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Nitrate reductase activity as an indicator of nitrate fixation and assimilation by tropical forest species on St. Thomas Island

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ABSTRACT: Measurements of nitrate in precipitation and nitrate reductase (NR) activity in green tissues of plants were taken in the remote crater lake area in Sao Tome Obo National Park. The nitrate concentrations in rainfall varied from 0.15 to 0.78 mg per dm^{-3}. Five tropical plant species (both endemic and common) growing under tropical forest canopy were investigated. NR activity in green tissues of plants was measured. NR activity differed greatly between the investigated plants. The lowest NR activity, close to null, was found in *Asplenium africanum* Desv. and *Cycloporus spissus* Desv. The highest was found in endemic *Begonia crateris* Exell., up to 1800 nmol of nitrite synthesized per g of dry mass per hour.

KEY WORDS: nitrate reductase activity, nitrogen deposition, nitrogen fixation, assimilation, tropical forests

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INTRODUCTION

Lack of available nitrogen is a widely known phenomenon in terrestrial ecosystems. Although free nitrogen may be fixed from the atmosphere by some prokaryotic organisms, both free- living and symbiotic (Kumar & Kumar 1988; Sinha & Kumar 1992), this form of nitrogen is not available to most vascular plant species. That is why nitrogen frequently has been found to be the inorganic element with the greatest effect on growth and productivity (Harper 1994). Nitrogen may be one of the most limiting nutrients, in addition to phosphorus, in tropical rain forest ecosystems. It has been demonstrated that dry and wet deposition of nutrients from the atmosphere can contribute a significant amount of nu-

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trients to rain forest (Stachurski 1987; Whitmore 1991; Silver 1994). Some plants are known to be able to absorb airborne nitrate compounds such as nitrogen oxides, nitrate, or gaseous nitric acid directly from atmospheric fallout (Norby 1989; Krywult *et al*. 1996; Krywult & Bytnerowicz 1997). Rain forests generally have developed on old soils that often date from the Tertiary (Whitmore 1991). Most of those soils are characterized by such unfavorable features as high salinity, the presence of toxic elements (e.g., aluminum), coarse sandy texture with excessive drainage, and finally very low fertility (Whitmore 1991). Soil substrate usually is formed slowly under dense vegetation and it may be degraded rapidly when exposed to direct rainfall or strong wind action under sparse vegetation (Lesack 1993). Although a tropical forest ecosystem may have continual input of nutrients from weathering bedrock, most of the nutrients are lost through deep erosion and are washed away by rain (Nykvist *et al*. 1994). The poor soil quality of these systems seems to be in contradiction with the luxuriant vegetation of those ecosystems. Little is known about the nutrient sources of tropical rain forests. Recent reports have suggested the following possible sources of nitrogen in the rain forest: mineral soil, nitrogen-fixing epiphyllous algae, and processes of decomposition, which in lowland lasts 4–12 months (Whitmore 1991; Carnejo *et al*. 1994; Silver 1994).

Usually, well-developed fine roots (Silver & Voght 1993) rapidly absorb nitrogen released from dead organic matter. All rain forests also gain some nitrogen from the atmosphere with rainwater (Visser 1964; Thornton 1965; Strigel *et al*. 1994). Electrical fixation of atmospheric nitrogen, photochemical fixation of atmospheric nitrogen, and photooxidation of ammonia are some of the important factors contributing to the presence of nitrates in rainwater.

Atmospheric nitrates may also originate from soil erosion (dust), volcanic eruptions, industrial contamination, meteor trails and atmospheric transport from oceans and large lakes (Visser 1964). The average concentration of nitrates in rainwater often varies in different areas, ranging from 2.1 to 6.1 mg/l (Gore 1968). This variation may be partly explained by differences in the distances from the closest nitrogen sources. Studies carried out in developed countries have confirmed that airborne nitrogen is directly assimilated by plants (Stachurski 1987; Krywult *et al*. 1994). According to previous studies, vascular plants can play an important role in fixation of nitrate from atmospheric fallout (Norby 1989; Krywult *et al*. 1996). Although nutrient cycling in the tropical rain forest has been studied, little is known about atmospheric deposition of nitrogen and the importance of the process to the condition of tropical rain forests. Nor is much known about special plant adaptations for using the airborne pool of nitrogen and other nutrients. Fixation of nitrates by vascular plants directly from atmospheric deposition might be an important source of this limited nutrient; plants may have physiological adaptations to carry out this process.

Our study was intended to *(i)* estimate the nitrate concentration in bulk precipitation in a remote area free of human-sourced pollution such as industrial emissions or vehicle traffic pollution, and *(ii)* measure nitrate reductase activity in different plant species as an indicator of potential fixation and assimilation of atmospheric nitrate by the plants of natural (primary) tropical rain forests.

MATERIALS AND METHODS

Study area

Saint Thomas Island is located in Guinea Bay (lat. 0°15′N, long. 7°35′E). The forest is a typically developed lowland rain forest on the southern and western sides, and mountain-type rain forest in the central part of the island, with undestroyed structure (primary forest). The Lagoa Amelia Crater Lake area where the research was done is situated about 1250 m a.s.l. in the central part of the island (Fig. 1). The climate is characterized as humid tropical climate with annual precipitation above 2000 mm. The air temperature in the Lagoa Amelia Crater Lake was nearly stable at 24–26°C during the investigation period. It rained daily during the early afternoon.

Plant material

The forest around Lagoa Amelia Crater Lake is typically developed mountain rain forest with a high frequency of pteridophytes. During the wet season when the research was performed, rain events are

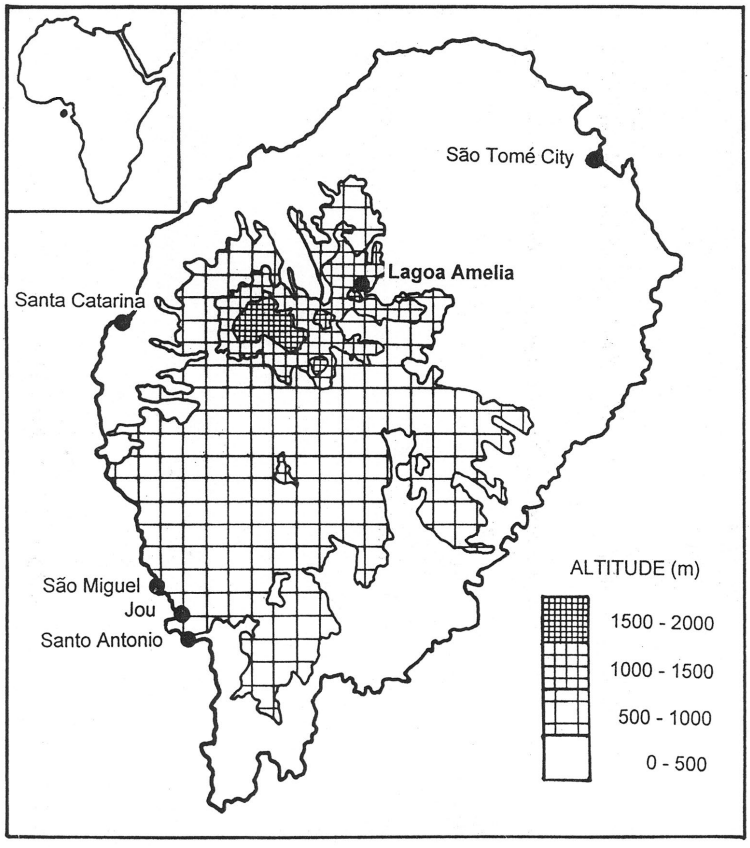

Fig. 1. Schematic vertical map of St. Thomas Island. Mountain chain in the central part of the island is an important precipitation-creating element. At the experimental site (Lagoa Amelia) in the central part of the island, precipitation is higher than 2000 mm annually.

frequent and occur mainly afternoons. Five species growing close to each other on the same slope under the forest canopy were chosen. *Cycloporus spissus* Desv. (Polypodiaceae) and the epiphytic *Asplenium africanum* Desv. (Aspleniaceae) represented the pteridophytes. *Potomorpha* sp. (Piperaceae), *Chinchona* sp. (Rubiaceae), and *Begonia crateris* Exell. (Begoniaceae) (Exell 1944) represented the angiosperms. These species were chosen because they are common in this place.

Estimation of nitrate input

Five bulk precipitation collectors were exposed to rainfall around the investigated area. Rainfall water collecting began before sampling material for NR activity, continued parallel to this experiment, and completed after NR activity measurements ended. Samples were collected at 3-day intervals. The volume of the water samples from the collectors was measured. The samples were mixed, stored in hermetic vials, and immediately mailed to Poland. Nitrate concentration was measured by ion chromatography (DIONEX 100i, USA).

Nitrate reductase activity

Nitrate reductase (NR) activity typically is assayed by measuring nitrite production in tissues that have been vacuum- infiltrated with buffered nitrate solution (Downs *et al*. 1993). In the present study the NR assay was adapted from a number of studies (Jaworski 1971; Chantarotwong *et al*., 1976; Al Gharbi & Hipkin 1984; Norby *et al.* 1989) with our own modifications (Krywult *et al*. 1996). Six to nine leaf circles (4 mm diameter) were collected randomly from previously selected plants. The leaves were collected always at the same time of day, between 2:00 p.m. and 3:00 p.m., directly after rain events. Immediately after collection the plant material was placed in test tubes with buffer solution (dibasic potassium phosphate, pH = 7.5) with 0.6% propanol-1, and evacuated under 0.33 atm for 10 min. Then a known amount of potassium nitrate was added and a sample of solution was taken to obtain the background level of nitrite. The samples were incubated for 2 h at 25^{o}C in a water bath in darkness.

After incubation the amount of synthesized nitrite was determined colorimetrically using sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride at 540 nm (Keeney and Nelson, 1982) with a spectrophotometer (Carl Zeiss, Germany). After incubation the leaf rings were oven-dried at 60^{o}C to dry mass.

Nitrate reductase activity is shown as the amount of nitrite produced (nmol) per gram of leaf tissue dry mass per hour. Three sessions of measurements were performed at 3-day intervals. Four plant individuals of each species were measured. The Kruskal-Wallis nonparametric ANOVA [one-way analysis of variance by ranks] and Dunn's multiple comparison test was used to analyze the differences between examined species.

Results

Nitrate concentration in rainfall

Concentrations of nitrate ions measured in precipitation (rainfall) during the investigation period varied from 0.15 to 0.78 mg (average 0.45, standard deviation = 0.22) per liter of rainfall (Fig. 2). The concentration was lowest in the biggest rainfall event (45 mm). Probably this lowest concentration was caused by high dilution in the large amount of falling rain, although no evidence of a correlation between the amount of water and the nitrate concentration was found.

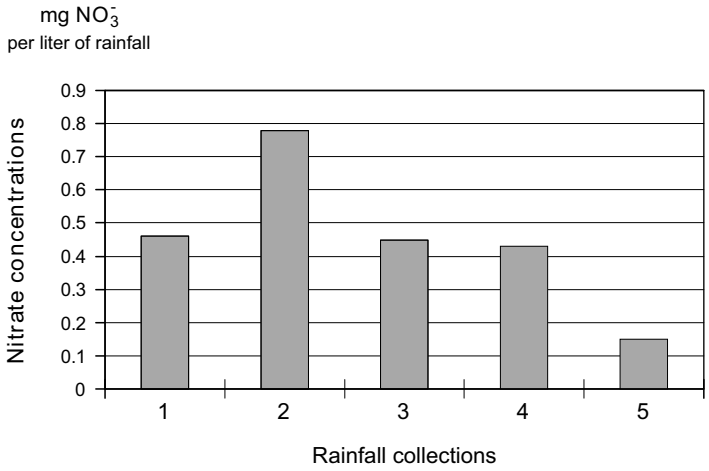

Fig. 2. Average concentration of nitrate in recorded rainfall collections during the experiment. Numbers 1–5 described rainfall collections in Lagoa Amelia crater lake area. Nitrate reductase activity was measured after collections 2, 3 and 4. Collections 1 and 5 were done before and after NR measurements. All collections were done at 3-day intervals.

Nitrate reductase activity

NR activity measured in vivo and shown as the amount of nitrite synthesized per gram of leaf dry mass per hour differed drastically between *Begonia crateris* and the other investigated plants. The highest enzyme activity demonstrated by *Begonia crateris* varied between 519 and 1796 nmol of synthesized nitrite per gram dry mass per hour. There were significant differences between some of investigated species $H = 38.05$; $p < 0.0001$. Differences occurred between *Begonia crateris* and *Asplenium africanum* [mean rank difference (MRD) = 30.96, $p < 0.001$], *Begonia crateris* and *Cyclosporus spissus* (MRD = 35.17, $p < 0.001$), and *Begonia crateris* and *Chinchona* sp. (MRD = 33.54, $p < 0.001$). No statistically significant differences in NR activity between *Begonia crateris* vs. *Potomorpha* sp. and the other investigated plant species were found. The lowest NR activity was in both pteridophyte species: from zero to 58 nmol of synthesized nitrite per gram dry mass per hour. *Chinchona* sp. also demonstrated very low NR activity (Fig. 3).

Discussion

The results obtained in this study show that the nitrate concentration in rainfall was lower than in industrialized areas but higher than that found at other unpolluted sites (Lindberg *et al.* 1986; Godzik 1997). Because air-polluting industry does not exist on the island, there is very little traffic, and villages are situated far away from Lagoa Amelia, the nitrate ion in the rainfall probably is due mainly to high electrical activity in the atmosphere. In that type of ecosystem the amount of nitrate provided with rainfall may play an important role in addition to nitrogen fixation by microorganisms in the whole pool of available

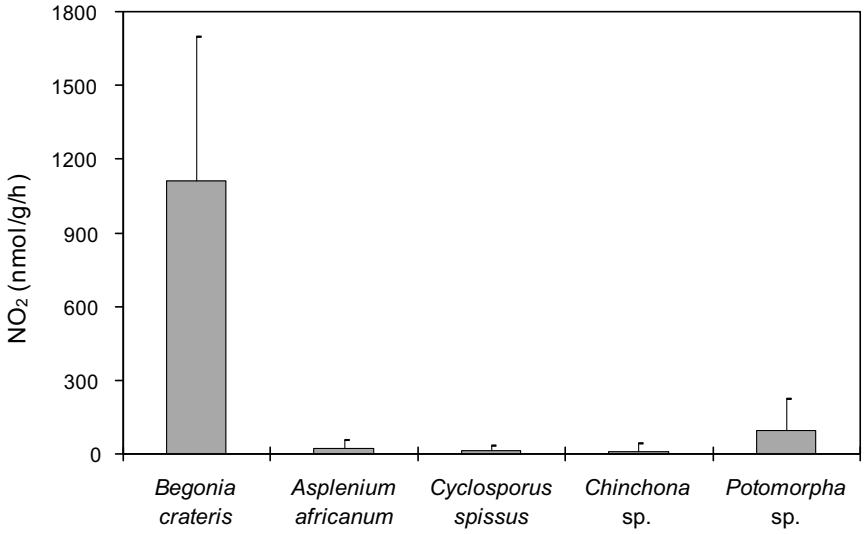

Fig. 3. Nitrate reductase activity demonstrated as amount of nitrite synthesized per gram of dry mass per hour measured in leaves of 5 investigated species at the Lagoa Amelia site in Sao Tome Obo National Park.

nitrogen (Mahendrappa *et al*. 1986; Kumar & Kumar 1988; Stryer 1997). The high decomposition rate, poorly fertilized soils and steep slopes with high daily outflow of water limit the available pool of nitrogen in soil. Nitrogen supply and NR activity play an important role in the growth dynamic of trees (Da Matta *et al*. 1999). In that situation, foliar absorption ability and assimilation of nitrate directly from precipitation should be important tools of plant competition for nitrogen.

To avoid the influence of interfering factors, the authors tried to find a site with similar light and soil conditions for all investigated plants during these studies. The climatic character of the site – constancy of temperatures during the investigation period, constant relative humidity around 98% – and minimal anthropogenic influence, gave an opportunity to examine the differences in NR activity between species at a natural site in similar conditions.

Recent ecophysiological experiments have found that the activity of the enzyme nitrate reductase (NR) in vascular plants is a good indicator of the presence of oxidized nitrogen compounds in the atmosphere (Norby 1989; Norby *et al*. 1989; Downs *et al*. 1993; Krywult *et al*. 1996; Krywult & Bytnerowicz 1997). Other factors may influence NR activity (Norby *et al*. 1989). Different species of plants may reduce nitrate in different parts (roots or shoots) or use different sources of nitrogen such as ammonia or nitrate from mycorrhizal processes (Pate 1980; Andrews 1986; Mahendrappa *et al*. 1986; Gniazdowska-Skoczek 1998). That may partially explain the large differences between the investigated plant species.

More studies are needed to explain these phenomenon. Determining NR levels and activity in plant tissues and estimating a plant's ability to use nitrogen compounds from

the atmosphere should prove useful in studies of the nutrient status and plant condition of various ecosystems.

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