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Leaf uptake of nitrogen dioxide (NO₂) in a tropical wet forest: implications for tropospheric chemistry

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Abstract Tropical forest soils are known to emit large amounts of reactive nitrogen oxide compounds, often referred to collectively as NO_y (NO_y = NO + NO₂ + HNO₃ + organic nitrates). Plants are known to assimilate and emit NO_y and it is therefore likely that plant canopies affect the atmospheric concentration of reactive nitrogen compounds by assimilating or emitting some fraction of the soil-emitted NO_y. It is crucial to understand the magnitude of the canopy effects and the primary environmental and physiological controls over NO_y exchange in order to accurately quantify regional NO_y inventories and parameterize models of tropospheric photochemistry. In this study we focused on nitrogen dioxide (NO₂), which is the component of NO_y that most directly catalyzes the chemistry of O₃ dynamics, one of the most abundant oxidative species in the troposphere, and which has been reported as the NO_y species that is most readily exchanged between plants and the atmosphere. Leaf chamber measurements of NO₂ flux were measured in 25 tree species growing in a wet tropical forest in the Republic of Panama. NO₂ was emitted to the atmosphere at ambient NO₂ concentrations below 0.53–1.60 ppbv (the NO₂ compensation point) depending on species, with the highest rate of emission being 50 pmol m⁻² s⁻¹ at <0.1 ppbv. NO₂ was assimilated by leaves at ambient NO₂ concentrations above the compensation point, with the maximum observed uptake rate being 1,550 pmol m⁻² s⁻¹ at 5 ppbv. No seasonal variation in leaf NO₂ flux was observed in this study and leaf emission and uptake appeared to be primarily controlled by leaf nitrogen and stomatal conductance, respectively. When scaled to the entire canopy, soil NO emission rates to the atmosphere

were estimated to be maximally altered ±19% by the overlying canopy.

Keywords Reactive nitrogen oxides · Leaf NO₂ uptake · Tropical forests · Tropospheric photochemistry

Introduction

Reactive nitrogen oxides (NO_y; NO_y primarily consists of NO, NO₂, HNO₃, and organic nitrates) have a central role in controlling the oxidative chemistry of the lower atmosphere, including (1) regulation of the photochemical production of ozone, a key atmospheric pollutant and greenhouse gas, (2) regulation of the concentration of hydroxyl radical (OH) and other HO_x species, and (3) regulation of the production of nitric acid and organic nitrates, both acid rain precursors (Crutzen 1983). Current atmospheric chemistry models utilize measured soil NO emission rates as a primary input and assume that photochemical transformations of NO to the other components of NO_y occur well above the influence from plant canopies (e.g., Crutzen and Zimmermann 1991). This practice ignores the possibility that plants can affect local photochemistry by assimilating and emitting certain forms of NO_y. Past studies of a tropical forest in Brazil suggested that up to 60% of the soil-emitted NO can be transformed and assimilated by the overlying canopy (Bakwin et al. 1990; Jacob and Bakwin 1991). Emission and uptake of NO_y in the tropics may be particularly important to atmospheric chemistry. Because photochemical reactions proceed most efficiently in the low-latitude humid tropics (Harriss et al. 1988), and since tropical areas are being developed for human use at a rapid rate, it is important to measure intact, undisturbed tropical systems to allow us to understand potential impacts on the atmosphere (Crutzen 1985; McElroy and Wofsy 1986). Additionally, Matson et al. (1998) have suggested atmospheric N deposition will increase in tropical forests, which could lead to increased soil NO emissions (Hall and Matson 1999).

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Worldwide, anthropogenic sources account for approximately two-thirds of total NO_y emissions to the lower atmosphere (primarily from NO production during biomass and fossil-fuel combustion), with most of the remainder produced biogenically by microbial production of NO in the soil (Crutzen 1983; Enhalt and Drummond 1982; Homolya and Robinson 1984; Logan 1983; Placet and Streets 1987; Robinson et al. 1968; Stedman and Shetter 1983). Once in the atmosphere, NO is rapidly oxidized to NO_2 (Fehsenfeld et al. 1992). The NO_2 that is formed is capable of photo-dissociation to NO and ground-state atomic oxygen [$\text{O} (^3\text{P})$], the latter of which can react with O_2 to form O_3 . Details of the chemical reactions involving the components of NO_y are complex. One can generalize, however, by stating that a considerable fraction of the reactive photochemistry that occurs is dependent on the presence of nitrogen oxides – emitted in the form of NO , oxidized to NO_2 , and subsequently converted to a variety of inorganic and organic nitrates (Crutzen 1979; Trainer et al. 1991).

The ability of vegetation to assimilate NO_2 from the atmosphere is well established. Controls over the uptake of NO_2 by plant leaves are associated with the diffusive process, reflecting the interplay between the NO_2 concentration gradient between the atmosphere and the intercellular air spaces of the leaf and the stomatal conductance to NO_2 transport (Johansson 1987; Thoene et al. 1991; Weber and Rennenberg 1996). Plant emission of NO_2 at low atmospheric concentrations has also been observed, indicating the existence of an NO_2 compensation point (Johansson 1987; Rondon et al. 1993; Weber and Rennenberg 1996; Wildt et al. 1997). Other factors that have been observed to influence NO_2 fluxes between leaves and the atmosphere include photon flux density (light intensity), temperature and relative humidity (Neubert et al. 1993; Weber and Rennenberg 1996). The relative importance and potential interactions between these controls has yet to be determined.

In the present study, we have focused on the leaf uptake dynamics of NO_2 in a wet tropical forest. Tropical ecosystems represent some of the largest sources of soil-emitted NO (Williams et al. 1992), and past modeling studies have suggested that tropical forest canopies can significantly influence the amount of soil emitted NO_y that is eventually transported to the atmosphere (Bakwin et al. 1990; Jacob and Bakwin 1991).

Specifically, our studies were conducted to address three fundamental questions:

1. Do leaves of different tropical species, and leaves in different vertical positions in the canopy, vary in their capacity to assimilate and emit NO_2 , and is this variability correlated with differences in stomatal conductance, photosynthesis rate, and/or leaf-level N content?
2. To what extent are these processes affected by seasonality?
3. Do leaves of different species exhibit different compensation points with respect to NO_2 and do these

compensation points lead to the canopy being a net source or sink for reactive N compounds?

Materials and methods

Plant material and fields sites

Field measurements were made on 25 tropical tree species growing in an old growth wet tropical forest on the Caribbean Coast of the Republic of Panama. Of the original 25 species, 16 were identified to have multiple individuals within the study area and were intensely studied. These species appeared to be the most common at the site and were representative of the surrounding forest. Measurements were made during two field campaigns representing the dry (15 February–10 March 1999) and wet (1 November–15 December 1999) seasons. Research was conducted at the Fort Sherman Canopy Crane, which is managed by the Smithsonian Tropical Research Institute for the United Nations Environmental Program. The canopy crane is located within Fort Sherman in a forest preserve site of approximately 120 km². The average annual rainfall at the site is approximately 3,500 mm, and all species are evergreen. The exact forest age is not known, but historical estimates suggest that it has not received intensive logging within the last 200 years. The average canopy height is approximately 40 m, with the tallest trees exceeding 44 m. The crane is 52 m high and has a radial length of 54 m. This length gives a horizontal coverage of approximately 9,000 m². The crane has 1.4 m² gondola, the position of which is controlled by a crane operator working at the top of the tower. The crane operator maintains contact with the gondola occupants by means of a two-way radio and can position the gondola anywhere within the reach of the crane.

NO_2 flux and photosynthesis measurements

Measurements of NO_2 flux were made with a leaf chamber which enclosed 6 cm² of leaf area and was connected to a portable gas-exchange system that measured all photosynthetic parameters (model LI-6400, LiCor, Lincoln, Neb.). An artificial light source was used, which consisted of small, red light-emitting diodes (LiCor). Several known NO_2 concentrations were delivered to the leaf chamber by diluting NO_2 from a cylinder (Scott Specialty Gases, Riverside, Calif.) with ultra-high purity 'zero air'. NO_2 concentration in the air that exited the chamber was measured with a chemiluminescence detector (model LMA-3, Scintrex/Unisearch, Concord, Ontario). The NO_2 detection limit was determined to be 5 pptv using standard gases produced by progressive dilution. The instrument has a linear range between 1 and 50 ppbv (Drummond et al. 1988). Additionally, when calibrated to a known sample gas immediately prior to measurement, as per our measurement procedure, the instrument has been found to respond linearly to NO_2 levels as low as 15 pptv (Bakwin et al. 1990). For measurements on leaves, the experimental protocol consisted of measuring the NO_2 concentration in the air exiting an empty leaf chamber before and after each measurement. The difference between the NO_2 concentrations of the empty chamber and the leaf-filled chamber was attributed to assimilation or emission of NO_2 by the leaf. All NO_2 -enriched air was passed through black PTFE-Teflon tubing enclosed in an opaque plastic sheath to avoid photochemical decomposition. Unless otherwise stated, chamber conditions were controlled at 350 ppm CO_2 , 65% relative humidity, 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, and 28 °C. The LI-6400 system incorporates thermo-electric heat exchangers mounted on the sides of the cuvette to control temperature, and a variable intensity red LED light source with a peak irradiance at 670 nm. Carbon dioxide and water vapor were measured using the LICOR open-path infrared gas analyzers. Several measurements were recorded when leaf internal CO_2 concentration (C_i) was stable, and exhibited values between 240 and 375 ppm CO_2 .

Measurement of leaf area and leaf N content

For determination of leaf N content, leaves were harvested and dried for 3–4 days at 54°C in a drying oven. The dried tissues were then frozen in liquid N₂ and ground to a powder in a mortar and pestle. Samples of 2–4 mg were weighed on a microbalance (Sartorius, Westbury, N.Y.) and analyzed for total N content using a carbon/nitrogen analyzer (model 20/20, Europa Scientific, Crewe, UK) at the University of Georgia, Athens, Analytical Chemistry Laboratory. All values are referenced to apple-leaf standards calibrated by the National Institute of Standards (Boulder, Colo.). Leaf areas were measured using a video leaf area meter (model A, Delta T Devices, Cambridge, UK).

Calculations and statistics

The flux of NO₂ (J_{NO_2} ; mol m⁻² s⁻¹) to or from a leaf was calculated as:

$$J_{\text{NO}_2} = f(C_0 - C_1) / A \quad (1)$$

where f (mol s⁻¹) is the air flow rate through the chamber, $C_0 - C_1$ (mol mol⁻¹) is the difference in NO₂ mole fraction between the outlet of the chamber containing a leaf (C_1) and the empty chamber (C_0), and A (m²) is the leaf area enclosed in the chamber.

Means were compared for significant differences using analysis of variance (ANOVA). Individual trees were used as the experimental unit and multiple measurements on an individual tree were averaged into a single independent sample. The existence of significant relationships between two variables was determined by simple linear and nonlinear regressions. Relationships between NO₂ flux, stomatal conductance, and photosynthetic rates were compared using a non-linear least squared regression of the form:

$$y = y_0 + a[\exp(-bx)] \quad (2)$$

where y is NO₂ flux, x is stomatal conductance or photosynthesis rate, and a and b are fitting parameters. This model has been used successfully to describe photosynthetic response to light intensity (Huxman et al 1998); a response similar to the relationship we observed between photosynthetic rate, stomatal conductance and NO₂ flux. All statistical tests were conducted with SAS statistical software (Little et al. 1991) using a significance level of 0.05.

Results

NO₂ uptake rates varied across sixteen tree species from 153 to 1550 pmol m⁻² s⁻¹ when measured at 5 ppbv NO₂ (Fig. 1). The highest uptake rates were observed in *Manilkara bidentata*, an overstory-dominant tree species. The lowest rates were observed in *Virola novi*, a lower canopy tree. In general, upper canopy species exhibited higher uptake rates compared to lower canopy species. Emission rates of NO₂ varied among the same 16 tree species between 1.3 and 90.7 pmol m⁻² s⁻¹ at <0.1 ppbv (Fig. 1). The highest emission rates were observed in *Lonchocarpus longifolium*. During the measurement period (1 November–15 December 1999) *L. longifolium* was undergoing an annual leaf senescence cycle, which may have affected NO₂ emission rates. Emission rates were not significantly different between upper canopy and lower canopy trees.

Rates of NO₂ emission and uptake were not significantly different between the wet and dry seasons in the two canopy dominant species, *Brosimum utile* and *M. bi-*

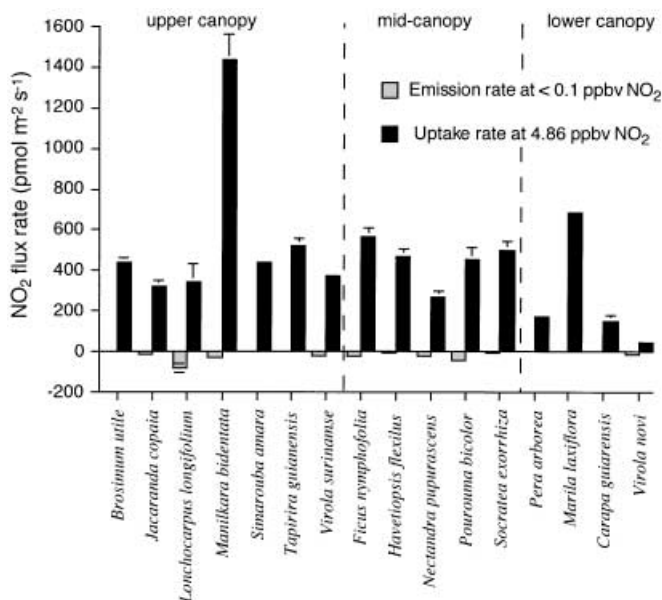


Fig. 1 Leaf NO₂ uptake and emission rates measured for sixteen tropical tree species. Each bar represents the mean of 15–45 measurements. Species are separated by dashed lines indicating relative canopy location. Error bars = ±1 SE. Error bars are not shown if smaller than the symbol used

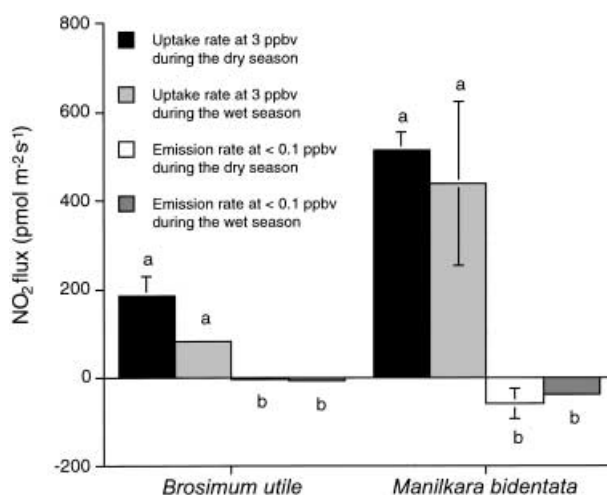


Fig. 2 Comparison of leaf NO₂ flux rates measured during the wet and dry seasons. Each bar represents the mean of 35–45 measurements. Error bars = ±1 SE. Error bars are not shown if smaller than the symbol used

dentata (Fig. 2). Inter-seasonal comparisons using other species were limited by lack of measurements at all cuvette NO₂ concentrations during the dry season. However, direct comparisons of measurements made on the same trees showed similar flux rates in both seasons. The one exception to this was *L. longifolium*, which exhibited higher emission rates and lower uptake rates in the wet season compared to the dry season. As has been previously stated, *L. longifolium* was undergoing leaf senescence during the wet season, which may have affected the comparison of NO₂ flux rates.

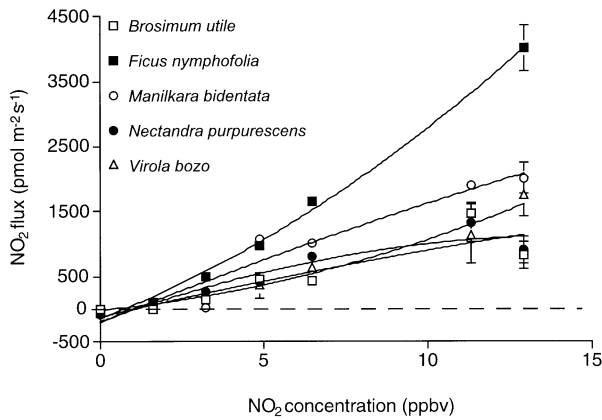


Fig. 3 The influence of NO_2 concentration on the flux of NO_2 . Each data point represents the mean of ten leaves. Error bars = $\pm 1\text{SE}$ ($n = 10$). Error bars are not shown if smaller than the symbol used. See Table 1 for NO_2 compensation points

Table 1 Compensation points with respect to NO_2 (Γ_{NO_2} ; ppbv, $F = 38.575$, $P < 0.0001$, $n = 10$), average maximum photosynthetic rate (A_{max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, $F = 19.343$, $P < 0.0001$, $n = 9-34$), and average leaf area specific nitrogen content (N_{leaf} ; mmol N m^{-2} , $F = 190.267$, $P < 0.0001$, $n = 8-130$), for five tropical tree species. Letters indicate significant differences (SNK test, $P < 0.05$)

Species	Γ_{NO_2}	A_{max}	N_{leaf}
<i>Brosimum utile</i>	0.52 ^a	7.75 ^b	157 ^a
<i>Ficus nympholia</i>	0.85 ^b	10.03 ^c	151 ^a
<i>Nectandra pupurescens</i>	1.09 ^c	4.12 ^a	148 ^a
<i>Virola novi</i>	1.23 ^c	4.08 ^a	241 ^c
<i>Manilkara bidentata</i>	1.60 ^d	8.09 ^b	224 ^b

The influence of NO_2 concentration on the flux of NO_2 to leaves was determined by sampling ten leaves each in five species at NO_2 concentrations between 0.1 and 13 ppbv (Fig. 3). At low NO_2 concentrations, many of the leaves were net sources. As the NO_2 concentration increased, the NO_2 flux changed from emission to uptake, and there was a significant, positive correlation between NO_2 concentration and NO_2 flux. The compensation point for NO_2 uptake was estimated to be between 0.52 and 1.60 ppbv across five species (Table 1). The compensation point with respect to NO_2 (Γ_{NO_2} ; Table 1) was generally related to leaf N content (N_{leaf} ; Table 1), but not to maximum rates of photosynthesis (A_{max} ; Table 1).

NO_2 uptake rates appear to be in part regulated by stomatal dynamics ($F = 23.9674$, $P < 0.0001$). As stomatal conductance increased from 0 to $0.25 \text{ mol m}^{-2} \text{ s}^{-1}$, rates of NO_2 uptake also increased (Fig. 4 a). However, at conductances $> 0.25 \text{ mol m}^{-2} \text{ s}^{-1}$, rates of NO_2 uptake remained constant (Fig. 4a). In contrast, leaf emission rates of NO_2 exhibited no obvious relationship with stomatal conductance (Fig. 4a). Leaves of *M. bidentata* (Fig. 4a, indicated data points) exhibited relatively higher rates of NO_2 uptake at a given conductance compared to all other species. These points were considered outliers and not included in the nonlinear regression analysis.

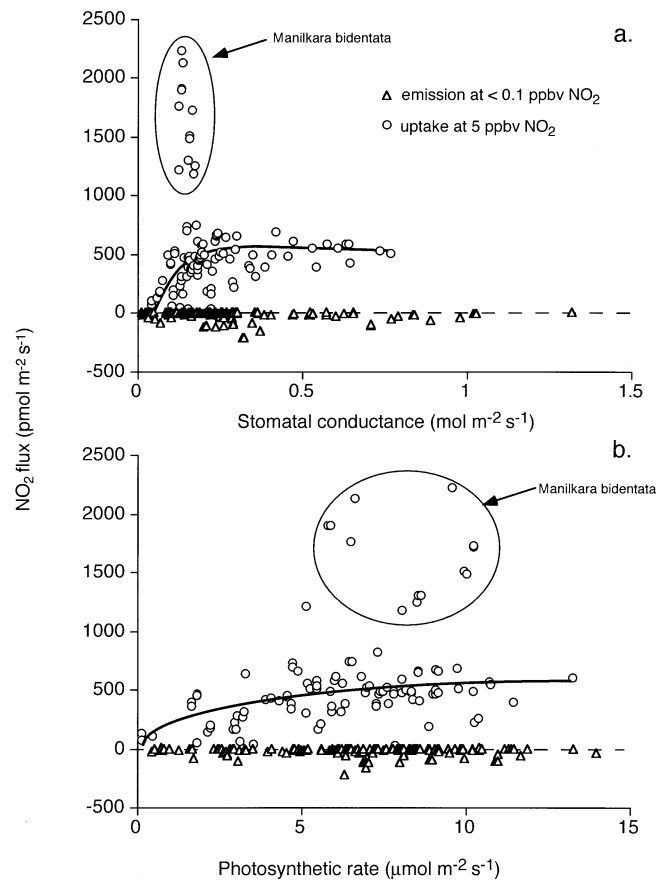


Fig. 4 Relationships between NO_2 uptake (circles) and emission (triangles) rates and **a** stomatal conductance and **b** photosynthetic rate. Each data point represents a single measurement

NO_2 uptake rates were related to rates of photosynthesis (Fig. 4b, $F = 25.6428$, $P < 0.0001$). At rates of photosynthesis $< 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$, rates of NO_2 uptake were $< 100 \text{ pmol m}^{-2} \text{ s}^{-1}$. However, at photosynthetic rates $> 2 \mu\text{mol m}^{-2} \text{ s}^{-1}$, NO_2 uptake rates varied between 100 and $2,200 \text{ pmol m}^{-2} \text{ s}^{-1}$. NO_2 emission rates exhibited no obvious relationship to photosynthetic rate (Fig. 4b). In a manner similar to stomatal conductances, leaves of *M. bidentata* exhibited relatively higher rates of NO_2 uptake at a given photosynthetic rate compared to all other species (Fig. 4b, indicated data points). Similar to conductances, these points were considered outliers and not included in the nonlinear regression analysis.

Leaf uptake rates of NO_2 increased with height within the canopy (Fig. 5a). In addition, leaf N concentrations, stomatal conductances and A_{max} increased with height within the canopy (Fig. 5b,c). In contrast, NO_2 emission rates were relatively constant throughout the vertical expanse of the canopy (Fig. 5a). Specific leaf area did not increase with canopy height when examined across species (data not shown).

Although the highest leaf N contents corresponded to the highest rates of leaf NO_2 uptake, there was no significant relationship (Fig. 6a; $r^2 = 0.307$, $P = 0.08$). In contrast, NO_2 emission rates by leaves were positively

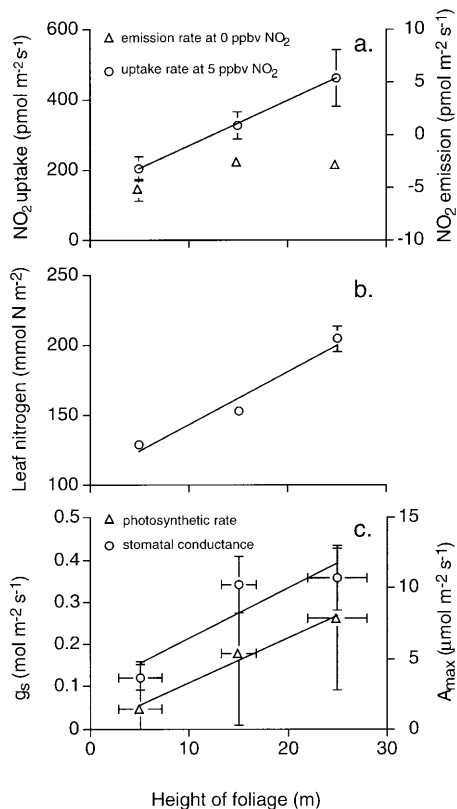


Fig. 5 Trends in **a** NO₂ uptake and emission rates, **b** leaf nitrogen content, and **c** stomatal conductance (g_s) and maximum photosynthetic rate (A_{max}) at increasing heights within the canopy. Each data point is the mean of 12–21 leaves (of different species) presenting foliage within a given height range above the forest floor. The height ranges examined were 0–10 m, 10–20 m, and 20–30 m. Error bars = ± 1 SE ($n = 12$ –21). Error bars are not shown if smaller than the symbol used

correlated with leaf N contents (Fig. 6b; $r^2 = 0.471$, $P < 0.05$).

During the wet-season field campaign (1 November–15 December 1999), we observed an individual of *L. longifolium* in the process of leaf senescence. Over a 30-day period, leaf NO₂ uptake rates decreased from 580 to 1.6 pmol m⁻² s⁻¹ (Fig. 7a). In contrast, leaf NO₂ emission rates were initially low (<10 pmol m⁻² s⁻¹), increased to the highest rates observed in this study (>200 pmol m⁻² s⁻¹), and then decreased (<1 pmol m⁻² s⁻¹) (Fig. 7b). Coincident with the changes in leaf NO₂ flux rate, leaf N contents (Fig. 7c) and maximum levels of photosynthesis (Fig. 7d) decreased.

Discussion

Tropical forest soils represent the largest source of soil-emitted NO and are second only to anthropogenic inputs in contributing to the flux of reactive N to the troposphere (Williams et al. 1992). Therefore, it is important to understand how the overlying plant canopy may alter the flux rate of NO_y compounds to the atmosphere. We

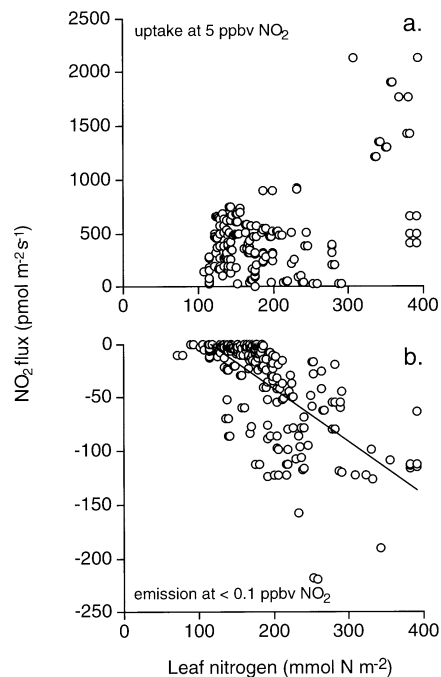


Fig. 6 Relationships between leaf nitrogen content and **a** leaf NO₂ uptake rate and **b** leaf NO₂ emission rate ($r^2 = 0.471$, $P < 0.05$). Each data point represents a single measurement

show in this study (Fig. 1) that NO₂ uptake rates are substantial in tropical forest trees and that there is variation among species in their ability to take up or emit NO₂. At NO₂ concentrations above the compensation point, the average uptake rate for NO₂ was similar in magnitude to that reported for other plant species (Hanson and Lindberg 1991; Okano et al. 1986; Weber and Rennenberg 1996), but the maximum uptake rates we recorded (>1000 pmol m⁻² s⁻¹) were somewhat higher than those reported for temperate trees (Ammann et al. 1995; Rondon et al. 1993; Rondon and Granat 1994; Thoene et al. 1996).

Soil fluxes of NO and within-canopy NO_y concentrations have been reported to be higher in the dry season than during the wet season in tropical wet forests (Bakwin et al. 1990; Kaplan et al. 1988). We therefore investigated if tropical tree leaves take up NO₂ at a higher rate in the dry season compared to the wet season. In the two species examined, uptake rates were similar in both seasons (Fig. 2). Seasonal variation in leaf NO₂ uptake has been reported in an agricultural corn system (Hereid and Monson 2000) and attributed to seasonal differences in stomatal conductance. It may be that because stomatal conductance did not vary between the wet and dry season in the species we examined (data not shown), we observed no seasonal variation in leaf NO₂ uptake.

We observed an increase in NO₂ uptake rate as the concentration of ambient NO₂ was increased (Fig. 3), which is consistent with previous studies (Johansson 1987; Rondon et al. 1993; Thoene et al. 1991; Weber

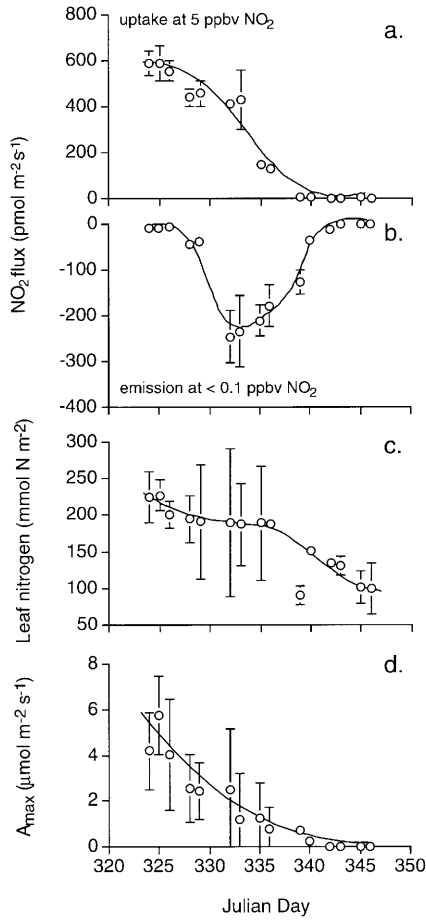


Fig. 7 A 30-day time course of measurements on a single individual of *Lonchocarpus longifolium* undergoing leaf senescence. Measurements include **a** leaf NO_2 uptake rates, **b** leaf NO_2 emission rates, **c** leaf nitrogen contents, and **d** maximum rates of photosynthesis (A_{\max}). Lines through data are hand drawn and not a statistical fit. Error bars = $\pm 1\text{SE}$ ($n=5$). Error bars are not shown if smaller than the symbol used

and Rennenberg 1996). The range of NO_2 compensation points measured for leaves in this study (0.52–1.60 ppbv) is comparable to the value of 1.15 ppbv reported in a past study of wheat leaves (Weber and Rennenberg 1996) and 1.60–1.90 in beech trees (Gessler et al. 2000; Kaplan et al. 1988), but is somewhat higher than the range reported for coniferous trees (0.1–0.7 ppbv; Rondon et al. 1993; Rondon and Granat 1994). NO_2 compensation points were generally related to leaf N concentration with the highest compensation points corresponding to the highest leaf N concentrations (Table 1).

Our observations support past studies that have reported the dependence of NO_2 uptake rate on stomatal conductance (Johansson 1987; Rondon et al. 1993; Thoene et al. 1991; Weber and Rennenberg 1996). However, we only observed evidence of stomatal control when conductances were below $0.5 \text{ mol m}^{-2} \text{ s}^{-1}$ (Fig. 4). At stomatal conductances between 0.5 and $1.5 \text{ mol m}^{-2} \text{ s}^{-1}$ NO_2 uptake rates no longer increased, and we infer that in addition to a stomatal diffusive resistance, a resis-

tance to NO_2 assimilation or emission exists within the mesophyll tissue. Possible internal resistances include the disproportionation reaction of NO_2 in the apoplast and scavenging of NO_2 in the leaf apoplast by ascorbate or other free-radical scavenging compounds.

To our knowledge, the roles of such mechanisms have not been defined with regard to the uptake/emission dynamics of NO_2 . However, in the most comprehensive effort to date Ramage et al. (1993) compared the behavior of NO and NO_2 using three models – one based solely on diffusive limitations, one based on diffusive limitations and the disproportionation reaction in the apoplast, and one based on diffusive limitations, apoplastic disproportionation and ascorbate free-radical scavenging. Although the attempt to validate the model was based on a limited set of measurements, the model with all three components replicated patterns most accurately.

The mechanism behind the unusually high NO_2 uptake rates we observed in *Manilkara bidentata* (Fig. 4, circled datapoints) is unknown. However, *M. bidentata* exhibited some of the highest leaf N concentrations of any species examined and this may, at least partially, explain the higher NO_2 uptake rates.

Leaf NO_2 uptake rates increased coincident with canopy height when averaged across all species (Fig. 5a). This appears to be the result of higher stomatal conductances found higher in the canopy (Fig. 5c). Interestingly, leaf NO_2 emission rates did not increase with canopy height and, on average, appear to have been relatively constant throughout the canopy (Fig. 5a). This may imply that while stomatal conductance exerts strong control over NO_2 uptake rate, it has little control over NO_2 emission rate.

Higher emission and uptake rates were related to leaf N concentration (Fig. 6). In the case of leaf NO_2 uptake, although the highest NO_2 uptake rates corresponded to the highest leaf N concentrations, no significant relationship existed between the two parameters (Fig. 6a). In previous studies, NO_2 uptake rate has been found to be highest in those leaves with the highest N concentration (Heried and Monson, unpublished data) or to have no significant relationship between plant tissue N concentration and NO_2 uptake rate (Rogers et al. 1979). In contrast, a significant relationship between leaf NO_2 emission rate and leaf N content was observed across all species in this study (Fig. 6b). The mechanisms controlling NO_2 emission from leaves are not well understood. However, previous studies have correlated NO_2 exchange rate to nitrate/nitrite reductase activity (Klepper 1991) and stomatal conductance (Johansson 1987; Thoene et al. 1991; Weber and Rennenberg 1996). Our data indicate NO_2 emission in these species is generally related to leaf N concentration (Fig. 6b), but not to stomatal conductances and maximum photosynthetic rates (Fig. 5c). Assuming a relationship between leaf N concentration and leaf nitrate/nitrite reductase activity (Gebauer et al. 1988), our results would be consistent with a positive correlation between NO_2 emission rate and leaf nitrate/nitrite reductase activity.

Table 2 Predicted alterations in NO₂ flux from a tropical wet forest. Values for soil NO flux (Bakwin et al. 1990), leaf area index (LAI) (Jipp et al. 1998; Larcher 1995; Smith et al. 1998), and an

assumed ambient NO₂ concentration. Literature derived values were obtained from similar wet forests in Brazil and Costa Rica

	[NO ₂] < compensation point	[NO ₂] > compensation point
[NO ₂] in canopy	0.40 ppbv	2.00 ppbv
Soil NO flux	150 pmol m ⁻² s ⁻¹	557 pmol m ⁻² s ⁻¹
Leaf emission at <0.40 ppbv NO ₂	3.63 pmol m ⁻² s ⁻¹	
Leaf uptake at 2 ppbv NO ₂		13.56 pmol m ⁻² s ⁻¹
LAI	5–10	5–10
Total emission	168–186 pmol m ⁻² s ⁻¹	421–489 pmol m ⁻² s ⁻¹
% alteration by canopy	+11–19	–12–19

At least in the single species we examined, leaf NO₂ emission rates appear to be enhanced during leaf senescence (Fig. 7b). This increase is most likely due to leakage of NO₂ during the breakdown and translocation of leaf N compounds during senescence. Such a mechanism is supported by our observation of decreased leaf N concentration (Fig. 7c) and photosynthetic rates (Fig. 7d) coincident with a transient increase in leaf NO₂ emission rate (Fig. 7b).

It is obvious that plants have the potential to assimilate a considerable fraction of soil-derived NO after oxidation within the canopy airspace to NO₂. Failure to take this influence into account may result in significant overestimation of the soil as a regional and global source of NO_y (e.g., Jacob and Bakwin 1991). We can illustrate the potential alteration of these fluxes using soil NO flux values reported in the literature and by assuming a range of canopy NO₂ concentrations (Table 2). Based on past measurements of soil NO production from a tropical forest in Brazil (Bakwin et al. 1990), it is reasonable to assume NO fluxes of 150–557 pmol m⁻² s⁻¹. Assuming this soil flux leads to an average within-canopy daytime NO₂ concentration of 0.40–2.00 ppbv, average leaf NO₂ uptake and emission rates would be 13.56 and 3.63 pmol m⁻² s⁻¹, respectively (Table 2). Multiplying these uptake rates by a range of reported LAI values (Jipp et al. 1998; Larcher 1995; Smith et al. 1998), the canopy is estimated to assimilate and emit 11.5–19.6 and 12.2–19.5% of the soil-emitted NO as NO₂, respectively, dependent on LAI.

It is apparent that NO₂ exchanges between plant canopies and the atmosphere are significant in magnitude and can potentially affect atmospheric chemistry dynamics. The results of this study demonstrate that much of the environmental regulation of NO₂ fluxes can be attributed to stomatal dynamics, though there is some evidence that mesophyll processes may also be important. Given the central role that NO₂ plays in the regulation of tropospheric O₃ formation, high priority should be given to elucidating the magnitude of canopy NO₂ uptake in a variety of ecosystems and the significance of such uptake to inventories of soil NO_y emissions to the atmosphere.

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