Griggs, R. F. 1937. Growth of liverworts on "nitrogen-free" sand. Amer. J. Bot. 24: 295-298.

Gundale, M. J., Deluca, T. H., and Nordin, A. 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Global Change Biol. 17: 2743-2753.

Heijmans, M. M. P. D., Berendse, F., Arp, W. J., Maaseliuk, A. K., Klees, H., Visser, W. De, and Breeman, N. van. 2001. Effects of elevated carbon dioxide and increased nitrogen deposition on bog vegetation in The Netherlands. J. Ecol. 89: 269-279.

Hemond, H. F. 1983. The nitrogen budget of Thoreau's bog. Ecology 64: 99-109.

Henriksson, E., Henriksson, L. E., Norrman, J. O., and Nyman, P. O. 1987. Biological dinitrogen fixation (acetylene reduction) exhibited by blue-green algae (Cyanobacteria) in association with mosses gathered on Surtsey, Iceland. Arct. Alp. Res. 19: 432-436.

Hobbie, S. E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol. Monogr. 66: 503-522.

Hoffman, G. R. 1966. Ecological studies of *Funaria hygrometrica* (L.) Hedw. in eastern Washington and northern Idaho. Ecol. Monogr. 36: 157-180.

Hoffman, G. R. 1974. Growth stimulation of *Marchantia polymorpha* from ilmenite basalt and volcanic ash. Bryologist 77: 632-636.

Honegger, R. 1980. Zytologie der blaualgen-hornmoos-symbiose bei *Anthoceros laevis* aus Island. Flora 170: 290-302.

Hooper, C. A. 1982. An experimental study of algal communities on *Sphagnum*. Unpubl. Ph. D. Dissertation, University of Michigan, 179 pp.

Huntley, B. J. 1971. Vegetation. In: Zindern Bakker, E. M. van, Winterbottom, J. M., and Dyer, R. A. (eds.). Marion and Prince Edward Islands; Report on the South African Biological and Geological Expedition, 1965-1966. A. A. Balkema, Capetown.

Jauhiainen, J., Wallen, B., and Malmer, N. 1998. Potential NH_4^+ and NO_3^- uptake in seven *Sphagnum* species. New Phytol. 138: 287-293.

Joenje, W. and During, H. J. 1977. Colonisation of a desalinating Wadden-polder by bryophytes. Plant Ecol. 35: 177-185.

Jones, K. and Wilson, R. E.. 1978. The fate of nitrogen fixed by a free-living blue-green alga. In: Granhall, U. (ed.). Environmental Role of Nitrogen-fixing Blue-green Algae and Asymbiotic Bacteria. Ecol. Bull. (Stockholm) 26: 158-163.

Jones, M. L. M., Oxley, E. R. B., and Ashenden, T. W. 2002. The influence of nitrogen deposition, competition and desiccation on growth and regeneration of *Racomitrium lanuginosum* (Hedw.) Brid. Environ. Pollut. 120: 371-378.

Joseph, M. and Meeks, J. C. 1987. Regulation of expression of glutamine synthetase in a symbiotic *Nostoc* strain associated with *Anthoceros punctatus*. J. Bacteriol. 169: 2471-2475.

Kielland, K. 1997. Role of free amino acids in the nitrogen economy of Arctic cryptogams. Ecoscience 4: 75-79.

Killian, C. 1923. Cultures d'Hepatiques. Soc. Biol. Paris (Compt. Rend.) 88: 746-748.

Kimura, J. and Nakano, T. 1990. Reconstitution of a *Blasia-Nostoc* symbiotic association under axenic conditions. Nova Hedw. 50: 191-200.

Kopáček, J. and Blažzka, P. 1994. Ammonium uptake in alpine streams in the High Tatra Mountains (Slovakia). Hydrobiologia 294: 157-165.

Koranda, M., Kerschbaum, S., Wanek, W., Zechmeister, H., and Richer, A. 2007. Physiological responses of bryophytes *Thuidium tamariscinum* and *Hylocomium splendens* to increased nitrogen deposition. Ann. Bot. 99: 161-169.

Kotanen, P. M. 2002. Fates of added nitrogen in freshwater Arctic wetlands grazed by snow geese: The role of mosses. Arct. Antarct. Alp. Res. 34: 219-225.

Kull, O., Aan, A., and Soelsepp, T. 1995. Light interception, nitrogen and leaf mass-distribution in a multilayer plant community. Funct. Ecol. 9: 589-595.

Lambert, R. L. and Reiners, W. A. 1979. Nitrogen-fixing moss associations in the subalpine zone of the White Mountains, New Hampshire. Arct. Alp. Res. 11: 325-333.

Leizerovich, I., Kardish, N., and Galun, M. 1990. Comparison between eight symbiotic, cultured *Nostoc* by recombinant DNA. Symbiosis 8: 75-85.

Lindo, Z. and Whiteley, J. A. 2011. Old trees contribute bio-available nitrogen through canopy bryophytes. Plant Soil 342: 141-148.

Line, M. A. 1992. Nitrogen fixation in the sub-Antarctic Macquarie Island. Polar Biol. 11: 601-606.

Lockwood, L. G. 1975. The influence of photoperiod and exogenous nitrogen-containing compounds on the reproductive cycles of the liverwort *Cephalozia media*. Amer. J. Bot. 62: 893-900.

Lovelock, J. 1988. The Ages of Gaia. Bantam Books, New York, 252 pp.

Machlis, L. 1962. The effects of mineral salts, glucose, and light on the growth of the liverwort, *Sphaerocarpos donnellii*. Physiol. Plant. 15: 354-362.

Madhusoodanan, P. V. and Dominic, T. K. 1996. Epiphytic Cyanobacteria on mosses from Western Ghats of Kerala. J. Econ. Tax. Bot. 20: 355-360.

Matsuda, Y., Shimada, T., and Sakamoto, Y. 1992. Ammonium ions control gametic differentiation and dedifferentiation in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 33: 909-914.

McKane, R., Johnson, L., Shaver, G., Nadelhoffer, K., Fry, B., Rastetter, E., Giblin, A., and Laundre, J. 1993. Differentiation in uptake of ^{15}N by depth, season, and chemical form in an arctic tussock tundra plant community. 78th Ann. ESA Meeting, 31 July - 4 August 1993. Bull. Ecol. Soc. Amer. Program and Abstracts, Suppl. vol 74(2): 354.

Meeks, J. C. 1990. Cyanobacterial-bryophyte associations. In: Rai, R. N. (ed.). Handbook of Symbiotic Cyanobacteria. CRC Press, Boca Raton, pp. 43-63.

Meeks, J. C., Enderlin, C. S., Wycoff, K. L., Chapman, J. S., and Joseph, C. M. 1983. Assimilation of $^{13}NH_4^+$ by *Anthoceros*

grown with and without symbiotic *Nostoc*. Planta 158: 384-391.

Meeks, J. C., Enderlin, C. S., Joseph, C. M., Chapman, J. S., and Lollar, M. W. L. 1985. Fixation of $[^{13}N]N_2$ and transfer of fixed nitrogen in the *Anthoceros-Nostoc* symbiotic association. Planta 164: 406-414.

Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R. J. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429: 292-294.

Miyazaki, T. and Satake, K. 1985. *In situ* measurements of uptake of inorganic carbon and nitrogen by the aquatic liverworts *Jungermannia vulcanicola* Steph. and *Scapania undulata* (L.) Dum. in an acid stream, Kashiranashigawa, Japan. Hydrobiologia 124: 29-34.

Moore, C. M., Mills, M. M., Achterberg, E. P., Geider, R. J., LaRoche, J., Lucas, M. I., McDonagh, E. L., Pan, X., Poulton, A. J., Rijkenberg, M. J. A., Suggett, D. J., Ussher, S. J., and Woodward, E. M. S. 2009. Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nat. Geosci. 2: 867-871.

Morimoto, K. and Maruyama, Y. 1982. Nitrogen fixation by *Sphagnum* and blue-green algae system in the Ozegahara moor. Ozegahara: Scientific Researches of the Highmoor in Central Japan, pp. 231-241.

Nakatsubo, T. and Ino, Y. 1986. Nitrogen cycling in an Antarctic ecosystem 1. Biological nitrogen fixation in the vicinity of Syowa Station Japan. Mem. Natl. Inst. Polar Res. Ser. E Biol. Med. Sci. 37: 1-10.

Nakatsubo, T. and Ino, Y. 1987. Nitrogen cycling in an Antarctic ecosystem 2. Estimation of the amount of nitrogen fixation in a moss community on East Ongul Island. Ecol. Res. 2: 31-40.

Nakatsubo, T. and Ohtani, S. 1991. Nitrogen-fixing (C_2H_2-reducing) Cyanobacteria epiphytic of moss communities in the alpine zone of Mt. Fuji. Proc. Natl. Inst. Polar Res. Symp. Polar Biol. 4: 75-81.

Nordin, A. and Gunnarsson, U. 2000. Amino acid accumulation and growth of *Sphagnum* under different levels of N deposition. Ecoscience 7: 474-480.

Nordin, A., Nasholm, T., and Ericson, L. 1998. Effects of simulated N deposition on understorey vegetation of a boreal coniferous forest. Funct. Ecol. 12: 691-699.

Orebamjo, T. O. and Stewart, G. R. 1975. Ammonium repression of nitrate reductase formation in *Lemna minor* L. Planta 122: 27-36.

Paffen, B. G. P. and Roelefs, J. G. M. 1991. Impact of carbon dioxide and ammonium on the growth of submerged *Sphagnum cuspidatum*. Aquat. Bot. 40: 61-71.

Pandey, K. D., Kashyap, A. K., and Gupta, R. K. 1992. Nitrogen fixation by Cyanobacteria associated with moss communities in Schirmacher Oasis, Antarctica. Israel J. Bot. 41: 187-198.

Paulissen, M. P., Ven, P. J. Van Der, Dees, A. J., and Bobbink, R. 2004. Differential effects of nitrate and ammonium on three fen bryophyte species in relation to pollutant nitrogen input. New Phytol. 164: 451-458.

Peirce, G. J. 1906. *Anthoceros* and its *Nostoc* colonies. Bot. Gaz. 42: 55-59.

Peters, G. A. 1991. *Azolla* and other plant-Cyanobacteria symbioses: Aspects of form and function. Plant Soil 137: 25-36.

Pressel, S., Matcham, H. W., and Duckett, J. G. 2007. Studies of protonemal morphogenesis in mosses. XI. *Bryum* and related genera; a plethora of propagules. J. Bryol. 29: 241-258.

Prakasham, R. and Rai, A. N. 1991. Evidence for the occurrence of a specific methylammonium transport system in the cultured cyanobiont of the *Anthoceros punctatus-Nostoc* association. J. Gen. Microbiol. 137: 1783-1788.

Rai, A. N., Borthakur, M., Singh, S., and Bergman, B. 1989. *Anthoceros-Nostoc* symbiosis: Immunoelectronmicroscopic localization of nitrogenase, glutamine synthetase, phycoerythrin and ribulose 1,5-biphosphate carboxylase/oxygenase in the cyanobiont and the cultured (free-living) isolate *Nostoc* 7801. J. Gen. Microbiol. 135: 385-395.

Rai, A. N., Soderback, E., and Bergman, B. 2000. Tansley Review No. 116. Cyanobacterium - Plant symbioses. New Phytol. 147: 449-481.

Renzaglia, K. 1982a. Apical development and the production of *Nostoc* auricles in *Blasia pusilla* L. Misc. Publ. Bot. Soc. Amer. 162: 4.

Renzaglia, K. S. 1982b. A comparative developmental investigation of the gametophyte generation in the Metzgeriales (Hepatophyta). Bryophyt. Biblio. 24: 1-253.

Ridgway, J. E. 1967. The biotic relationship of *Anthoceros* and *Phaeoceros* to certain Cyanophyta. Ann. Missouri Bot. Gard. 54: 95-102.

Rodgers, G. A. 1978. The effect of some external factors on nitrogenase activity in the free-living and endophytic *Nostoc* of the liverwort *Blasia pusilla*. Physiol. Plant. 44: 407-411.

Rodgers, G. A. and Henriksson, E. 1976. Associations between the blue-green algae *Anabaena variabilis* and *Nostoc muscorum* and the moss *Funaria hygrometrica* with reference to the colonization of Surtsey. Acta Bot. Islandica 4: 10-15.

Rosswall, T. and Granhall, U. 1980. Nitrogen cycling in a subarctic ombrotrophic mire. In: Sonesson, M. (ed.). Ecology of a Subarctic Mire. Ecol. Bull. (Stockholm) 30: 209-234.

Rudolph, H. J. and Voigt, J. U. 1986. Effects of NH_4^+-N and NO_3^--N on growth and metabolism of *Sphagnum magellanicum*. Physiol. Plant. 66: 339-343.

Rudolph, H., Deising, H., and Voigt, J. U. 1987. The tolerance of raised bog species in respect to inorganic nitrogen. Symp. Biol. Hung. 35: 71-80.

Salisbury, F. B. and Ross, C. W. 1978. Plant Physiology. Wadsworth Publ. Co., Inc., Belmont, Calif. 2nd Edn.

Sanville, W. 1988. Response of an Alaskan wetland to nutrient enrichment. Aquat. Bot. 30: 231-243.

Satake, K. 1980. Limnological studies on inorganic acid lakes in Japan. Jap. J. Limnol. 41: 41-50.

Saxena, D. K. 1981. Role of *Anthoceros* as a bio-fertilizer. XIII International Botanical Congress Abstracts, Sydney vol. 292.

Schuler, J. F., Diller, V. M., Fulford, M., and Kerstein, H. J. 1955. Plant Physiol. 30: 478-482. Cited in: Brown, D. H. 1982. Mineral nutrition. In: Smith, A. J. E. (ed.). Bryophyte Ecology. Chapman & Hall, London, pp. 383-444.

Schuster, R. M. 1992a. The Hepaticae and Anthocerotae of North America East of the Hundredth Meridian. Vol. V. Field Museum of Natural History, Chicago, Ill.

Schuster, R. M. 1992b. The Hepaticae and Anthocerotae of North America East of the Hundredth Meridian. Vol. VI. Field Museum of Natural History, Chicago, Ill.

Schuurkes, J. A. A. R., Kok, C. J., and Den Hartog, C. 1986. Ammonium and nitrate uptake by aquatic plants from poorly buffered and acidified waters. Aquat. Bot. 24: 131-146.

Schwoerbel, J. and Tillmanns, G. C. 1964. Untersuchungen uber die Stoffwechseldynamik in Fliebgewassern. Arch. Hydrobiol. (Suppl.) 28: 259-267.

Schwoerbel, J. and Tillmanns, G. C. 1972. Adaptation to ammonia *in situ* by submerged macrophytes. Arch. Hydrobiol. (Suppl.) 42, 1: 139-141.

Schwoerbel, J. and Tillmanns, G. C. 1974. Assimilation of nitrogen from the medium and nitrate reductase activity in submerged macrophytes: *Fontinalis antipyretica* L. Arch. Hydrobiol. Suppl. 47: 282-294.

Schwoerbel, J. and Tillmanns, G. C. 1977. Uptake of nitrate from the water and activity of nitrate reductase by *Fontinalis antipyretica* L. under light and dark conditions. Arch. Hydrobiol. (Suppl.) 48: 412-423.

Selkirk, P. M. 1979. Effect of nutritional conditions on sexual reproduction in *Riccia*. Bryologist 82: 37-46.

Sheridan, R. P. 1991. Nitrogenase activity by *Hapalosiphon flexuosus* associated with *Sphagnum erythrocalyx* mats in the cloud forest on the volcano La Soufriere Guadeloupe French West Indies. Biotropica 23: 134-140.

Simola, L. K. 1975. The effect of several protein amino acids and some inorganic nitrogen sources on the growth of *Sphagnum nemoreum*. Physiol. Plant 35: 194-199.

Simola, L. K. 1979. Dipeptide utilization by *Sphagnum fimbriatum*. J. Hattori Bot. Lab. 46: 49-54.

Singh, H. V. and B. R. Chaudhary, B. R. 1990. Nutrient effects on the formation of oogonia in *Oedogonium hatei* (Chlorophyta). Phycologia 29: 332-337.

Sironval, C. 1947. Expériences sur les stades de développement de la forme filamenteuse en culture de *Funaria hygrometrica* L. Bull. Soc. Bot. Belg. 29(1-2): 48-78.

Smith, V. R. 1984. Effects of abiotic factors on acetylene reduction by Cyanobacteria epiphytic on moss at a subAntarctic island. Appl. Environ. Microbiol. 48: 594-600.

Smith, V. R. 1993. Effect of nutrients on carbon dioxide assimilation by mosses on a sub-Antarctic island. New Phytol. 123: 693-697.

Smith, V. R. and Ashton, P. J. 1981. Bryophyte-Cyanobacteria associations on sub-Antarctic Marion Island: Are they important in nitrogen fixation? S. Afr. T. Nav. Antarkt., Deel 10/11: 24-26.

Smith, V. R. and Russell, S. 1982. Acetylene reduction by bryophyte-Cyanobacteria associations on a subAntarctic island. Polar Biol. 1: 153-157.

Snyder, J. M. and Wullstein, L. H. 1973a. Nitrogen fixation in granite outcrop pioneer systems. Bryologist 76: 197-199.

Snyder, J. M. and Wullstein, L. H. 1973b. The role of desert cryptogams in nitrogen fixation. Amer. Midl. Nat. 90: 257-265.

Soares, A. and Pearson, J. 1997. Short-term physiological responses of mosses to atmospheric ammonium and nitrate. Water Air Soil Pollut. 93: 225-242.

Solheim, B., Endal, A., and Vigstad, H. 1996. Nitrogen fixation in Arctic vegetation and soils from Svalbard, Norway. Polar Biol. 16: 35-40.

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

Sood, S. 1974. In vitro studies in Marchantiales. II. Effect of mineral nutrients, chelates and organic nitrogenous sources on the growth and sexuality in *Riccia crystallina*. Phytomorphology 24: 186-197.

Southorn, A. L. D. 1977. Bryophyte recolonization of burnt ground with particular reference to *Funaria hygrometrica*. II. The nutrient requirements of *Funaria hygrometrica*. J. Bryol. 9: 361-374.

Steinberg, N. A. and Meeks, J. C. 1987. Phototrophic and heterotrophic nitrogenase activity by the Cyanobacterium *Nostoc* in symbiosis with the bryophyte *Anthoceros*. Plant Physiol. (Bethesda) 83 (4 suppl.): 24.

Steinberg, N. A. and Meeks, J. C. 1989. Photosynthetic CO_2 fixation and ribulose bisphosphate carboxylase/oxygenase activity of *Nostoc* strain UCD 7801 in symbiotic association with *Anthoceros punctatus*. J. Bacteriol. 171: 6227-6233.

Steinberg, N. A. and Meeks, J. C. 1991. Physiological sources of reductant for nitrogen fixation activity in *Nostoc* strain UCD 7801 in symbiotic association with *Anthoceros punctatus*. J. Bacteriol. 173: 7324-7329.

Stewart, W. D. P. 1967. Nitrogen fixing plants. Science 158: 1426-1432.

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

Stewart, W. D. P. and Rodgers, G. A. 1978. Studies on the symbiotic blue-green algae of *Anthoceros*, *Blasia* and *Peltigera*. Ecol. Bull. 26: 247-259.

Strengbom, J., Nordin, A., Näsholm, T., and Ericson, L. 2001. Slow recovery of boreal forest ecosystem following decreased nitrogen input. Funct. Ecol. 15: 451-457.

Syrett, P. J. and Morris, I. 1963. The inhibition of nitrate assimilation by ammonium in *Chlorella*. Biochim. Biophys. Acta – Specialized Section on Enzymological Subjects 67: 566-575.

Touffet, J. 1971. Apercu phytosociologique et écologique syr les tourbières des pentes du Finistère. Bot. Rhedonica 2(2): 77-80.

Trainor, F. R. 1959. A comparative study of sexual reproduction in four species of *Chlamydomonas*. Amer. J. Bot. 46: 65-70.

Twenhöven, F. L. 1992. Competition between two *Sphagnum* species under different deposition levels. J. Bryol. 17: 71-80.

Vanderpoorten, A. 2000. Hydrochemical determinism and molecular systematics in the genus *Amblystegium* (Musci). Application to the biomonitoring of surface waters. Dissertation de docteur en sciences agronomiques et ingénierie biologique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium, 99 pp.

Vieira, A. R., Gonzalez, C., Martins-Loução, M. A., Branquinho, C. 2009. Intracellular and extracellular ammonium (NH_4^+) uptake and its toxic effects on the aquatic biomonitor *Fontinalis antipyretica*. Ecotoxicology 18: 1087-1094.

Vlassak, K., Paul, E. A., and Harris, R. E. 1973. Assessment of biological nitrogen fixation in grassland and associated sites. Plant Soil 38: 637-649.

Voth, P. D. and Hamner, K. C. 1940. Responses in *Marchantia polymorpha* to nutrient supply and photoperiod. Bot. Gaz. 102: 169-205.

Wanek, W. and Pörtl, K. 2008. Short-term 15 N uptake kinetics and nitrogen nutrition of bryophytes in a lowland rainforest, Costa Rica. Funct. Plant Biol.: 35: 51-62.

Wann, F. B. 1925. Some of the factors involved in sexual reproduction of *Marchantia polymorpha*. Amer. J. Bot. 12: 307-318.

West, N. J. and Adams, D. G. 1997. Phenotypic and genotypic comparison of symbiotic and free-living Cyanobacteria from a single field site. Appl. Environ. Microbiol. 63: 4479-4484.

Wilson, R. E. 1975. Growth and nitrogen fixation by a species of *Nostoc* found in association with the moss *Gymnostomum recurvirostrum* on soil hummocks by Slapstone Sike, Upper Teesdale. M. Sc. Thesis, Univ. Lancaster.

Wong, F. C. Y. and Meeks, J. C. 2002. Establishment of a functional symbiosis between the Cyanobacterium *Nostoc*

punctiforme and the bryophyte *Anthoceros punctatus* requires genes involved in nitrogen control and initiation of heterocyst differentiation. Microbiology 148(1): 315-323.

Zackrisson, O., DeLuca, T. H., Gentili, F., Sellstedt, A., and Jäderlund, A. 2009. Nitrogen fixation in mixed *Hylocomium splendens* moss communities. Oecologia 160: 309-319.

Zimicki, J. 1976. The role of *Sphagnum* moss in the immobilization of nitrogen in montane ecosystems. Unpublished report for the Mellon Foundation, Dartmouth College, Hanover, N.H.