

Circadian rhythms constrain leaf and canopy gas exchange in an Amazonian forest

Christopher E. Doughty,¹ Michael L. Goulden,¹ Scott D. Miller,¹ and Humberto R. da Rocha²

Received 28 April 2006; revised 19 June 2006; accepted 28 June 2006; published 8 August 2006.

[1] We used a controlled-environment leaf gas-exchange system and the micrometeorological technique eddy covariance to determine whether circadian rhythms constrain the rates of leaf and canopy gas exchange in an Amazonian forest over a day. When exposed to continuous and constant light for 20 to 48 hours leaves of eleven of seventeen species reduced their photosynthetic rates and closed their stomata during the normally dark period and resumed active gas exchange during the normally light period. Similarly, the rate of whole-forest CO₂ uptake at a predetermined irradiance declined during the late afternoon and early morning and increased during the middle of the day. We attribute these cycles to circadian rhythms that are analogous to ones that have been reported for herbaceous plants in the laboratory. The importance of endogenous gas exchange rhythms presents a previously unrecognized challenge for efforts to both interpret and model land-atmosphere energy and mass exchange. **Citation:** Doughty, C. E., M. L. Goulden, S. D. Miller, and H. R. da Rocha (2006), Circadian rhythms constrain leaf and canopy gas exchange in an Amazonian forest, *Geophys. Res. Lett.*, 33, L15404, doi:10.1029/2006GL026750.

1. Introduction

[2] Research on the controls of ecosystem gas exchange has focused on the direct effects of light, moisture availability and other aspects of the physical environment on plant physiology [Jones, 1992; Lambers *et al.*, 1998; Sellers *et al.*, 1997; Bonan, 2002]. The possibility that plant circadian rhythms [McClung, 2001; Johnson *et al.*, 1998; Webb, 2003], associated with the up and down regulation of activity by endogenous biological clocks, constrain the rates of land-atmosphere exchange over a day has received scant attention. This lack of attention is surprising since there is strong evidence that circadian rhythms control many processes in C₃ plants [Harmer *et al.*, 2002] including stomatal conductance [Mansfield and Heath, 1963; Stalfelt, 1963] and leaf photosynthesis [Hennessey and Field, 1991; Williams and Gorton, 1998; Dodd *et al.*, 2005]. Nonetheless, previous work on photosynthetic circadian rhythms has focused on short-lived herbaceous plants growing in pots in the laboratory, and we are unaware of observations of photosynthetic circadian rhythms in the field. Moreover,

we are unaware of surveys that document the occurrence of photosynthetic circadian rhythms in other plant functional types such as large trees, or of efforts to establish the role of circadian rhythms in controlling whole-ecosystem gas exchange over a day.

[3] We used a controlled-environment leaf gas-exchange system and the micrometeorological technique eddy covariance to determine whether circadian rhythms constrain the rates of leaf and canopy gas exchange in an Amazonian forest over a day. We focused on Amazonian forest because tropical forests play key roles in the global hydrological, energy, and carbon cycles. Understanding of the controls on tropical forest gas exchange lags that for temperate ecosystems, even though tropical forest accounts for ~45% of global terrestrial evapotranspiration [Hetherington and Woodward, 2003] and at least 30% of global terrestrial primary production [Field *et al.*, 1998].

2. Methods

[4] Measurements were made between June 2000 and August 2004 at the LBA (Large-Scale Biosphere-Atmosphere Experiment in Amazonia) km 83 and 67 sites in the Tapajós National Forest, Pará, Brazil [Goulden *et al.*, 2004; Saleska *et al.*, 2003]. The vegetation was semideciduous closed tropical forest with canopy emergents on flat upland terrain.

[5] The gas exchange by 56 leaves (Table 1) exposed to constant conditions for 20 to 48 hours in light and 6 leaves in darkness was measured with a LiCor 6400 gas exchange system. Plants were identified following Ribero *et al.* [1999]. We were unable to identify some plants to species after consulting experts on the local flora, and some of these species may be unknown to science. Most of the illuminated measurements were made at a Photon Flux Density (PPFD) of 100 μmol m⁻² s⁻¹, a leaf temperature of 30°C, a chamber CO₂ concentration of 370 μmol mol⁻¹, and either a constant chamber vapor pressure or a constant flow through the chamber. Some runs were made at a PPFD of 1000 μmol m⁻² s⁻¹ or without temperature control or without CO₂ control. Repeated measurements on *Micropholis sp.* indicated the occurrence of an endogenous gas exchange rhythm was independent of the configuration of the gas exchange system and the environmental conditions within the chamber. The neighboring leaves on the branch being tested were kept in darkness, and a larger section of the branch was shaded under a tarp, throughout each run. Leaves were accessed on the ground or from scaffolding.

[6] We used the eddy covariance technique to measure the rates of whole ecosystem CO₂ exchange from June 2000 to August 2004 [Goulden *et al.*, 2004]. Eddy covariance is

¹Department of Earth System Science, University of California, Irvine, California, USA.

²Department of Atmospheric Sciences, University of São Paulo, São Paulo, Brazil.

Table 1. Occurrence of Endogenous Gas Exchange Rhythms

	Family	Leaves Sampled	Functional Group
Plants with no apparent endogenous rhythm ^a			
<i>Chimarrhis turbinata</i>	Rubiaceae	6	Subcanopy
<i>Protium punctulatum</i> Macbr.	Burseraceae	3	Canopy
Plants with an endogenous rhythm in stomatal conductance but not photosynthetic capacity ^b			
<i>Distachya huber</i>	Cecropiaceae	4	Pioneer
<i>Serjania</i> sp.	Sapindaceae	1	Liana
<i>Lecythis</i> sp.	Lecythidaceae	4	Subcanopy
<i>Prionostemma aspera</i>	Hippocrateaceae	1	Liana
Plants with an endogenous photosynthetic capacity rhythm ^c			
<i>Derris amazonica</i>	Leguminosae-Papilionoideae	2	Liana
<i>Duguetia flagellaris</i>	Ammonaceae	2	Understory
<i>Eschweilera amazonica</i>	Lecythidaceae	3	Canopy
<i>Micropholis</i> sp.	Sapotaceae	16	Canopy
<i>Lecythis lurida</i>	Lecythidaceae	3	Canopy
<i>Copaifera duckei</i>	Leguminosae-Caesalpinioideae	3	Canopy
<i>Manilkara huberi</i>	Sapotaceae	6	Canopy
Plants with a sharp photosynthetic decline and no subsequent recovery ^d			
<i>Minuartia quianensis</i> Aubl.	Olacaceae	2	Canopy
<i>Sclerolobium paraense</i>	Leguminosae-Caesalpinioideae	4	Subcanopy
<i>Faramea platyneura</i>	Rubiaceae	1	Subcanopy
<i>Arrabidaea</i> sp.	Bignoniaceae	1	Liana

^aPhotosynthesis, stomatal conductance, and C_i remained constant or declined gradually over time.

^bPhotosynthesis, stomatal conductance, and C_i declined during the normally dark period and recovered during the next normally light period.

^cPhotosynthesis and stomatal conductance declined and C_i increased during the normally dark period and recovered during the next normally light period.

^dPhotosynthesis and stomatal conductance declined with no subsequent recovery during the next normally light period.

a micrometeorological technique that measures the net turbulent exchange of CO_2 by a patch of forest upwind of a meteorological tower. The area sampled varies with the meteorological conditions, decreasing to ~ 10 ha during daytime and increasing to as much as 1000 ha on atmospherically-stable nights. The fluxes were averaged for 30-minute periods and corrected for changes in CO_2 storage between the measurement height and the ground.

3. Results and Discussion

[7] We exposed 62 leaves on 17 plants representing 17 species to constant irradiance and temperature for 20 to 48 hours while continuously measuring the rate of gas exchange. Seven of the species exhibited an endogenous rhythm in photosynthetic capacity, and four additional species exhibited an endogenous rhythm in stomatal conductance but not photosynthetic capacity (Table 1). For example, the net CO_2 Assimilation (A_n) by *Micropholis* sp., a canopy tree, decreased after 1300 Local Time (LT), remained near zero during the evening, and recovered beginning before dawn (Figure 1). The A_n decline during the normally-dark period was not a result of stomatal closure and a lower intercellular CO_2 concentration (C_i). *Micropholis*'s photosynthesis increased during daytime, when C_i was low, and declined at night, when C_i was high. The concentration of CO_2 within the chamber was held constant during these measurements, and the change in C_i resulted from, rather than caused, the photosynthetic trend.

[8] A limitation to our approach is that a single leaf was held in constant conditions while most of the rest of the plant was exposed to ambient conditions. The study leaf's water potential presumably declined during daytime and recovered at night in concert with the water potential of nearby leaves (Figure 2a), raising the possibility that hy-

draulic communication imposed a spurious 24-hour gas exchange cycle on the investigated leaf. However, hydraulic coupling cannot explain the observed gas exchange trend since the associated changes in leaf water potential would be expected to decrease leaf photosynthesis during daytime, whereas the opposite trend was observed. Moreover, photosynthetic recovery typically started before dawn (Figures 1 and 2), indicating that it was not initiated by exposure of the remainder of the plant to light.

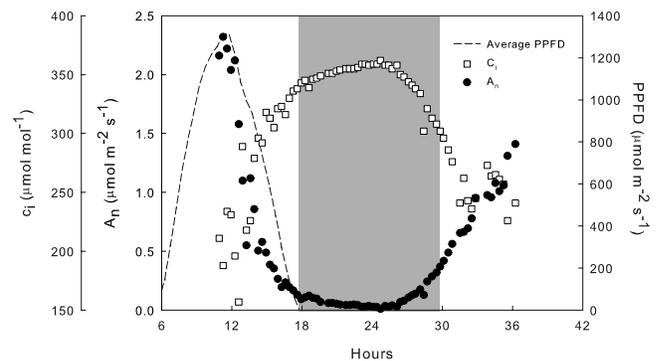


Figure 1. Leaf net CO_2 exchange (A_n , filled circles) and calculated intercellular CO_2 concentration (C_i , open squares) by a *Micropholis* sp. leaf exposed to continuous light for 24 hours (PPFD $100 \mu\text{mol m}^{-2} \text{s}^{-1}$, leaf T 26°C , relative humidity $\sim 70\%$, chamber CO_2 $370 \mu\text{mol mol}^{-1}$). The gas exchange measurements were made on a canopy leaf growing ~ 30 -m above ground level in an Amazonian tropical forest. The dashed line shows the average incident PPFD at the site; the shaded area shows the normally-dark period.

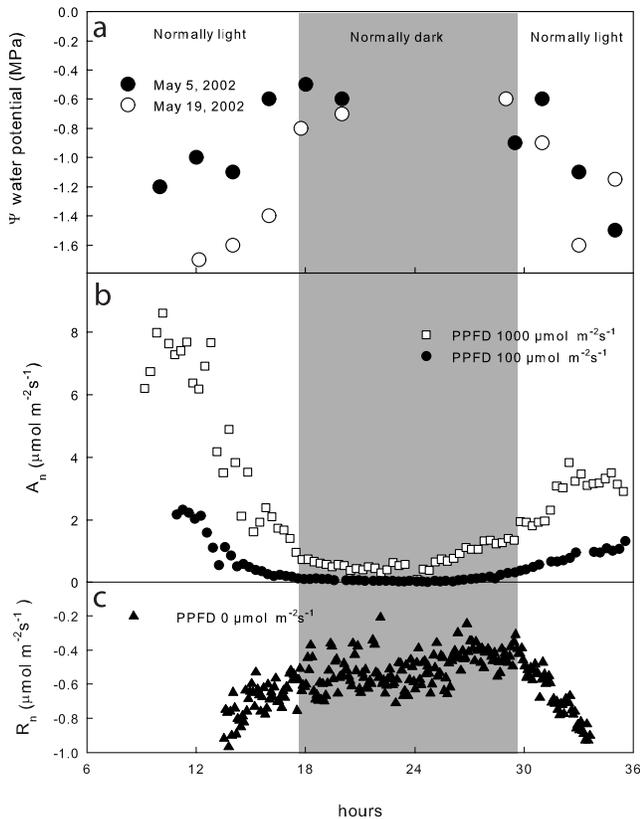


Figure 2. (a) Leaf water potential for *Micropholis sp.* leaves exposed to ambient conditions. (b) Leaf CO₂ exchange (A_n) by *Micropholis sp.* leaves in continuous light for 24 hours (filled circles PPFD 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf T 26 °C, relative humidity ~70%, chamber CO₂ 370 $\mu\text{mol mol}^{-1}$; open squares PPFD 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf T 26 °C, relative humidity ~75%, chamber CO₂ ~370 $\mu\text{mol mol}^{-1}$). (c) Leaf respiration by a *Micropholis sp.* leaf in constant darkness for 24 hours (filled triangles PPFD 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf T 30 °C, relative humidity ~65%, chamber CO₂ 370 $\mu\text{mol mol}^{-1}$). Shaded area shows the normally-dark period.

[9] *Micropholis*'s photosynthesis was always strongly controlled by light, though the amplitude of the light response changed with time of day (Figure 2). The photosynthetic uptake at PPFDs of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ declined after 1300LT and recovered beginning before 0600LT (Figure 2b). Dark respiration also declined (became less negative) beginning shortly after 1300LT and recovered beginning around 0600LT (Figure 2c). There was no obvious difference in period or phase between light intensities. The similar trend at all light levels suggests a general up-regulation of metabolism during the normally light period and down-regulation during the normally dark period.

[10] Co-occurring species at the Tapajós National Forest differ in the degree to which endogenous factors control leaf gas exchange (Table 1). Two species exhibited no obvious endogenous gas exchange trend. Four other species exhibited a marked decline in gas exchange during the first afternoon without subsequent recovery. Four other species exhibited an endogenous trend in stomatal conductance,

which, in turn, caused photosynthesis to decline during the normally dark period even though the biochemical capacity to fix carbon apparently remained constant. The final seven species, including *Micropholis sp.*, exhibited endogenous rhythms in the biochemical capacity to fix carbon. The patterns of gas exchange were generally consistent between different leaves on a plant and between different times of the year for a plant. The occurrence of an endogenous rhythm was not confined to a single plant functional group, but was observed in an understory plant, three lianas, a pioneer, and six canopy or subcanopy trees. The rhythms we observed for *Micropholis* and other plants appear similar to, and in some cases more extreme than, the gas exchange rhythms that have been attributed to circadian oscillators in other species [Hennessey and Field, 1991; Williams and Gorton, 1998; Dodd et al., 2005].

[11] We analyzed 16,138 half-hour measurements of whole-forest CO₂ exchange (Net Ecosystem Exchange; NEE) made at the LBA km-83 site from 2000 to 2004 to determine whether ecosystem gas exchange also exhibits an endogenous rhythm. NEE at a PPFD of 500 to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ increased from an uptake rate of 3.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 645LT to a maximum of 16.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 945LT before declining to 0.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 1615LT (Figure 3b). The changing relationship between irradiance and NEE (Figure 3a) paralleled that observed at the leaf-level (Figure 2b), and the diurnal NEE changes might have resulted from an endogenous gas exchange rhythm similar to that observed for individual leaves. However, several possible alternative explanations for the NEE observations merit consideration as well.

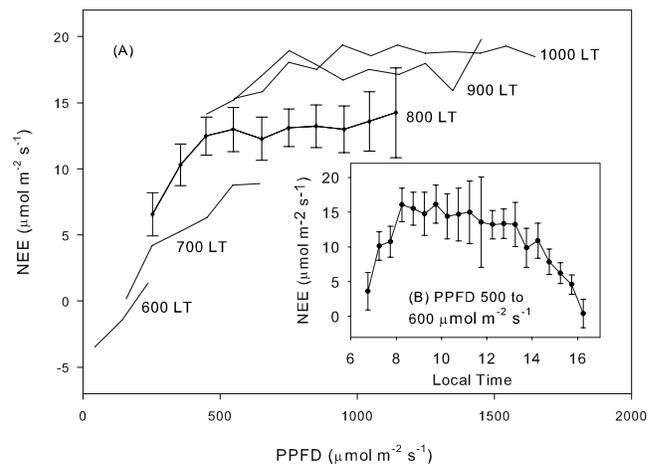


Figure 3. (a) Net Ecosystem CO₂ exchange (NEE; uptake shown as a positive flux) measured by eddy covariance as a function of incident PPFD. Light curves are averages ($n > 60$) within 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ wide PPFD bins for one-hour intervals centered on the Local Time (LT) shown. Ninety five percent confidence intervals are shown for the 730–830 curve; confidence intervals for the other times are similar. NEE was screened to exclude periods with a friction velocity (U^*) $< 0.2 \text{ m s}^{-1}$. (b) Average NEE ($n > 20$) and 95% confidence intervals for half-hour intervals with a PPFD of 500 to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as a function of Local Time.

[12] Increased soil respiration due to afternoon warming might have caused part of the afternoon NEE decline. However, automated chamber observations at the site showed that soil respiration increased by less than $1 \mu\text{mol m}^{-2}\text{s}^{-1}$ from morning to afternoon [Goulden *et al.*, 2004]. Alternatively, decreased afternoon photosynthesis due to increased stress might have caused part of the afternoon NEE decline. However, it is unlikely that increased stress alone caused the dramatic NEE reduction from 1300 to 1700LT since air temperature and vapor saturation deficit were nearly constant, evapotranspiration was decreasing, and leaf water potential was recovering during this period (Figure 2a) [da Rocha *et al.*, 2004]. Moreover, the diurnal change in NEE for the subset of observations with a PPFD of 400 to $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ and measurable rainfall within the preceding 2 hours was similar to that for observations with a PPFD of 400 to $700 \mu\text{mol m}^{-2}\text{s}^{-1}$ and no rain within 24 hours (plots not shown). Recent rain and the resulting wet canopy would be expected to decrease stress, whereas NEE declined by $\sim 11 \mu\text{mol m}^{-2}\text{s}^{-1}$ from 1230 to 1630LT on both wet and dry afternoons. Finally, a midday increase in the ratio of diffuse to direct beam radiation might have increased photosynthesis. However, this effect was probably small since the midday light environment was dominated by patchy sunlight, which has only a minor effect on NEE [Rocha *et al.*, 2004]. Moreover, most studies indicate that the ratio of diffuse to direct light peaks early and late in the day [Campbell and Norman, 1998], and diurnal changes in light quality should have maximized rather than suppressed NEE at a given irradiance during these periods [Gu *et al.*, 2002].

[13] We believe the diurnal NEE shifts are far larger than can be accounted for by the combined effects of soil respiration, stress, and light quality. We acknowledge that whole ecosystem exchange observations of the type analyzed may be confounded by multicollinearity and are unlikely to provide the definitive proof of an endogenous circadian rhythm that is possible under tightly controlled laboratory conditions. Similarly, we note that the logistical difficulty of fieldwork in a tropical forest canopy prevented us from fully documenting that the rhythms we observed at the leaf level have all the hallmarks of circadian oscillators [McClung, 2001; Johnson *et al.*, 1998; Webb, 2003]. Nonetheless, we emphasize that the diurnal changes in CO_2 exchange we observed for the whole forest (Figure 3) appear very similar to those that we observed for individual leaves (Figures 1 and 2), which, in turn, appear very similar to the circadian rhythms that have been intensively investigated in the laboratory [Hennessey and Field, 1991; Williams and Gorton, 1998; Dodd *et al.*, 2005]. The predawn increase in leaf-level CO_2 uptake (Figures 1 and 2), and the morning increase in whole-forest CO_2 uptake (Figure 3), are particularly difficult to explain if gas exchange is driven solely by the direct effect of the physical environment on physiology. In fact, a biological-clock-driven circadian rhythm is the only established mechanism we know of that can account for all of our observations.

[14] Our observations extend previous laboratory studies to show that endogenous gas exchange rhythms are common in canopy trees and understory plants growing naturally in a tropical forest. The adaptive significance of circadian gas exchange rhythms is poorly understood. One

possibility is that the nocturnal decline in photosynthesis reflects a general down regulation of metabolism that conserves energy. *Micropholis*'s rates of photosynthesis and respiration change in concert (Figures 2b and 2c), raising the possibility that leaves adjust their metabolism in anticipation of the average light conditions. Hence, a leaf's light response might change from one that is typical for a sun leaf during the middle of the day to one that is typical for a shade leaf during the early morning, late afternoon, and night.

[15] The local climate at the site may have promoted the evolutionary development of circadian rhythms. The site is 3° south of the equator, resulting in a nearly constant 12-hour photoperiod. The daily maximum air temperature was always within a few degrees of 29°C from 2000 to 2004, and most plants were sufficiently deeply rooted to avoid water stress, resulting in nearly constant rates of midday canopy gas exchange year-round [Goulden *et al.*, 2004]. This predictable environment, especially with respect to the aspects of the climate that directly control photosynthesis, may have selected for the anticipatory, biological clock-driven regulation of photosynthetic capacity. The size of tropical trees may also have led to adaptations that increase coordination among leaves and allow large plants to act in a physiologically integrated manner.

[16] The endogenous photosynthesis patterns coincide with the natural photoperiod, and the diel pattern of gas exchange predicted assuming constant physiology under current conditions may not differ markedly from that predicted after accounting for the endogenous cycle [Williams and Gorton, 1998]. Some researchers may emphasize this issue and conclude that endogenous rhythms are unimportant; a perspective that we believe misses the point. Both modeling and observational research on ecosystem gas exchange have focused almost entirely on the direct effects of the physical environment on plant physiology. We have shown that the exclusive focus on the direct physical controls on physiology is unwarranted, and that tropical plants possess endogenous rhythms that are poorly understood with respect to both mechanism and adaptive significance. We conclude that the gas exchange at our study site is strongly co-limited over the day by the plants' physiological capacity as regulated by circadian rhythms and by the direct effect of the physical environment on physiology. Both factors are important in controlling the diel patterns of gas exchange, and the goal for plant physiologists, micrometeorologists and modelers is to build a broad understanding of tropical forest physiology that accounts for both the endogenous and exogenous controls on land-atmosphere exchange.

[17] **Acknowledgments.** We thank Helber Freitas, Michela Figueira, Augusto Maia, and Albert de Sousa for field help; Clara Tinoco, Jim Randerson, Michael Keller, Jeff Chambers, Adrian Rocha, Diane Pataki, and the late Tim Hennessey for comments, suggestions, and ideas; Tomas Domingues and Jim Ehlerhinger for advice and equipment loan; UFPA in Santarém and Belém for help identifying species; and the LBA-ECO Santarém field office for support. This work was funded by NASA as a component of LBA-ECO.

References

- Bonan, G. B. (2002), *Ecological Climatology: Concepts and Applications*, Cambridge Univ. Press, New York.
- Campbell, G. S., and J. M. Norman (1998), *An Introduction to Environmental Biophysics*, Springer, New York.

- da Rocha, H. R., et al. (2004), Seasonality of water and heat fluxes over a tropical forest in eastern Amazonia, *Ecol. Appl.*, *14*, S22–S32.
- Dodd, A. N., et al. (2005), Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage, *Science*, *309*, 630–633.
- Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski (1998), Primary production of the biosphere: Integrating terrestrial and oceanic components, *Science*, *281*, 237–240.
- Goulden, M. L., et al. (2004), Diel and seasonal patterns of tropical forest CO₂ exchange, *Ecol. Appl.*, *14*, S24–S54.
- Gu, L., D. Baldocchi, S. B. Verma, T. A. Black, T. Vesala, E. M. Falge, and P. R. Dwyer (2002), Advantages of diffuse radiation for terrestrial ecosystem productivity, *J. Geophys. Res.*, *107*(D6), 4050, doi:10.1029/2001JD001242.
- Harmer, S. L., et al. (2002), Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock, *Science*, *290*, 2110–2113.
- Hennessey, T. L., and C. B. Field (1991), Circadian-rhythms in photosynthesis-oscillations in carbon assimilation and stomatal conductance under constant conditions, *Plant Physiol.*, *96*, 831–836.
- Hetherington, M., and F. I. Woodward (2003), The role of stomata in sensing and driving environmental change, *Nature*, *424*, 901–908.
- Johnson, C. H., M. Knight, A. Trewavas, and T. Kondo (1998), *Biological Rhythms and Photoperiodism in Plants*, edited by P. Lumsden and A. Millar, pp. 1–34, Oxford Univ. Press, New York.
- Jones, H. G. (1992), *Plants and Microclimate*, Cambridge Univ. Press, New York.
- Lambers, H., F. S. Chapin III, and T. L. Pons (1998), *Plant Physiological Ecology*, Springer, New York.
- Mansfield, T. A., and O. V. S. Heath (1963), Studies in stomatal behaviour. 9. Photoperiodic effects on rhythmic phenomena in *xanthium pennsylvanicum*, *J. Exp. Bot.*, *14*, 334–352.
- McClung, C. R. (2001), Circadian rhythms in plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, *52*, 139–162.
- Ribero, J. E. et al. (1999), *Flora da Reserva Ducke* (in Portuguese), 799 pp., Dep. for Int. Dev., Manaus, Brazil.
- Rocha, A. V., et al. (2004), Photosynthetic and water use efficiency responses to diffuse radiation by an aspen-dominated northern hardwood forest, *For. Sci.*, *50*, 793–801.
- Saleska, S. R., et al. (2003), Carbon in Amazon forests: Unexpected seasonal fluxes and disturbance-induced losses, *Science*, *302*, 1554–1557.
- Sellers, P. J., et al. (1997), Modeling the exchanges of energy, water, and carbon between continents and the atmosphere, *Science*, *275*, 502–509.
- Stalfelt, M. G. (1963), Diurnal dark reactions in stomatal movements, *Physiol. Plantarum*, *16*, 756–766.
- Webb, A. A. R. (2003), The physiology of circadian rhythms in plants, *New Phytol.*, *160*, 281–303.
- Williams, W. E., and H. L. Gorton (1998), Circadian rhythms have insignificant effects on plant gas exchange under field conditions, *Physiol. Plantarum*, *103*, 247–256.

H. R. da Rocha, Department of Atmospheric Sciences, University of São Paulo, São Paulo, SP Brazil.

C. E. Doughty, M. L. Goulden, and S. D. Miller, Department of Earth System Science, University of California, Croul Hall, Irvine, CA 92697–3100, USA. (mgoulden@uci.edu)