Nitrogen fixation associated with *Scirpus atrovirens* and other nonnodulated plants in Massachusetts

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Received March 13, 1978

KANA, T. M., and J. D. TJEPKEMA. 1978. Nitrogen fixation associated with *Scirpus atrovirens* and other nonnodulated plants in Massachusetts. Can. J. Bot. **56**: 2636–2640.

Nitrogen fixation was measured using the acetylene reduction technique in soil cores of 13 nonnodulated, herbaceous plant species growing in mesic and wetland habitats. Six species that grew in a well-drained portion of an old field exhibited low rates of N_2 fixation (7 g $N \cdot ha^{-1} \cdot day^{-1}$). A bulrush, *Scirpus atrovirens*, which grew in an area of compacted soil in the old field, showed a moderate rate of N_2 fixation (30–100 g $N \cdot ha^{-1} \cdot day^{-1}$). These old field species exhibited a lag of only a few hours before a constant rate of acetylene reduction occurred. The estimated rates for the six wetland species were generally higher, and there was a lag such that the activity was still increasing after 20 h of incubation. N_2 fixation in cores of *S. atrovirens* was primarily associated with the roots and adhering soil. Incubation of the cores in N_2 for 24 h caused a fourfold increase in the acetylene reduction rate over that in air, although the rates during the first 4 h were similar.

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Les auteurs ont mesuré, à l'aide de la technique par réduction de l'acétylène, la fixation de l'azote dans des cylindres provenant de 13 espèces de plantes herbacées provenant d'habitats mésiques et d'habitats humides. Six espèces provenant d'une partie bien drainée d'un vieux champ ont réalisé de faibles taux de fixation d'azote (7 g $N \cdot ha^{-1} \cdot jour^{-1}$). Le *Scirpus atrovirens*, qui poussait dans une portion de sol compacté dans le vieux champ a réalisé une fixation modérée de l'azote (30–100 g $N \cdot ha^{-1} \cdot jour^{-1}$). Ces espèces des vieux champs n'ont une phase latente que de quelques heures avant qu'on observe un taux constant de réduction de l'acétylène. Les valeurs estimées pour les six espèces de sols humides étaient généralement plus élevées et la période latente est telle que l'activité augmente encore après 20 h d'incubation. La fixation de N_2 dans les cylindres du *S. atrovirens* est principalement associée aux racines et au sol qui y adhère. Lorsque les cylindres sont incubés en présence de N_2 pendant 24 h, on observe une forte augmentation (×4) dans le taux de réduction comparativement à ce qu'on observe dans l'air, bien que les taux soient identiques pendant les 4 premières h.

[Traduit par le journal]

Introduction

Nitrogen fixation is associated with the roots of a variety of plant species that lack nitrogen-fixing root nodules. For plants growing in well-drained soils, the rates observed are often negligible, although high rates of up to 100 kg $N \cdot ha^{-1} \cdot year^{-1}$ have been reported (Balandreau *et al.* 1976; Day, Neves, *et al.* 1975; De-Polli *et al.* 1977; Dobereiner *et al.* 1972; Kapustka and Rice 1976; Knowles and O'Toole 1975; Koch 1977; Lockyer and Cowling 1977; Nelson *et al.* 1976; Tjepkema and Burris 1976; Vlassak and Jain 1976; Vlassak *et al.* 1973). In contrast, measurements of wetland plants have indicated that high rates of nitrogen fixation are usually associated with their roots (Bristow 1974; Day, Harris, *et al.* 1975; Green and Edmisten 1974; Hanson 1977; Lee and Yoshida 1977; Patriquin and Knowles 1972; Tjepkema and Evans 1976; Trolldenier 1977; Yoshida and Ancajas 1973). But only a moderate number of plant species and habitats have been investigated, and few of the variables that might affect the measured rate have been studied. There are indications that some of the measured rates may not be good estimates of the true *in situ* rates (Eskew and Ting 1977; Okon *et al.* 1977; Tjepkema and van Berkum 1977).

This paper reports substantial nitrogen fixation associated with several wetland plants, low or nil rates for several plants in a well-drained old field, and an intermediate rate for a bulrush growing in

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areas of compacted soil in the old field. The characteristics of nitrogen fixation by the bulrush were examined in detail.

Materials and Methods

The sampling sites were in the vicinity of the Harvard Forest in north central Massachusetts. The old field species and *Scirpus atrovirens* were growing in a field that was mowed once each year in August. *Scirpus atrovirens* was growing in a compacted, somewhat depressed strip in the field, formed as a result of vehicular traffic. The soil pH in the *S. atrovirens* stand was 5.5. The wetland species were sampled at two sites. *Juncus balticus, Typha latifolia*, and *Carex scoparia* were growing in a poorly drained roadside ditch, whereas *Lysimachia terrestris, Scirpus polyphyllus*, and *Lythrum salicaria* were growing in a streamside freshwater marsh.

Undisturbed soil cores were obtained with a 6.8-cm-diameter stainless-steel cylinder unless otherwise noted. Plant shoots were removed and each core was placed in a 700-ml canning jar with a lid containing a serum stopper. For the experiment investigating the effect of core size on nitrogenase activity, the 12 × 12 cm cores were dug with a shovel and incubated in 4.9 ℓ plastic buckets with airtight lids. The 1.9- and 5.6-cm-diameter cores were obtained with cylindrical coring devices and incubated in the 700-ml jars. The cores of the field species were from 10–15 cm deep and contained the complete or nearly complete rooting zone. The cores of the wetland species were 15 cm deep but many of these cores did not contain the complete vertical rooting zone. Cores were always dug in the morning and brought into the laboratory prior to the injection of acetylene. The cores were incubated in the dark at soil temperature (17 to 19°C). Incubation always commenced within 1 h but typically within less than 0.5 h after digging the cores.

To study the effect of N_2 on acetylene reduction, the incubation jars containing the soil cores were flushed with N_2 three times over a period of 5 min. No rigorous attempt was made to exclude all O_2.

Nitrogenase activity was measured by the acetylene reduction technique as described previously (Tjepkema and Burris 1976). Acetylene-free controls were used to determine endogenous ethylene production, but no measurable amounts were found. Acetylene reduction rates were converted to N_2 fixation rates (grams N_2 per hectare per day) assuming a theoretical ratio of 3 mol acetylene reduced for each mole N_2 reduced.

Results

Nitrogenase activities of the seven species sampled from the field site are shown in Table 1. Uniformly low activity was associated with all species except *S. atrovirens*. The activity of *S. atrovirens* (58 ± 9 (SE) g $N \cdot ha^{-1} \cdot day^{-1}$) was about 20 times greater than the average of other field species tested, so it was chosen for more detailed study.

The effect of core diameter on nitrogenase activity associated with *S. atrovirens* is shown in Table 2. The cores of 1.9-, 5.6-, and 6.8-cm diameter were assayed on 27 June 1977, whereas the 12 × 12 cm cores and additional 6.8-cm cores were assayed on 9 July 1977. Due to differences in the acetylene reduction rates in the 6.8-cm cores between the two sampling dates, the data have been standardized by assuming equal rates for the 6.8-cm cores on the two dates and by representing the activity of the 12 × 12 cm cores as 100%. There was a large reduction in activity as core diameter decreased. It is not clear whether this was due to the larger ratio of surface area to volume in the smaller cores or to their lesser degree of intactness.

TABLE 1. N_2 fixation associated with field species in Massachusetts[a]

Species	N_2 fixation rate ± SE, g $N \cdot ha^{-1} \cdot day^{-1}$
Arrhenatherum elatius	0.5±0.3
Chrysanthemum Leucanthemum	4.7±2.2
Hieracium pratense	2.1±0.3
Potentilla canadensis	3.8±1.2
Rumex acetocella	0.6±0.6
Scirpus atrovirens	58.2±9.3
Solidago juncea	7.1±3.8

[a]Sampling dates: 14–15 June 1977.

TABLE 2. Effect of core size on N_2 fixation associated with *S. atrovirens*[a]

Core size, cm	Relative activity per square centimetre, % of maximum
12 × 12	100
6.8 diameter	81
5.6 diameter	30
1.9 diameter	25

[a]Sampling dates: 27 June and 9 July 1977; see text for explanation.

The effects of N_2 and air on acetylene reduction activity are different (Fig. 1). Whereas activity in air is constant within 4 h of acetylene injection, activity in N_2 increased for approximately 16 h until it reached a rate nearly four times higher than in air. Average rates were 71.5 and 279.6 g $N \cdot ha^{-1} \cdot day^{-1}$ for air and N_2 respectively. The rates of acetylene reduction were similar in air and N_2 during the first 4 h of incubation.

To determine the depths at which the activity occurs, soil cores were cut at 1-, 11-, and 21-cm depths and the nitrogenase activity associated with each level was measured (Table 3). It is apparent that surface organisms such as blue-green algae contributed only a small percentage of the total activity. Greater than 90% of the activity was found below 1 cm and above 11 cm. This corresponded closely to the zone of root growth.

To further localize the site of nitrogen fixation, intact cores were measured for nitrogenase activity in air and N_2 and then separated into fractions of unwashed roots and root-free soil. The amount of activity retained after core fractionation (Table 4)

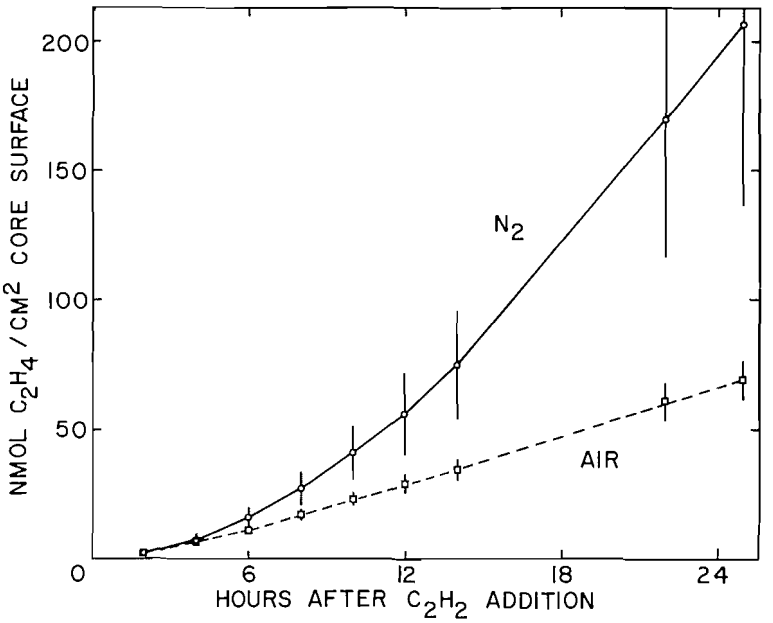

FIG. 1. Effect of N_2 and air on ethylene production in *S. atrovirens* cores. Vertical bars represent ± one SE.

was 8 and 22% for fractions incubated in air and N_2 respectively. Of the retained activity, most was located in the root fraction (88% in air and 77% in N_2). The lag in the rate of ethylene evolution observed in the intact cores in N_2 (similar to that shown in Fig. 1) was not observed in any of the fractions during the incubation period of 10 h.

Several factors suggest that anaerobiosis may exist in the compacted soil where *S. atrovirens* grew. First, the normal habitat of this species has been given as swamps and wet meadows (Gleason and Cronquist 1963), which are sites which favor plants that can grow in anaerobic soils. Secondly, cortical and surface staining, characteristic of ferric hydroxide, was observed along those roots possessing aerenchyma. This occurs in reducing soils when oxygen diffusion out of the root is restricted (Armstrong and Boatman 1967). Finally, large soil peds, which are up to about 3 cm^3 and are traversed by small roots lacking aerenchyma, existed throughout the soil. The centers of these peds may be anaerobic when the soil is moist or wet.

The higher nitrogenase activity associated with this anaerobic soil prompted us to sample other local plants growing on poorly aerated soils. Six species from nearby wetlands were sampled with the 6.8-cm coring device. Each species was growing in an area where the water table was at or above the soil surface. Estimated rates of N_2 fixation in the cores incubated in air are shown in Table 5. The rates ranged from about 20 g $N \cdot ha^{-1} \cdot day^{-1}$ for *Lysimachia terrestris*, a dicot, to about 180 g $N \cdot ha^{-1} \cdot day^{-1}$ for *Typha latifolia*, a monocot. Several of these species (see Table 5) exhibited a lag in ethylene evolution and did not reach constant rates within 20 h. Thus the reported rates are only preliminary estimates.

TABLE 3. N_2 fixation associated with *S. atrovirens* at different soil depths[a]

Depth, cm	N_2 fixation rate ± SE, g $N \cdot ha^{-1} \cdot day^{-1}$	% of total activity
Loose surface organics	0.5±0.3	0.6
0–1[b]	5.2±4.1	6.0
1–11	80.1±20.3	92.8
11–20	0.5±0.5	0.6

[a]Sampling date: 16 June 1977.
[b]Very little disturbance of this layer occurred despite the thinness.

TABLE 4. N_2 fixation in root and soil fractions of *S. atrovirens* cores[a]

Incubation atmosphere	Rates in intact cores,[b] g $N \cdot ha^{-1} \cdot day^{-1}$	Activity in root plus soil fractions, g $N \cdot ha^{-1} \cdot day^{-1}$	Activity in roots, %
Air	38.6	3.2	88
N_2	126.2	27.6	77

[a]Sampling date: 9 July 1977.
[b]Mean of four replicates.

TABLE 5. N_2 fixation associated with six wetland species in central Massachusetts

Habitat	Species	N_2 fixation rate ± SE, g $N \cdot ha^{-1} \cdot day^{-1}$
Roadside depression[a]	*Carex scoparia*	177 ± 58[c]
	Juncus balticus	65 ± 9[c]
	Typha latifolia	181 ± 33[c]
Freshwater marsh[b]	*Lysimachia terrestris*	20 ± 5
	Lythrum salicaris	27 ± 4
	Scirpus polyphyllus	100 ± 16

[a]Sampling date: 3 August 1977.
[b]Sampling date: 21 July 1977.
[c]Steady-state ethylene evolution was not achieved during 20 h of incubation. Rates were calculated from the average rate of ethylene production between 10 and 20 h.

Discussion

In this study it was found that wetland species of plants generally had much higher rates of nitrogenase activity associated with their roots than did species growing in the mesic soils of the field site. Unfortunately, the lag in the rate of ethylene evolution observed during the incubation of the wetland species made it difficult to interpret the steady-state rate. Tjepkema and Evans (1976) have shown, for *Juncus balticus* growing in an Oregon estuary, that increasing the diffusion rate by increasing the pC_2H_2 does not reduce the lag. It is possible that the disturbance of the soil allows proliferation of the N_2-fixing bacteria thus creating artificially high rates. Tjepkema and Evans (1976) have discussed this and alternative explanations for the lag. Because a lag may occur, it is important to be aware of the time course of ethylene evolution.

Scirpus atrovirens from the field site was chosen for more detailed study because it displayed relatively high nitrogenase activity and only a minimal lag in ethylene production when cores were incubated in air (Fig. 1). The nitrogenase activity seemed to be associated with the roots, since the greatest activity was observed in the root fraction (Table 4) and in the zone of root growth (Table 3). Anaerobic conditions favored the activity (Fig. 1 and Table 3) but since there was a lag of 16 h before maximal rates were observed for cores incubated in N_2 (Fig. 1), it is possible that these rates are artificially high due to the creation of favorable conditions.

From our work and that of others, we suggest that most observations of nitrogenase activity in soil cores from the root zone of nonnodulated plants fall into two categories. The first category is for plants growing in wet soils. For these, the measured steady-state nitrogenase activity is usually high, although there is a lag of 18 h or more before high rates are observed (Day, Harris, *et al.* 1975; Tjepkema and Evans 1976). Also, the activity is maximal when the cores are incubated in N_2 (Tjepkema and Evans 1976). The other category is for plants growing in mesic or dry soils. Here the nitrogenase activity is usually low, there is little or no lag (Tjepkema and Burris 1976; Tjepkema and van Berkum 1977), and incubating the cores in N_2 markedly decreases the rate (Tjepkema and Burris 1976; Dobereiner *et al.* 1972). *Scirpus atrovirens* seems to belong to the wetland category, since the soil gave indications of anaerobiosis and maximal rates were observed in N_2. But there was only a short lag before maximal activity was observed. This may be because the anaerobic conditions in the soil resulted from compaction rather than from saturation of the soil with water. Because of this, taking the soil cores may have disturbed the oxygen relationships less than for wetland soils, where water may drain from the larger pores in the soil when the cores are removed.

Acknowledgments

This research was supported in part by research grant DEB 77-02249 from the United States National Science Foundation and by the Maria Moors Cabot Foundation for Botanical Research of Harvard University.

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