

BIOLOGICAL DINITROGEN FIXATION IN TWO CEDAR GLADE COMMUNITIES OF MIDDLE TENNESSEE

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ABSTRACT--The mineral substrates of the cedar glades of middle Tennessee support abundant growth of colonies of *Nostoc* sp. This study assessed the biological dinitrogen (N_2) fixation by *Nostoc* sp. as well as heterotrophic dinitrogen fixers in two representative cedar glade communities. The environmental factors of air and soil temperature, soil moisture, soil pH, and soil ammonium were measured. Results showed that one site had a significantly higher soil pH (7.3 ± 0.1), a significantly higher rate of dinitrogen fixation activity, and a significantly lower seasonal mean soil ammonium level. The other site had a significantly lower pH (6.4 ± 0.2), a significantly lower rate of dinitrogen fixation activity, and a significantly higher seasonal mean soil ammonium level. Other environmental factors (air and soil temperature and soil moisture) showed no significant differences between the two sites. The soil moisture content appeared to be the major factor in regulating the seasonal pattern of dinitrogen fixation. The soil pH appeared to impact the overall amount of dinitrogen fixation activity.

There are approximately 15 counties in Middle Tennessee located in a basin surrounded by the Interior Low Plateau. Of these counties, 10 contain open areas characterized by the presence of rock at or near the surface (Quarterman, 1950). These open areas, or "glades," occurring on Lebanon Limestone of the Stones River Series are bounded by cedar woods. Quarterman (1950) described six different types of communities typical of the glades region: rock, gravel and grass glades, shrub thickets, cedar and cedar-hardwood stands. The vegetation of these glades has been extensively described by Harper (1926), Freeman (1933), Quarterman (1950), and Baskin et al. (1968).

Quarterman (1950) and Martin and Sharp (1983) noted the extensive growths of mats of *Nostoc commune* (Vauch) on exposed soil and rock surfaces. These mats are similar to those reported in relict grassland sites of southern Ohio (DuBois and Kapustka, 1983), also located on a dolomitic substratum. Biological dinitrogen (N_2) fixation by *Nostoc* sp. and other cyanobacteria has been reported as an important source of nitrogen input into natural terrestrial ecosystems, contributing between 1 and 39 kg N/ha/year (Day et al., 1975; Kapustka and Rice, 1976; Stewart et al., 1978; DuBois and Kapustka, 1983). The optimal growth and dinitrogen fixation by cyanobacteria requires light (Hardy et al., 1973), pH 7 to 8 (Stewart, 1974), temperature between 19° and 30°C (Steyn and Delwiche, 1970; Rychert and Skujins, 1974; Stewart, 1974), and a soil water potential greater than -1 bar (Rychert and Skujins, 1974; DuBois and Kapustka, 1983).

The objective of the present study was to assess the biological dinitrogen fixation activity in two representative cedar glades of middle Tennessee. To that end, assessments were made of the colony cover of *Nostoc* sp., the rates of biological dinitrogen fixation by colonies of *Nostoc* sp. and heterotrophic dinitrogen fixers, and the environmental factors of air and soil temperatures, soil water, soil pH, and soil ammonium.

MATERIALS AND METHODS

Site Description--Two sites were selected for this study. The first site was in the Cedars of Lebanon State Park (CL) located in Wilson Co.,

Tennessee, approximately 27 km north of the city of Murfreesboro, at 36°5'00"N latitude and 86°18'36"W longitude. This site was a combination of gravel and grass glades based upon the glade types identified by Quarterman (1950).

The second site selected was in the Stones River National Battlefield (SR) located in the northwestern corner of the city of Murfreesboro in Rutherford Co., Tennessee, at 35°52'30"N latitude and 86°25'48"W longitude. This site was a combination of gravel and grass glades with a light shrub thicket near one edge of the study area. Each site was sampled on a biweekly schedule starting on 21 March 1990 and continuing until 15 November 1990. Sites were sampled on alternating weeks, and all samplings occurred between 1000 and 1200 h.

Determination of Nostoc sp. Cover--During the spring site visits (March through May), the percent cover of colonies of *Nostoc* sp. was determined using point-frame analysis (Whitman and Siggeirsson, 1954). Fifty frames of 10 pins each were used at 2-m intervals along a 100-m transect. *Nostoc* sp. cover was expressed as the percentage of the 500 pins contacting colonies of *Nostoc* sp.

Acetylene Reduction Assay for Dinitrogen Fixation Activity--Dinitrogen fixation activity was measured using the acetylene reduction assay (ARA) technique (Balandreau and Dommergues, 1973). Field ARAs were conducted in situ using clear plexiglass cylinders (DuBois and Kapustka, 1983). On days when the soil was too dry (hard) to insert the plexiglass cylinders, soil cores (2.5 cm in diameter or 4.9 cm² of surface area by 1 cm in depth) were collected and placed in 25-ml serum bottles. Cylinders were placed (or soil samples were collected) every 10 m along a 100-m transect through the site. Acetylene reduction assays at these locations were used to determine total dinitrogen fixation at the site.

Heterotrophic dinitrogen fixation was determined by collecting soil cores (following the removal of the top 0.5 cm of soil) at 20-m intervals along the transect (DuBois and Kapustka, 1983). Dinitrogen fixation activity by colonies of *Nostoc* sp. was measured by performing ARAs in 10 plexiglass cylinders subjectively placed over the healthiest colonies in the site (DuBois and Kapustka, 1983). Generation of acetylene, assay acetylene concentration, and analysis of ethylene were

performed as described by DuBois and Kapustka (1983). The theoretical conversion factor of 3 mols acetylene reduced equal 1 mol dinitrogen reduced was used, and the data were expressed as micrograms of nitrogen per square meter per hour.

Soil Analyses—Soil samples were collected at each end and at the midpoint of the 100-m transect. Samples were kept on ice for transport to the laboratory where they were subsequently refrigerated at 4°C. Samples were used for the analysis of soil ammonium, soil pH, and soil dehydrogenase (reducing) activity.

For analysis of soil ammonium, 10 g of each soil sample were thoroughly mixed with 100 ml of deionized water. One milliliter of 10 M NaOH was added while the solution was stirring. An ammonium ion selective electrode connected to an Orion microprocessor pH/mV meter (Model 811) was used to determine ammonium concentration by comparing millivolt readings to those of a standard curve. Results are expressed as nanomoles of ammonium per gram of soil.

To determine soil pH, 10 g of each soil sample were thoroughly mixed with 100 ml of 10 mM CaCl₂. A pH electrode was inserted into the solution, and the pH was determined with an Orion microprocessor pH/mV meter.

Soil dehydrogenase (reducing) activity was determined using the procedure of Stevenson (1959) with modifications by Casida et al. (1964). Results are expressed as micrograms of formazan per gram of soil per hour.

The percent soil moisture (w/w) was determined by weighing 5 g of soil collected in the field, allowing it to air dry for a minimum of 48 h at room temperature (or until a stable reading was obtained), and reweighing. The difference in weights was expressed as percent soil water.

Air and soil temperatures were recorded during the ARAs. Air temperatures were recorded in the shade and soil temperatures were recorded at a depth of 2 to 3 cm.

Statistical Analyses—Seasonal patterns were compared using correlation analysis. Mean values for the various parameters were compared between the two sites using oneway analysis of variance (ANOVA). Both statistical procedures were part of the Minitab statistical computing system (Minitab, Inc., 1985).

RESULTS

The mean percent cover of colonies of *Nostoc* sp. for CL was 12.2%, ranging from 7.2 to 18.0% at individual samplings. For SR, the mean percent cover of colonies of *Nostoc* sp. was 6.0%, ranging from 4.8 to 8.0% at individual samplings. Although these values represent percent cover over a 100-m transect, the distribution of colonies of *Nostoc* sp. often was patchy in some areas, while other areas showed no visible colonies of *Nostoc* sp.

The two sites showed similar patterns of air and soil temperatures (Fig. 1) with the soil temperature typically 2 to 4° greater than the air temperature during any given sampling. Air and soil temperatures were between 15 and 25°C at the beginning and end of the study. The highest sampling air temperature was 38°C (SR; 6 July), and the highest soil temperature was 43°C (CL; 4 September). Only one sampling had a temperature recorded below 10°C (SR; air; 8 November). There was no significant difference ($P > 0.05$, ANOVA) in air or soil temperatures between the two sites (Table 1).

The mean percent soil moisture during the spring ranged between 15 and 30% (Fig. 2A). The mean percent soil moisture at CL had the first greatest decline in early June, while mean percent soil moisture at SR had the first greatest decline in late June. During July and August, both sites were relatively dry (<10%) with the exception of two samplings at CL. By October and November, the mean percent soil moisture at both sites had returned to the level of 15 to 20%. There was no significant

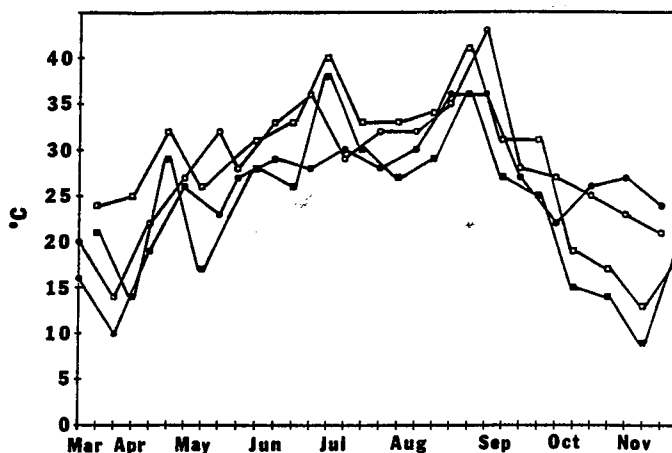


FIG. 1. Air (closed symbols) and soil (open symbols) temperatures for 1990 at sites at Cedars of Lebanon State Park, Wilson Co., Tennessee (circles), and Stones River National Battlefield, Rutherford Co., Tennessee (squares).

difference ($P > 0.05$, ANOVA) in mean percent soil moisture between the two sites (Table 1).

The mean soil pH was dramatically different between the two sites (Fig. 2B). At CL, mean soil pH ranged from 7.0 to 7.5, whereas, at SR, the range was between 6.1 and 6.8. Although there were modest fluctuations in mean soil pH at each site, soil pH at CL always remained neutral to slightly alkaline, and soil pH at SR was always slightly acidic. There was a significant difference ($P > 0.01$, ANOVA) in mean soil pH between the two sites (Table 1).

TABLE 1. Oneway analysis of variance in seasonal averages (\pm SD) of environmental factors and dinitrogen fixation activity between the sites at Cedars of Lebanon State Park, Wilson Co., Tennessee (CL), and Stones River National Battlefield, Rutherford Co., Tennessee (SR), for 1990.

Parameter	Site		F-ratio
	CL	SR	
Air temperature (C)	25.8 \pm 6.3	23.8 \pm 8.1	0.67
Soil temperature (C)	28.2 \pm 6.8	28.3 \pm 8.0	0.00
Percent soil moisture (w/w)	16.5 \pm 9.6	13.8 \pm 6.9	0.88
Soil pH	7.3 \pm 0.1	6.4 \pm 0.2	218.60**
Soil reducing activity (μ g formazan/g soil/h)	38.0 \pm 8.3	20.8 \pm 9.9	31.15**
Dinitrogen fixation activity (μ g N/m ² /h)	97.9 \pm 167.2	12.4 \pm 18.3	4.39*
Soil ammonium (μ mol/g soil)	0.49 \pm 0.48	1.01 \pm 0.70	6.52*

* $P \leq 0.05$.

** $P \leq 0.01$.

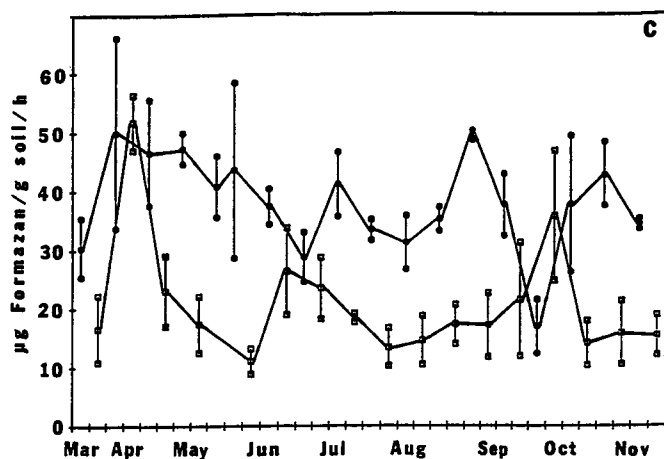
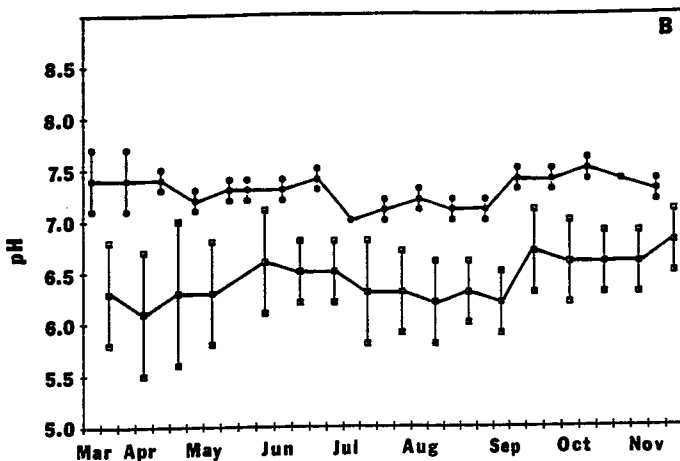
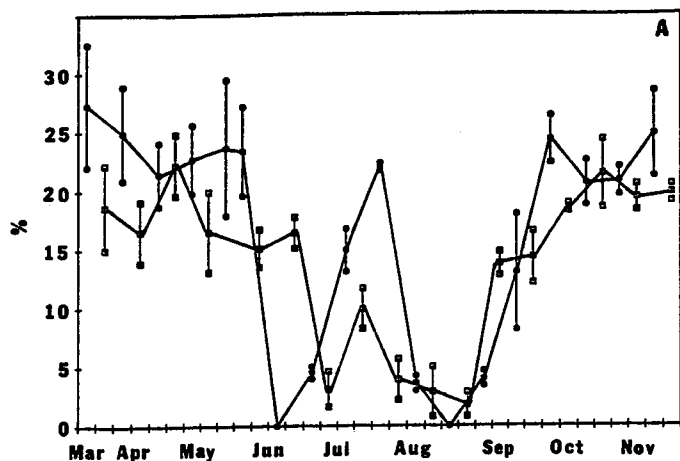


FIG. 2. Percent soil moisture (A), soil pH (B), and soil dehydrogenase (reducing) activity (C) for 1990 at sites at Cedars of Lebanon State Park, Wilson Co., Tennessee (circles), and Stones River National Battlefield, Rutherford Co., Tennessee (squares).

Soil dehydrogenase (reducing) activity was significantly higher ($P < 0.01$, ANOVA) at the CL site than at the SR site (Fig. 2C; Table 1). Both sites showed higher rates of reducing activity during the early portion of the study.

Dinitrogen fixation (acetylene reduction) activity along the transects at each site is shown in Fig. 3A. Although both sites showed the maximum rate of activity during May, the CL site had a much greater peak rate ($596.0 \pm 259.3 \mu\text{g N/m}^2/\text{h}$) than did the SR site ($69.7 \pm 98.9 \mu\text{g N/m}^2/\text{h}$). The CL site also had a significantly greater ($P < 0.05$, ANOVA) seasonal mean rate of dinitrogen fixation activity than the SR site (Table 1). The dinitrogen fixation activity attributable to colonies of *Nostoc* sp. is shown in Fig. 3B. The rates obtained from the subjectively-placed cylinders were adjusted by multiplying by the percent cover of colonies of *Nostoc* sp. for the respective sampling date. Again, the CL site had the greater amount of activity. The ARAs performed on the soil cores (heterotrophic dinitrogen fixation) showed no activity.

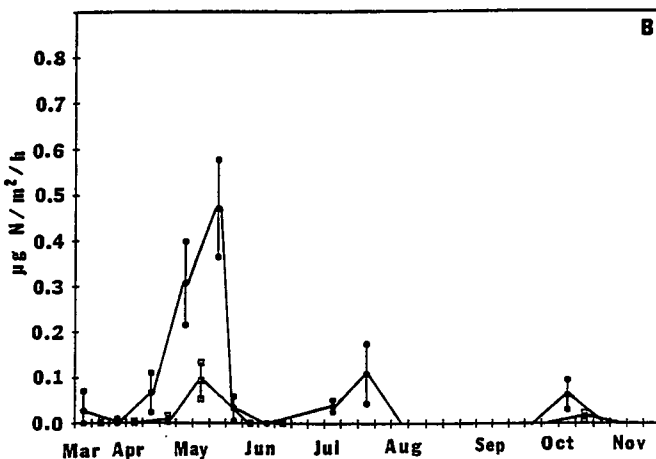
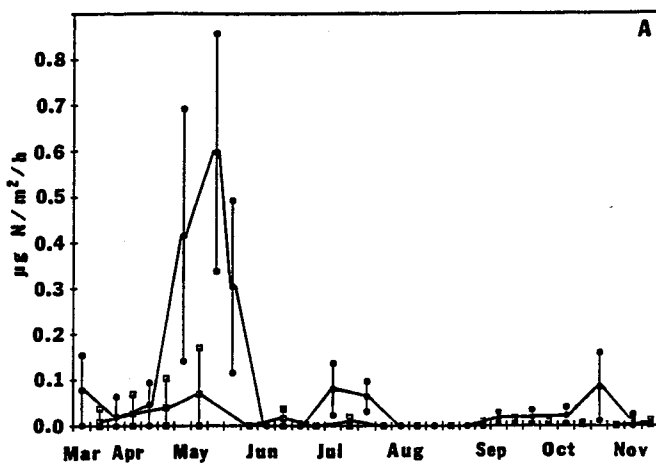


FIG. 3. Total dinitrogen fixation (acetylene reduction) activity (A) and *Nostoc* sp. (cyanobacterial) dinitrogen fixation (acetylene reduction) activity (B) for 1990 at sites at Cedars of Lebanon State Park, Wilson Co., Tennessee (circles), and Stones River National Battlefield, Rutherford Co., Tennessee (squares). Values are expressed as mean \pm SD.

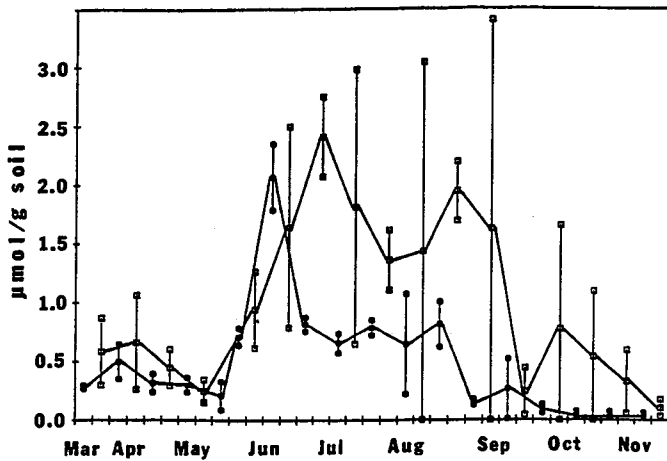


FIG. 4. Soil ammonium levels for 1990 at sites at Cedars of Lebanon State Park, Wilson Co., Tennessee (circles), and Stones River National Battlefield, Rutherford Co., Tennessee (squares). Values are expressed as mean \pm SD.

Soil ammonium levels for the two sites are shown in Fig. 4. At both sites, soil ammonium levels remained relatively low early in the study. By the end of May, both sites showed dramatic increases in soil ammonium. The soil ammonium level at the SR site remained high until late September, whereas the soil ammonium level at the CL site peaked in mid-June and decreased substantially by the end of June. A second decrease in the level of soil ammonium at CL occurred by early September. Soil ammonium level at the CL site (Fig. 4) peaked 2 weeks after the peak in dinitrogen fixation activity (Fig. 3A). When this 2-week delay is taken into account, there is a significant ($P < 0.01$) correlation ($r = 0.7912$) between soil ammonium and dinitrogen fixation at the CL site. This would indicate that the soil ammonium at the CL site is a result of dinitrogen fixation and not a result of processes such as decomposition. Although there was a 3-week delay in the same activity at the SR site, there was not a significant correlation between the delayed soil ammonium levels and dinitrogen fixation levels. This is most probably due to the continued high levels of soil ammonium after the peak.

DISCUSSION

Both sites selected were typical of most gravel and grass glades regions of Middle Tennessee. Colonies of *Nostoc* sp. were present throughout the sites, especially in low areas that formed temporary pools after a rain. Although there were fluctuations in the percent cover of *Nostoc* sp. (7.2 to 18.0% for CL; 4.8 to 8.0% for SR), there did not appear to be any pattern to these fluctuations. DuBois and Kapustka (1983) attributed fluctuations in colony cover of *Nostoc* sp. in a relict prairie in Ohio to changes in air and soil temperatures and soil water potential. These environmental factors may well have contributed to the fluctuations seen in cover of *Nostoc* sp. in the present study; however, the data do not clearly support this. However, the CL site had, on the average, twice the cover (12.2%) as the SR site (6.0%). In a study of a relict prairie bounded by cedars in southern Ohio (DuBois and Kapustka, 1983), the average seasonal colony cover of *Nostoc* sp. was also approximately 12%, similar to the CL site in the present study.

The air and soil temperatures and percent soil moisture were similar for the two sites. There was no significant difference in any of these parameters between the sites.

Soil dehydrogenase activity (reducing activity) was significantly higher in the CL site; yet, it did not show any seasonal pattern in that site. The SR site, however, showed a marked peak of activity early in the study. Overall, the rates of activity in the present study are comparable to those of others (Annala and Kapustka, 1982; Huffman et al., 1986).

The two sites were similar in air and soil temperatures and soil moisture, however, there appear to be significant differences in soil pH, dinitrogen fixation activity, and soil ammonium levels. The dinitrogen fixation activity peaks during times of higher soil water content and decreases dramatically during drier conditions. This pattern is similar to that found in southern Ohio (DuBois and Kapustka, 1983) and supports the conclusion that soil water greatly impacts the seasonal pattern of dinitrogen fixation.

The CL site was slightly alkaline (pH 7.0 to 7.5), while, the SR site was slightly acidic (pH 6.1 to 6.8). This factor may well have had an impact on the actual rates of dinitrogen fixation activity in the two sites. Firstly, the results (Fig. 3) show that the majority of the biologically fixed nitrogen in these sites was contributed by colonies of *Nostoc* sp. (and possibly other cyanobacteria as well). Secondly, it has been well documented that dinitrogen-fixing cyanobacteria prefer a neutral to slightly alkaline pH environment (Fogg et al., 1973; Stewart, 1974; Stewart et al., 1978). Even though the CL site had a far greater amount of dinitrogen fixation activity, the SR site showed twice the seasonal mean of soil ammonium (1.01 $\mu\text{mol/g}$ soil) than the CL site (0.49 $\mu\text{mol/g}$ soil). The more acidic soils of the SR site may be more efficient at retaining the soil ammonium than are the alkaline soils of the CL site. The alkaline soils of the CL site may be promoting a greater loss of soil ammonium by conversion of the ammonium ion to ammonia gas and, hence, loss to the atmosphere. This may be evident since the soil ammonium levels rose substantially at both sites only 1 to 2 weeks after the substantial increase in dinitrogen fixation activity. The soil ammonium level in the CL site decreased approximately 2 weeks after the decrease in dinitrogen fixation activity. However, the soil ammonium level in the SR site (although fluctuating) remained relatively high throughout most of the growing season (until mid-September). It would be difficult to determine from these data if the soil pH of these sites is directly affecting the growth and dinitrogen fixation activity of the cyanobacteria or affecting the levels of soil ammonium, which in turn affects the cyanobacterial dinitrogen fixation activity. Either way, the soil pH may be playing a role in regulating the overall amount of dinitrogen fixation activity in the cedar glades, whereas the soil moisture appears to impact the seasonal pattern of dinitrogen fixation. The glades with slightly acidic soils would have a low amount of dinitrogen fixation activity; yet, they would be able to maintain higher soil ammonium levels throughout the growing season. The glades with alkaline soils would promote greater growth and dinitrogen fixation by cyanobacteria; yet, they would not have the ability to retain the soil ammonium throughout the growing season.

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