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NONSYMBIOTIC AND SYMBIOTIC NITROGEN FIXATION IN A WEAKLY MINEROTROPHIC PEATLAND¹

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ABSTRACT

The acetylene reduction assay was used to measure nonsymbiotic and symbiotic nitrogen fixation in a weakly minerotrophic peatland throughout the ice-free season. Nonsymbiotic nitrogen fixation was found in surface materials and subsurface peat. In surface materials, nitrogenase activity measured in the field contributed about 0.6 kg N ha⁻¹ yr⁻¹, was closely associated with *Sphagnum*, but was not correlated with temperature between 12 and 27 C. No cyanobacteria were found in association with *Sphagnum*. In subsurface peat, nitrogenase activity measured in situ contributed no more than 0.4 kg N ha⁻¹ yr⁻¹ and was closely correlated with temperature between 7 and 21 C. There were uncertainties in these measurements due to presence of ethylene oxidizing activity and a long time lag. Symbiotic nitrogen fixation was found only in actinomycete-induced root nodules of *Myrica gale* L. Legumes were absent and the few lichens present lacked nitrogenase activity. Based on acetylene reduction assays, *Myrica gale* fixed about 35 kg N ha⁻¹ yr⁻¹. Nitrogenase activity in *Myrica gale* showed a strong seasonal pattern which varied little during three consecutive years even though water levels varied substantially. Nitrogen input to the peatland from nonsymbiotic nitrogen fixation was only 15% the amount contributed by bulk precipitation. Symbiotic fixation, in contrast, contributed approximately six times the amount in bulk precipitation.

RELATIVELY LITTLE IS KNOWN about annual inputs of nitrogen into peatlands (mires) via biological dinitrogen fixation. Related studies concerning flooded soils, especially rice paddies, have been more numerous and have recently been reviewed (Buresh, Casselman and Patrick, 1980). In peatlands nitrogenase activity occurs in heterotrophic bacteria, free-living cyanobacteria (blue green algae), quasi-symbiotic cyanobacteria associated with *Sphagnum*, lichens, actinorhizal (actinomycete-nodulated) plants, and legumes. Nitrogenase activity due to heterotrophic bacteria is present in a wide variety of peatlands in Europe and North America (Granhall and Selander, 1973; Blasco and Jordan, 1976; Waughman and Bellamy, 1980). Nitrogenase activity due to free-living and quasi-symbiotic cyanobacteria, as well as lichens containing cyanobacteria, has also been found in Europe and North America (Granhall and Selander, 1973; Alexander and Schell, 1973; Blasco and Jordan, 1976; and others). Actinorhizal plants, namely *Myrica gale* and *Alnus* spp., also grow in both Euro-

pean and North American peatlands, where they can have substantial nitrogenase activity (Akkermans, 1971; Akkermans and van Dijk, 1976; Sprent, Scott and Perry, 1978; Schwintzer, 1979). In addition a legume, *Lathyrus palustris*, occurs in some strongly minerotrophic peatlands in North America (Curtis, 1959).

In the present study, I examined all components of a weakly minerotrophic peatland containing vigorous stands of *Myrica gale* for nitrogenase activity to determine the relative amounts of nitrogen fixed by *Myrica gale* and other agents of biological nitrogen fixation. Seasonal variations in nitrogenase activity were also examined.

MATERIALS AND METHODS—Study area—The study site is located in Tom Swamp near Harvard Pond (42°30'N, 72°12'W, elevation approximately 252 m) in the Harvard University Forest at Petersham, Massachusetts. Most observations were made in a 0.7-ha area dominated by *Myrica gale* and designated "Open Mat" in an earlier study (Schwintzer, 1979). This site is located in an extensive open peatland (about 400 m × 400 m) on a partially floating mat at the northern end of the lake. Measurements of subsurface nitrogenase activity in the peat were made in adjacent parts of the open peatland lacking *Myrica gale*.

The vegetation of the Open Mat site is dominated by *Myrica gale* (mean cover 53%) and

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Chamaedaphne calyculata (12%). The ground layer covering the hummocks and hollows on the mat surface consists primarily of *Sphagnum* spp. (mean cover 35%) and leaf and woody litter. The peat is weakly minerotrophic and has acidic (pH 3.9) shallow ground waters low in metal ions (Schwintzer, 1979). Further details of the Open Mat site including information on other studies in the area are given by Schwintzer (1979).

Methods—All measurements of nitrogenase activity were made with the acetylene reduction technique (Hardy, Burns and Holsten, 1973), which has proven to be reliable when correctly used, except under some specialized circumstances (Buresh et al., 1980; Knowles, 1981).

Peat surface: Nitrogenase activity associated with *Sphagnum* spp., litter and other surface materials was measured in the field on sunny days. Samples were taken at randomly selected points along three 2-m-wide belt transects widely spaced within the Open Mat site. Cores of surface materials (5.5 cm diameter; 5 cm deep) were removed with minimal disturbance and placed in 245-ml glass canning jars having metal lids fitted with a rubber septum. The jars, with the samples in their original orientation, were placed on the ground surface where the samples had been removed and were injected with acetylene (10% by volume). Gas samples were removed at the end of the 45-min incubation period, brought to the laboratory and injected into a Carle model 9500 gas chromatograph having a flame ionization detector as described previously (Schwintzer, 1979).

The temperature of the surface materials was measured with a thermistor probe immediately before they were placed in the jars and again at the end the incubation period.

Annual nitrogen fixation in the surface materials was estimated by assuming that the mean measured nitrogenase activity prevailed for 24 hr per day for 200 days (the approximate ice-free period) and a C_2H_4 to N_2 conversion factor of 4.0. The actual conversion factor may be greater and is probably variable. Some recent measurements include 4.5 in the *Sphagnum*-cyanobacteria quasi-symbiosis (Basilier, 1980), 3.4–7.0 in decaying wood depending on length of incubation (Silvester, 1981) and 5.3–19.6 in lichens depending on season and environment (Millbank, 1981) and 3.5 in surface *Sphagnum* peat (Chapman and Hemond 1982).

Subsurface peat: Nitrogenase activity associated with the subsurface peat was measured in peat cores in the laboratory and in situ.

Samples were taken in midmorning at two areas of the open peatland adjacent to the Open Mat and lacking *Myrica gale* but otherwise similar to the Open Mat site. The vascular vegetation was clipped at ground level and surface materials to 5 cm deep were removed to eliminate overlap with the peat surface measurements. Cores 18 cm in diameter and extending 4 cm below the water table were cut. The above-the-water-table portion of the cores ranged from 0.5 to 10 cm in thickness depending on microrelief and the hydrology of the wetland.

For laboratory experiments the cores were cut at the bottom and placed in 5-l plastic tubs equipped with snap-on plastic lids fitted with two vents and a serum stopper. Water from the peatland was added to the original depth, and the cores were returned to the laboratory and placed in an incubator. The lids were sealed, the desired gasses were added depending on the experiment, and 1-ml gas samples were removed periodically and injected into the gas chromatograph. The gas volume in each tub was determined by displacement with water. Acetylene reduction was measured in the presence of 10% acetylene by volume. Ethylene oxidation was measured in the absence of acetylene with an initial ethylene concentration of 4 ppm (v/v). Controls containing 4 cm of tap water and lacking peat were used to correct for the disappearance of ethylene due to adsorption on the tub, solution in water, and leakage. Endogeneous ethylene production was measured in air.

For in situ experiments, the cores were left attached at the bottom and enclosed in inverted 5-l plastic tubs sealed below by the water table. The tubs were equipped with two vents, a serum stopper and a 17 × 17-cm Saran bladder. The bladders adjusted the gas pressure with changing temperatures, thus keeping the water level and aeration regime constant during the experiment. After preparation of the cores, the Saran bladders were filled with 400 ml of air, and 500 ml acetylene was added through one of the vents while atmospheric pressure was maintained by gas escaping from the second vent. The vents were sealed and aluminum shades were placed over the tubs to minimize temperature changes. Gas samples were taken at the end of 22 and 30 hr, returned to the laboratory and injected into the gas chromatograph. The measured acetylene concentration at 30 hr was approximately 10%. Subsurface temperatures were monitored with a recording thermometer. Rates of acetylene reduction were calculated from the change in ethylene concentration between 22 and 30 hr, a gas volume of 3,900 ml (the volume in the

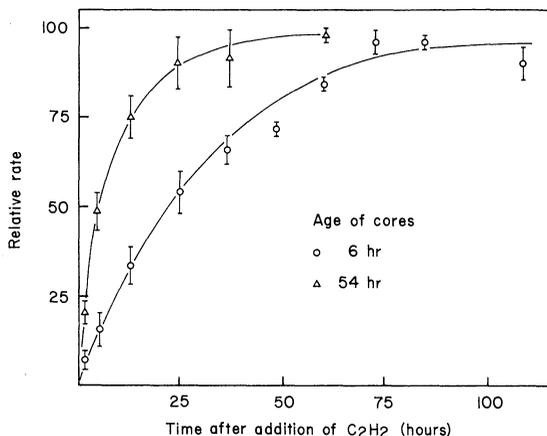


Fig. 1. Effect of the age of subsurface peat cores at the time of acetylene addition on the rate of acetylene reduction. Values are $\bar{x} \pm SE$; $n = 5$. Relative rates were obtained by setting the maximum rate for each core equal to 100. The maximum rates were $10.7 \pm 2.1 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ hr}^{-1}$ ($\bar{x} \pm SE$; $n = 5$) for cores aged 6 hr and 10.5 ± 2.2 for cores aged 54 hr. Measurements were made in the laboratory at a temperature of 16 C.

tubs above the water table) and a surface area of 254 cm².

I measured nitrogenase activity between 20 and 30 hr after the addition of acetylene because a preliminary laboratory experiment (Fig. 1) showed that the acetylene-dependent phase of the long time lag following addition of acetylene commonly seen in waterlogged soils (e.g., Waughman and Bellamy, 1972; Tjepkema and Evans, 1976; Lee, Alimagno and Yoshida, 1977) is largely complete and the system has not had time to change radically from its original condition. In Fig. 1, the much more rapid increase in acetylene reduction rates in cores aged 54 hr at the time of acetylene addition shows that the lag seen in fresh cores (aged 6 hr) consisted of at least two components, one dependent on acetylene and the other acetylene-independent. The acetylene-dependent component of the lag probably involves diffusion of acetylene to the sites of nitrogenase activity and release of ethylene to the atmosphere (Watanabe, Lee and Alimagno, 1978). Stimulation of nitrogenase activity by acetylene during extended incubation may also contribute to this component (David and Fay, 1977; David, Apte and Thomas, 1978; Silvester, 1981). The acetylene-independent component probably reflects metabolic activity. It may involve a variety of processes (Tjepkema and Evans, 1976; Waughman, 1976) including reduction of pO₂ at various microsites to levels more favorable to nitrogenase activity and pro-

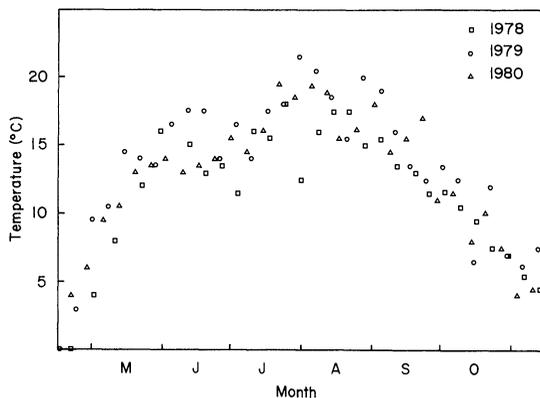


Fig. 2. Peat temperature at 15 cm below the surface in a Massachusetts peatland. Measurements were made at 9 a.m. as described by Schwintzer (1979).

liferation of nitrogen fixing bacteria in response to changes in pO₂ or newly available substrates.

Annual nitrogen fixation in the subsurface peat was calculated as follows: Annual nitrogenase activity per m² was taken as the area under a smoothed curve fitted by eye to the data in Fig. 5. Nitrogenase activity was assumed to become measurable about May 1 when the subsurface temperature exceeded 7 C in 1980 (Fig. 2). A value of 4.0 was used for the C₂H₄ to N₂ conversion factor as in the calculation of annual surface fixation.

Myrica gale nodules: Nitrogenase activity in *Myrica gale* nodules was measured at the Open Mat site at approximately 3-wk intervals from May through October in 1978 (Schwintzer,

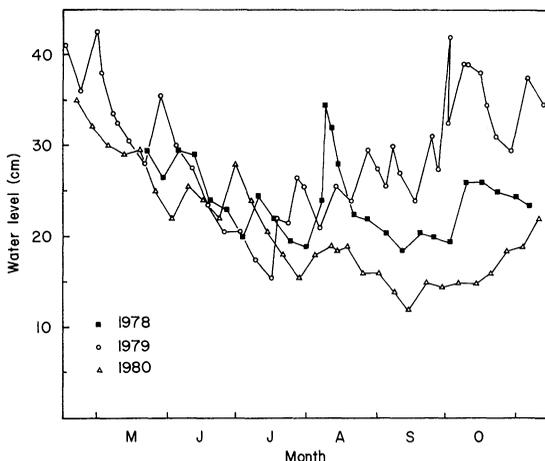


Fig. 3. Water level in a Massachusetts peatland. Measurements were made at 9 a.m. relative to a fixed point with an assigned level of 100 cm as described by Schwintzer (1979).

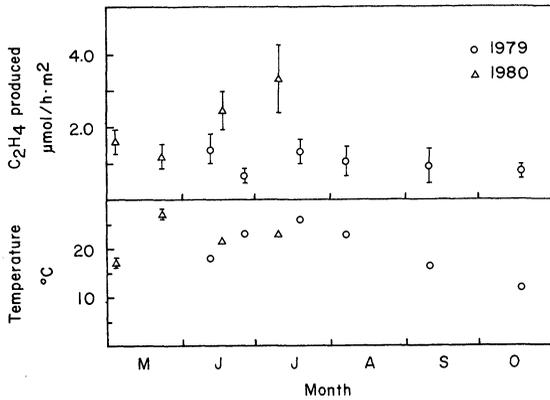


Fig. 4. Nitrogenase activity in surface materials in the light and corresponding surface temperatures in a Massachusetts peatland. Values are $\bar{x} \pm SE$; $n = 15$ (1979), 20 (1980).

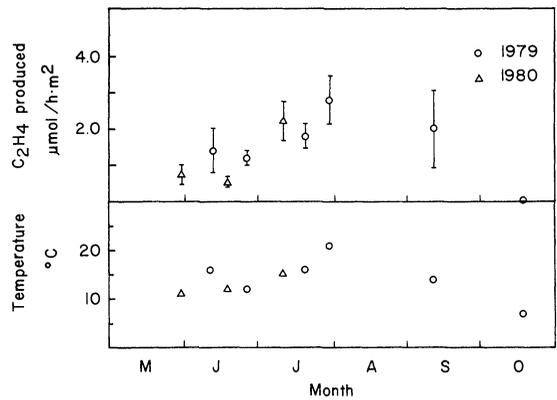


Fig. 5. Nitrogenase activity in the subsurface peat measured in situ and corresponding subsurface temperatures in a Massachusetts peatland. Values are $\bar{x} \pm SE$; $n = 6$.

1979), 1979 (Schwintzer, Berry and Disney, 1982) and 1980 (Schwintzer and Tjepkema, 1983). The annual nitrogenase activity per gram of nodule was taken as the area under the seasonal nitrogenase activity curve. The annual rate of nitrogen fixation by *Myrica gale* was then calculated using a nodule biomass of 104 kg DW ha⁻¹ as measured in 1978 (Schwintzer, 1979) and the theoretical C₂H₄ to N₂ conversion factor of 3.0. A similar value (3.14) has been measured in field-collected nodules of *Comptonia peregrina*, a closely related species (Fessenden, Knowles and Brouzes, 1973).

RESULTS—Peat temperatures (Fig. 2) were similar during the 1978–1980 growing seasons except in the spring of 1979 when the peat thawed and warmed up about 2 weeks earlier. Water levels (Fig. 3) declined from high levels in spring until mid-July during all 3 years. They differed substantially, however, in late summer and fall with very high levels occurring in 1979 and very low levels in 1980.

Nonsymbiotic nitrogen fixation—Peat surface: During field incubation the temperature

of the materials inside the glass incubation vessels rose above that of adjacent unenclosed materials. The average increase within the jars measured at the end of the incubation was 4.3 ± 0.4 C ($\bar{x} \pm SE$; $n = 116$). This was probably unimportant because temperature had little effect on nitrogenase activity (see below).

Nitrogenase activity associated with surface materials was low from early May through late October and showed no clear seasonal pattern (Fig. 4). Corresponding temperatures of the unenclosed surface ranged from 12 to 27 C and showed only a weak seasonal pattern (Fig. 4) due to rapid warming of the surface on sunny days and greater penetration of sunlight through the shrubs early and late in the season. Mean nitrogenase activity was not correlated with surface temperature ($r = 0.20$, ns). These nitrogenase activities were calculated to result in an annual addition of nitrogen to the peatland of 0.6 kg N ha⁻¹ yr⁻¹.

Most of the nitrogenase activity of the surface materials was associated with *Sphagnum* spp. (Table 1). The highest activities were found in samples composed primarily of *Sphagnum*,

TABLE 1. Relationship between *Sphagnum* content and nitrogenase activity in surface samples

Sample composition ^a	No. of samples having nitrogenase activity ($\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ hr}^{-1}$) of:		
	<0.5	0.5–2.0	>2.0
$\geq 75\%$ <i>Sphagnum</i>	9	40	37
<75% <i>Sphagnum</i>	24	6	1
No <i>Sphagnum</i>	24	11	0

^a Many samples contained leaf and twig litter and some contained other mosses in addition to *Sphagnum*.

TABLE 2. Ethylene evolution and disappearance in peat cores in the absence of acetylene in the laboratory

Parameter measured Date	Temperature (C)	No. of cores	$\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ hr}^{-1}$ ($\bar{x} \pm SE$)
Ethylene evolution (ethylene absent initially)			
5/20–5/22/80	15	6	0.013 \pm 0.002
9/3–9/5/80	17	5	0.027 \pm 0.004
Ethylene disappearance due to peat (4 ppm initial ethylene)			
5/21–5/22/80	15	6	0.15 \pm 0.04
9/3–9/5/80	17	5	0.22 \pm 0.11

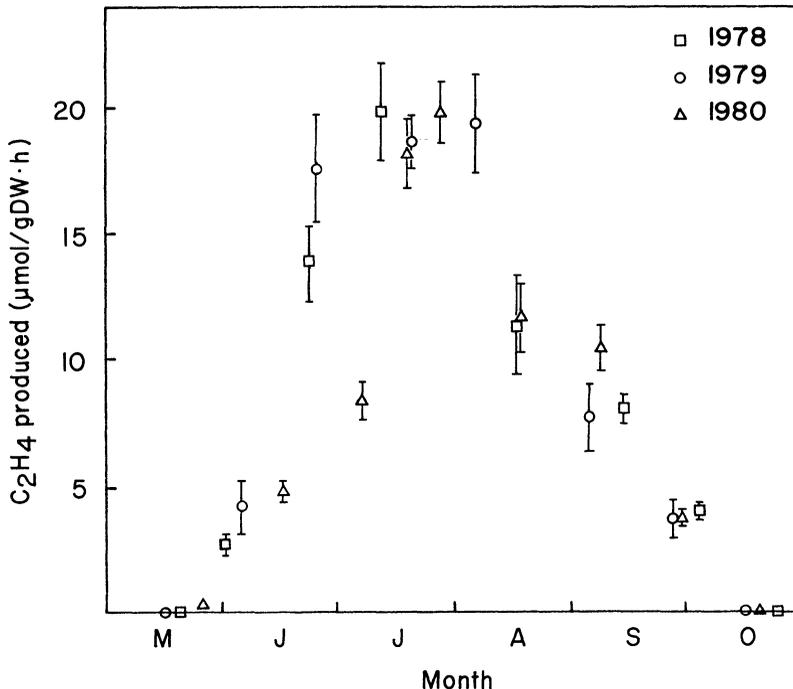


Fig. 6. Nitrogenase activity in excised *Myrica gale* nodules at prevailing soil temperatures in a Massachusetts peatland. Values are $\bar{x} \pm SE$; $n = 20$. Data are from the following: 1978 (Schwintzer, 1979), 1979 (Schwintzer et al., 1982), and 1980 (Schwintzer and Tjepkema, 1983) except for the value for 7/19/80.

but not all samples rich in *Sphagnum* had high activities. Samples lacking *Sphagnum* had only low activities. Several of the most active *Sphagnum* samples were examined microscopically for cyanobacteria on three different dates, but none were found.

Subsurface peat: Ethylene was evolved at very low rates in subsurface peat in the absence of acetylene, and added ethylene disappeared at low rates (Table 2). This pattern strongly suggests the presence of ethylene oxidizing microorganisms. Ethylene disappearance was associated with the peat, since much lower rates of ethylene loss occurred in the minus peat controls.

Nitrogenase activity associated with the subsurface peat measured in situ was low from late May through October and showed a weak seasonal pattern (Fig. 5). Mean nitrogenase activity was closely correlated with subsurface temperatures ($r = 0.91$, $P < 0.001$), which ranged from 7 to 21 C. The annual addition of nitrogen to the peatland due to subsurface nitrogenase activity was calculated to be $0.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

Symbiotic nitrogen fixation—The peatland lacked legumes and only a few widely scattered lichens were found. None had appreciable nitrogenase activity in the light and none contained cyanobacteria.

In *Myrica gale* nodules (Fig. 6), both the seasonal pattern of nitrogenase activity and the annual nitrogenase activity were very similar in the 3 years they were measured. Nitrogenase activity appeared in mid-May, peaked in July or early August and disappeared in the second half of October. Annual nitrogen fixation was $34.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 1978, $37.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 1979 and $30.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 1980. The value for 1980 is probably too low because the nodules were completely excised from the roots. This resulted in a 14% reduction in activity in mid-July but did not affect activity in early June (Schwintzer and Tjepkema, 1983).

DISCUSSION—Measured nitrogenase activities in the surface materials were very low and were largely associated with *Sphagnum*. These activities were probably due to heterotrophic bacteria because no cyanobacteria were found by microscopic examination and the pH of 3.9 at this site is unfavorable for cyanobacteria. Others have also failed to find cyanobacteria in association with *Sphagnum* at pH below 4.0 (Granhall and Hofsten, 1976; Lambert and Reinert, 1979). Presence of cyanobacteria cannot be completely ruled out, however, without a more thorough search.

My estimate of annual fixation of $0.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in surface materials is probably best

considered an upper limit on the annual nitrogen fixation by surface materials during the measurement period because all measurements were made during the daytime and at relatively high light intensities, conditions presumably favoring nitrogenase activity. A similar estimate of $0.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was obtained for nitrogenase activity due to heterotrophic bacteria associated with *Sphagnum* in the White Mountains of New Hampshire (Lambert and Reiners, 1979).

Measured nitrogenase activities in the subsurface peat were also very low and are subject to uncertainty because of procedural difficulties. The in situ method used here was designed to measure both aerobic and anaerobic nitrogenase activity with the least possible disturbance of the peat to minimize changes in nitrogenase activity due to substrate disturbance and altered oxygen relations (e.g., Waughman, 1976; Kana and Tjepkema, 1978). However, the weight of the experimenters caused the peat to sink and resulted in a temporary displacement of the water table.

Further uncertainty is introduced by presence of a long time lag (see Methods) and endogenous ethylene production. The subsurface peat probably has significant endogenous ethylene production because it has appreciable ethylene oxidizing activity and measurable rates of ethylene evolution (Table 3; Witty, 1979; Knowles, 1981). Interestingly, Hemond (1983) has also obtained strong indirect evidence of endogenous ethylene production and oxidation in subsurface peat. In the presence of acetylene, endogenously produced ethylene adds to the ethylene produced by nitrogenase and can lead to serious overestimation of nitrogenase activity when the rates are less than $100 \text{ g N ha}^{-1} \text{ day}^{-1}$ (Witty, 1979). The maximum rates observed here were only $5 \text{ g N ha}^{-1} \text{ day}^{-1}$ (using a conversion factor of 4.0).

Given the difficulties discussed above, my estimate of annual nitrogen fixation of $0.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the subsurface peat is uncertain. However, the true rate is unlikely to be higher because most of the factors discussed lead to overestimation of nitrogenase activity.

The measured nitrogenase activity in the subsurface peat was closely correlated with temperature. Consequently, temperature was probably a major controlling factor and was largely responsible for the shape of the seasonal curve. In contrast, nitrogenase activity in surface materials was not correlated with temperature indicating that another factor, possibly moisture, was of major importance. Moisture content has been shown to be important in controlling nitrogenase activity as-

sociated with mosses in Alaska (Billington and Alexander, 1978).

In general nonsymbiotic nitrogenase activity in peatlands increases with increasing minerotrophy (and hence pH) from low rates, $0.3\text{--}1.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, in ombrotrophic and very weakly minerotrophic peatlands to much higher rates, $21\text{--}115 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, in moderately to strongly minerotrophic peatlands (see summary of annual rates of nitrogen fixation in peatlands in Chapman and Hemond [1982], and also Waughman and Bellamy [1972]). The only exception to this pattern found to date is a moderate rate of nitrogenase activity, $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, found in an ombrotrophic (pH 3.8) bog in eastern Massachusetts (Chapman and Hemond, 1982). The total nonsymbiotic nitrogen fixation found here at the weakly minerotrophic (pH 3.9) Tom Swamp site was $1.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ with 0.6 kg N contributed by surface materials and 0.4 kg N by subsurface peat. These values fit the general pattern very well.

Significant nitrogen fixation by rhizosphere-associated bacteria might be expected in minerotrophic peatlands because flooded soil systems are favorable environments for associative nitrogen fixation (Kana and Tjepkema, 1978; Buresh et al., 1980) and minerotrophic, open peatlands have even lower levels of soluble combined nitrogen than other wetlands (Waughman and Bellamy, 1980; Schwintzer and Tomberlin, 1982). The in situ method used here measured rhizosphere-associated nitrogenase activity as well as that of free-living bacteria. Similar in situ methods have been used to measure rhizosphere-associated nitrogenase activity in rice (Lee et al., 1977; Boddy, Quilt and Ahmad, 1978; Bladensperger, 1980; and others). Contrary to expectation, the measured nitrogenase activities were low and show that there is little or no associative fixation in this peatland. Absence of significant associative fixation may be due to the low pH of the site.

Myrica gale nodules fixed substantial amounts of nitrogen on an annual basis. Contrary to previous expectation the shape of the seasonal nitrogenase activity curve (Fig. 6) and the annual fixation rate were remarkably similar during all 3 years in spite of substantial differences in peatland water levels (Fig. 3). Water level might be expected to affect nitrogenase activity because it strongly affects aeration in the nodule environment. Most living *Myrica gale* roots and nodules were located in the relatively thin layer ($<30 \text{ cm}$) of peat above the midsummer water table (Schwintzer, 1979; Schwintzer and Lancelle, 1983) and the majority were submersed when the relative

water level shown in Fig. 3 exceeded 30 cm. In addition water level indirectly reflects the amount of cloudy and rainy weather which presumably reduces production of photosynthate. Peat temperatures were similar, however, during the three seasons except in late April and early May (Fig. 2). Substantial agreement in the seasonal nitrogenase activity curve in 2 consecutive years was also observed in *Alnus viridis* in France (Moiroud and Capellano, 1979) but the pattern was more variable in *Myrica gale* in Scotland (Sprent et al., 1978). Factors affecting the shape of the seasonal nitrogenase activity curve including temperature, shoot phenology, and nodule phenology have been discussed elsewhere (Schwintzer, 1979; Schwintzer, Berry and Disney, 1982).

Myrica gale probably makes an important contribution to the nitrogen budgets of many of the sites where it occurs. This species is widely distributed in wetlands and along shores in the northern United States, Canada and Europe where it forms extensive stands and is a dominant plant in a number of plant associations (Schwintzer, 1979). In peatlands *Myrica gale* is usually restricted to minerotrophic sites (fens) although it is also found on ombrotrophic bogs near the ocean (Gorham, 1957; Damman, 1977, 1978). This species tolerates a wide range of pH and occurs at values as low as 3.7 (Bond, 1951) and as high as 7.0 (Schwintzer, 1978). Its roots are frequently well noduleated, but lack nodules in some locations especially on drier soils (Bond, 1976; Schwintzer and Lancelle, 1983).

The measured nitrogen inputs by biological nitrogen fixation apply to the Open Mat site portion of the peatland, an area dominated by *Myrica gale* (see Study Area). Here the total input was about 36 kg N ha⁻¹ yr⁻¹ with about 35 kg N contributed by *Myrica gale* and 1.0 kg N by nonsymbiotic nitrogen fixation. The remainder of the peatland has not been described in detail but is variable with respect to presence of trees, extent of *Sphagnum* cover, and density of *Myrica gale*. Rates of nitrogen fixation presumably vary from about 1 kg N ha⁻¹ yr⁻¹ in areas lacking *Myrica gale* to 36 kg N in areas with dense stands of *Myrica gale*. The contribution of nitrogen fixation to the total nitrogen budget of the peatland cannot be completely determined because data on other inputs are incomplete. However, the addition of 36 kg N ha⁻¹ yr⁻¹ at the Open Mat site is large compared to inputs by bulk precipitation, which probably added about 6.5 kg N based on measurements at nearby Hubbard Brook (Bormann, Likens, and Melillo, 1977). But the addition of 1.0 kg N by biological ni-

trogen fixation in areas lacking *Myrica gale* is small compared to inputs by bulk precipitation. Inputs by impaction of aerosols are likely to be small, contributing <1 kg N ha⁻¹ yr⁻¹, based on measurements in nearby forests (Tjepkema, Cartica and Hemond, 1981). Finally hydrologic inputs may be significant, but their size cannot be estimated from the available data.

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