Nitrogen fixation in forests of central Massachusetts

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The acetylene reduction method was used to measure nitrogen fixation in soil cores of 16 cm diameter and 16 cm depth that included A and B horizon soil, roots, and decaying litter. Forty-three combinations of location and associated tree species were sampled. The rate of nitrogen fixation for most soil cores was 1 g N ha^{-1} day^{-1} or less, which extrapolates to less than 0.2 kg N ha^{-1} $year^{-1}$. The highest rates, with values of up to 23 g N ha^{-1} day^{-1}, were observed in old fields being invaded by trees. The time course of acetylene reduction was usually linear for a 24-h period, most of the activity was in the upper 15 cm of soil, and the maximum rates were observed in midsummer. No significant nitrogen fixation was observed in preliminary measurements of decaying woody litter or of aerial surfaces of trees and rocks. It is suggested that nitrogen fixation is not a significant input of nitrogen for the forests studied. If so, there may be significant unrecognized nitrogen inputs to forests, such as dry absorption of ammonia from the atmosphere.

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L'auteur a utilisé la méthode par réduction de l'acétylène pour évaluer la fixation d'azote dans des cylindres de sol ayant 16 cm de diamètre et 16 cm de hauteur, comportant les horizons A et B et incluant les racines et la litière en décomposition. Quarante-trois combinaisons de localités et d'espèces d'arbres associés ont été échantillonnées. Dans la plupart des sols, le taux de fixation a été estimé à 1 g N ha^{-1} $jour^{-1}$ ou moins, ce qui par extrapolation représente moins de 0.2 kg N ha^{-1} $année^{-1}$. Les taux les plus élevés atteignant 23 g N ha^{-1} $jour^{-1}$ ont été observés dans de vieux champs en voie d'être envahis par le forêt. La réduction de l'acétylène en fonction du temps est habituellement linéaire au cours de 24 h; l'activité se retrouve surtout dans les 15 premiers cm du sol, les taux maximums ayant lieu au milieu de l'été. Aucune fixation significative n'a été observée lors de mesures préliminaires conduites dans de la litière ligneuse en décomposition ainsi qu'à partir des surfaces aériennes des arbres et des pierres. D'après l'auteur, la fixation d'azote ne constituerait pas un apport important d'azote dans ces forêts. Si tel est le cas, il pourrait exister des apports méconnus d'azote dans les milieux forestiers, telle que l'absorption d'ammoniaque à partir de l'atmosphère.

[Traduit par le journal]

Introduction

Nitrogen is the mineral nutrient which is taken up in the largest quantities by forest trees, and additions as fertilizer often increase the rate of tree growth (1). Although large quantities of nitrogen are present in forest soils, there is probably a significant rate of nitrogen loss due to leaching, denitrification, tree removal, and forest fires. Thus soil nitrogen levels would gradually decline if these losses were not balanced by nitrogen inputs to the forest. A detailed analysis of the nitrogen budget for the Hubbard Brook Experimental Forest in New Hampshire suggests that the major inputs of nitrogen are from precipitation and nitrogen fixation (2). Nitrogen fixation was estimated to be 14 kg N ha^{-1} $year^{-1}$ and was calculated from the rate of nitrogen accretion, outputs, and other known inputs, rather than by direct measurement. A number of researchers have made direct measurements of nitrogen fixation in forests and some have found rates that are small compared with the nitrogen input in precipitation (3, 4, 5, 6, 7). But others have reported substantial activity. Nitrogen fixation of from 8 to 20 kg N ha^{-1} $year^{-1}$ has been estimated for the soil and canopy of Douglas fir in England (8), 10 kg N ha^{-1} $year^{-1}$ for swamp forest soils in South Carolina (9), 12 kg N ha^{-1} $year^{-1}$ for an oak–hickory forest in North Carolina (10), and 2 to 10 kg N ha^{-1} $year^{-1}$ for lichens growing on Douglas fir in Oregon (11). Substantial nitrogen fixation has also been reported for mixed woodland in England (12), decaying litter in coniferous forests in New Zealand (13), and various reports of significant phyllosphere activity have been reviewed by Ruinen (14).

But there have been no investigations of nitrogen fixation in the forests of northeastern United States, except for a study of nitrogen fixation by decaying woody litter at Hubbard Brook in New Hampshire (15), and preliminary measurements

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(with negative results) of nitrogen fixation in the rhizosphere, phyllosphere, and organic and mineral soil horizons at the same location (15). Also, most of the previous studies have included only a few sampling sites and tree species, and the nitrogenase assay has often been done with soil samples that were substantially disrupted. In the present study I have minimized disruption by using much larger diameter soil cores than have most other workers and have sampled 43 different combinations of location and tree species.

Materials and Methods

All measurements were made at the Harvard Forest in central Massachusetts in 1977. The soils sampled developed from glacial tills, derived from granitoid and schistose rocks. Well-drained soils are most common and have been classified as Entic Haplorthods or Entic Fragiorthods (16). Moist soils are Aquentic Fragi- or Hapl-orthods, while wet soils are several different subgroups of Inceptisols. For well-drained soils, the horizon thicknesses are approximately as follows: O, 3 cm; A, 5 cm; B, 60 cm. The soil pH of the A horizon, measured in water, varied from 3.7 to 5.3. Rainfall occurred at regular intervals during the sampling period, and the soil was always moist when sampled. The soil temperature at 8 cm varied from about 14°C in June, to 18°C in August, to 8°C in October.

Nitrogen fixation was measured by the acetylene reduction method (17), using modifications of the procedure described earlier (18). Undisturbed soil cores (original soil structure intact) were taken with a shovel and trimmed to a diameter of about 16 cm and a depth of 14 to 18 cm. The cores included the O, A, and usually part of the B horizon. The cores were placed in polyethylene tubs of 4.9-L volume and gas samples were removed via serum stoppers fitted into the snap-on lids. The leakage rate from the containers was negligible for 1- or 2-h incubations, as evidenced by a 3% or less decrease in acetylene concentration, but for longer periods the lids had to be sealed on with adhesive plastic tape. Acetylene was generated from calcium carbide and added to the air in the tubs to give about 0.1 atm (1 atm = 101.325 Pa) of acetylene in the gas phase surrounding the soil sample. The cores were incubated either at the site of collection at air temperature for a 1.5- to 2-h period, or for longer periods in an incubator at soil temperature. Acetylene and ethylene were measured by gas chromatography of gas samples taken at 0.5 and 1.5 h (or sometimes longer) after addition of acetylene, and the acetylene reduction rate was calculated from the increase in ethylene peak height. Acetylene reduction rates were converted to nitrogen fixation rates using an assumed ratio of $3\,C_2H_2 : 1\,N_2$ (17). At most locations, six replicate cores were taken within an area of about 30 m^2. Within this area, all trees were of the same species, and the cores were always immediately adjacent to only this species. Acetylene was added to five of the cores and the sixth was used as a control to measure the rate of ethylene evolution in the absence of acetylene. An ethylene peak was usually not detectable in the control samples. The maximum values observed corresponded to a nitrogen fixation rate of about 0.3 g N ha^{-1} day^{-1}.

Nested sample plots were used to measure nitrogen fixation by woody litter. Litter samples with diameters greater than 3.0 cm were sampled in 1 × 7.6 m plots. If possible, all litter from a plot was incubated in a single plastic tub of 4.9-L volume, the same as used for soil cores. If the volume was too large, a representative subsample was taken with a saw. Woody litter samples with diameters less than 3.0 cm were collected from 1-m^2 plots, with all of the litter from a plot being placed in a single plastic tub. Measurements were made when the wood was wet from heavy rains. On 26 July the litter was incubated outdoors with acetylene for 3.3 h at 18°C, and on 22 September it was incubated indoors at 20°C for 32 h.

Samples of moss, lichens, and bark from rocks and tree trunks were incubated in a 7 × 13 cm layer in 700-mL glass bottles, while small branches and their attached leaves were incubated in a 30× 50 cm layer in Saran bags. Collections were made after a 1-day period of rain, while leaf and bark surfaces were still wet. Both bags and bottles were placed in a growth chamber at 25°C and about 500 ft-c (1 ft-c = 10.764 lx) of light for 5 h and at 20°C in the dark for 12 h.

Results

The rate of acetylene reduction was measured as a function of time for soil cores taken under *Fraxinus americana*, *Rhus typhina*, *Acer rubrum*, *Quercus rubra*, *Populus tremuloides*, *Acer saccharum*, and *Carya ovata*. With the exception of the cores taken under *Acer rubrum*, a linear time course was observed, although a lag of up to 40 min may have occurred, since the first measurements were made 40 min after C_2H_2 addition (*F. americana* and *R. typhina*, Fig. 1). *Acer rubrum* was growing in a wet soil and there was a lag of about 4 h before linear rates were observed (Fig. 1). These results indicate that the length of the incubation period had little effect on the accuracy of the measurements, except for the soil cores taken under *A. rubrum*, where too short an incubation period may have led to an underestimation of the rate in some measurements. Previous work has also shown that about 30 min is sufficient for C_2H_2 diffusion into soil cores and C_2H_4 diffusion out. Tjepkema and Burris (18) found that rates of

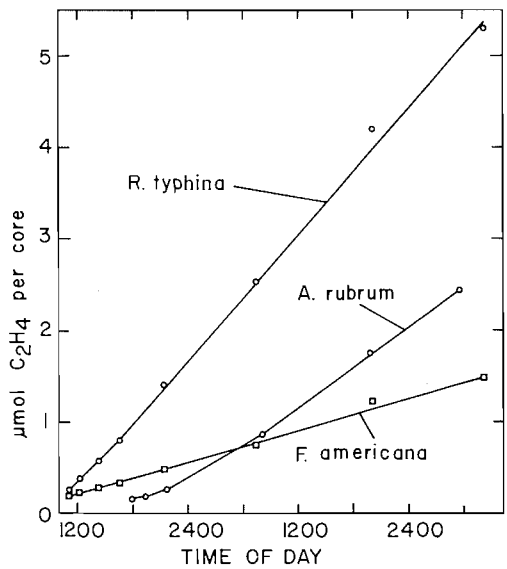

FIG. 1. Acetylene reduction to ethylene as a function of time after the addition of acetylene. Each point is the mean of five replicates.

TABLE 1. Variation of nitrogenase activity with soil depth (grams N per hectare per day)

Species	Date	Depth, cm	Mean	SE	No. of samples
Fraxinus americana	26 Aug. 77	0–15	1.3	0.4	3
		15–30	0.13	0.02	3
		30–45	0.16	0.03	3
Quercus rubra	19 July 77	0–15	0.9	0.26	5
		15–30	0.29	0.06	5

acetylene reduction associated with prairie grasses were linear at 15 min after the addition of acetylene for soil cores of both 6.8 and 12 cm diameter. Tjepkema and van Berkum (19) found that 1.5 h was required for acetylene to become 50% equilibrated at a depth of 20 cm from the soil surface, with 5 h being required for 90% equilibration. In the present experiments, the average distance to the surface of the cores was only 2.3 cm (8 cm maximum), so that about 40 min for the equilibration of C_2H_2 and C_2H_4 between the soil and external gas phase appears to be reasonable.

The effect of core depth on acetylene reduction activity was examined for soil cores under *F. americana* and *Q. rubra* (Table 1). A large fraction of the activity appears to be in the upper 15 cm of soil, so that deeper cores are not necessary for estimating the rate of nitrogen fixation in these soils. Depth effects were not examined for other species, but Day *et al.* (12) have reported high nitrogenase activity at depths of 50 cm in some wet soils. Since nitrogenase activity was examined only in the upper 16 cm of wet soils in the present study, it may have been underestimated. But forested wet soils are limited in extent at the Harvard Forest and included only the *Betula alleghaniensis* site and three of the seven *Acer rubrum* sites.

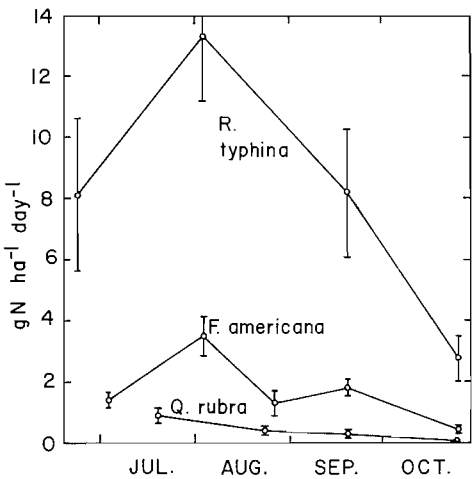

FIG. 2. Seasonal variation of nitrogenase activity in root–soil cores at three locations. The vertical bars indicate ± 1 SE; $n = 5$, except for *F. americana* on 26 August, where $n = 3$.

Nitrogenase activity in Harvard Forest soils was maximal during the summer season, with the rate being lower in spring and fall, and probably negligible in the winter (Fig. 2). Thus if one measures a low rate of nitrogen fixation at a given site on a summer date, under conditions of adequate soil moisture, it is probable that the yearly rate of nitrogen fixation is also low.

The nitrogenase activity found in forest soils of the Harvard Forest is summarized in Table 2. In many of the samples, there was no measurable acetylene reduction during the period of measurement (1.5 h or more). For these samples, the maximum rate that could have occurred without detection is indicated in Table 2 and was 2 g N ha^{-1} day^{-1} or less. The highest nitrogenase activity was found in old fields that had been invaded by *Fraxinus americana*, *Rhus typhina*, *Populus tremuloides*, *P. grandidentata*, and *Betula populifolia*. The trees were about 10–25 years old and the mean rate of nitrogen fixation at a given location was as much as 23 g N ha^{-1} day^{-1}. But the rates observed in older stands of trees were much lower, with a rate of less than 1 g N ha^{-1} day^{-1} being observed for most sites and tree species. The highest rate found in a forest stand on mesic soils was 3.5 g ha^{-1} day^{-1}, while 7.6 g N ha^{-1} day^{-1} was found under *Acer rubrum* growing on a wet site.

Other possible sites of nitrogen fixation in the forest were considered in preliminary experiments. The results of Table 3 suggest that woody litter is not an important site of nitrogen fixation, since at the more active of the two sites measured, the total rate by all sizes of litter was only 1.1 g N ha^{-1} day^{-1}. But there was a wide variability in rate between plots, which was especially a problem for fallen trunks, since only one (*Castenea dentata*) was found on the 11 plots measured.

Preliminary measurements were also made of nitrogen fixation by organisms such as lichens, blue-green algae, and bacteria on the aerial surfaces of trees and rocks. Although only a few samples were taken, the data of Table 4 indicate that nitro-

TABLE 2. Estimated rates of nitrogen fixation in soils under various tree species at the Harvard Forest (grams N per hectare per day)

Species	No. of sites	No. of dates	No. of cores	Range of mean nitrogenase activity	Range of dates
Rhus typhina	3	6	28	<2–13.3	10 June – 25 Oct.
Populus grandidentata	1	1	2	11	11 June
P. tremuloides	4	4	20	4.3–15.4	11 June – 23 Aug.
Betula populifolia	4	4	20	<0.8–9.5	11 June – 23 June
B. papyrifera	3	3	15	<0.7–<0.8	16 June – 3 July
B. alleghaniensis	1	1	5	<1.5	10 July
Fraxinus americana	2	5	23	0.4–23	23 June – 25 Oct.
Prunus serotina	1	1	5	<0.5	3 July
Acer rubrum	7	9	44	<0.6–7.6	16 June – 19 Sep.
A. saccharum	1	1	5	0.2	23 Aug.
A. pensylvanicum	1	1	5	<1.1	15 June
Quercus rubra	3	7	35	0.1–<1.0	15 June – 25 Oct.
Fagus grandifolia	2	1	10	<1.1	15 June
Carya ovata	1	1	5	1.0	23 Aug.
Castanea dentata, *Betula lenta*	1	1	5	<0.6	9 June
Pinus strobus	3	3	15	<0.3–<0.8	18 May – 16 June
Pinus resinosa	2	2	10	<0.5–<0.8	6 June – 16 June
Picea glauca	1	1	5	<0.8	18 June
Tsuga canadensis	2	2	10	<0.5–<1.1	6 June – 15 June

TABLE 3. Nitrogen fixation in woody litter (grams N per hectare per day)

Overstory trees	Date	Stem diameter, cm	No. of plots	Mean	SE
Quercus rubra	26 July 1977	0–3	6	0.018	0.006
		>3	6	0.06	0.03
Quercus rubra, *Pinus strobus* *Acer rubrum*, *Betula lenta*	22 Sep. 1977	0–3	5	0.4	0.18
		>3	5	0.06	0.025
Castanea dentata	22 Sep. 1977	40 cm	1	0.6	—

TABLE 4. Nitrogen fixation by organisms on the aerial surfaces of trees and rocks (grams N per hectare per day)

Sample type and species	No. of samples	Mean	SE
Leaves and small branches			
Quercus rubra,* *Acer rubrum*,* *Betula populifolia*, *Rhus typhina*, *Fraxinus americana*, *Pinus strobus*	8	0.14	0.036
Tree trunks			
Quercus rubra,* *Acer rubrum*, *Rhus typhina*, *Fraxinus americana*	5	0.04	0.021
Junction of tree trunk and soil			
Quercus rubra,* *Acer rubrum*, *Fraxinus americana*	4	2.2×10^{-4}	1.3×10^{-4}
Exposed rocks; in stands of:			
Quercus rubra,* *Acer rubrum*, *Rhus typhina*, *Fraxinus americana*	5	0.003	0.003

*Two samples were taken for these species; one sample was taken for each of the other species.

gen fixation by these organisms is also an insignificant contribution to nitrogen inputs in the forests of central Massachusetts.

Discussion

The data presented in this paper strongly suggest that nitrogen fixation in the forest soils of central Massachusetts is not significant in terms of the overall nitrogen budget. Fixation rates for most soil cores were 1 g N ha^{-1} day^{-1} or less. Owing to low soil temperatures in the colder months, this activity is probably maintained for no longer than 150 days per year, so that the yearly rate of nitrogen fixation would be no more than 0.15 kg N ha^{-1} $year^{-1}$. This amount of nitrogen is negligible compared with the 6.5 kg N ha^{-1} $year^{-1}$ observed in precipitation at Hubbard Brook, New Hampshire (2), which is probably similar to that received at the Harvard Forest. It is possible that the acetylene reduction technique might underestimate the true nitrogen fixation rate, but the only known example of this is for nitrogen fixation by methane-oxidizing bacteria, where acetylene inhibits the oxidation of methane (20). Methane-oxidizing bacteria are probably not important in forest soils. Higher nitrogen fixation rates were observed under trees that were invading old fields, but the annual rate of nitrogen fixation was still very low, about 1.0 kg N ha^{-1} $year^{-1}$.

Sources of nitrogen fixation other than in soils have not been thoroughly considered in the present research. But no nodulated plants are present in the forest, except for *Alnus rugosa* in wet places in old fields, *Comptonia peregrina* in very scattered locations in forest openings, and *Amphicarpa bracteata* in a few wet areas. In the experiments of Tables 3 and 4, nitrogen fixation by decaying woody litter and organisms on tree and rock surfaces was briefly considered. The data indicate that neither is an important source of nitrogen fixation, although the rate of nitrogen fixation may sometimes be as great as that in the soil. The low rates of nitrogen fixation in woody litter are consistent with the findings of Roskoski in New Hampshire (15).

The low pH of soil and rainfall may be factors limiting nitrogen fixation at the Harvard Forest. The highest rates of nitrogen fixation were observed in old field sites where the soil pH in the A horizon was about 5.2 while the highest rate in older forests was for a site under *Fraxinus americana* with a pH of 4.8, and the pH at the remaining sites of lower activity ranged from 3.8 to 4.6. The acidity of precipitation at the Harvard Forest has not been measured but a mean pH value of 4.1 has been reported for a 10-year period at Hubbard Brook, New Hampshire, which is about 125 mi (1 mi = 1.609 km) north of the Harvard Forest (21). This acidity appears to be substantially due to air pollution by sulfur dioxide, and this level of acidity and (or) sulfur dioxide might suppress nitrogen fixation by lichens (22, 23).

The rate of nitrogen fixation in soils at the Harvard Forest appears to be generally less than 0.2 kg N ha^{-1} $year^{-1}$. If it is confirmed that nitrogen fixation in the phyllosphere and woody litter is also negligible, then the only known input of nitrogen to the forest would be from precipitation, and this input is relatively small. If the preceding is true, losses of nitrogen, such as by denitrification and leaching, must also be small or the nitrogen content of the soil would gradually decline. Another possibility is that there are unrecognized sources of nitrogen input. One that deserves further investigation is the dry absorption of ammonia from the atmosphere by tree leaves and the soil. It has been shown that this is possible in some systems and that the rate may be significant (24).

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