Chlorophyll fluorescence varies more across seasons than leaf water potential in drought-prone plants

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ABSTRACT

Among the effects of environmental change, the intensification of drought events is noteworthy, and tropical vegetation is predicted to be highly vulnerable to it. However, it is not clear how tropical plants in drought-prone habitats will respond to this change. In a coastal sandy plain environment, we evaluated the response of six plant species to water deficits across seasons, the relationship between their morpho-physiological traits, and which traits would be the best descriptors of plants' response to drought. Regardless of leaf succulence and phenology, responses between seasons were most strongly related to chlorophyll fluorescence. In this study we have demonstrated that a better comprehension of how tropical species from drought-prone habitats cope with changes in water availability can be based on seasonal variation in leaf water potential and chlorophyll fluorescence. Temporal variation in leaf water potential and chlorophyll fluorescence was found useful for differentiating between groups of sandy soil species that are responsive or unresponsive to water availability. However, chlorophyll fluorescence appeared to be a more sensitive descriptor of their seasonal and short-term responses.

Key words: drought, functional traits, plant-water relations, photoinhibition, seasonal habitats.

INTRODUCTION

In the current scenario of large-scale environmental change, the search for functional traits which will allow us to predict the response of communities and species to changes in conditions and resources has been identified as one of the 100 fundamental questions in ecology (Sutherland et al. 2013). Among the effects of environmental change, the intensification of drought events is noteworthy (McDowell et al. 2008, 2011), and several studies have identified plant physiological traits that may be descriptors for the responses of plants to water availability (Bartlett et al. 2012, Choat et al. 2012, Bhaskar and Ackerly 2006). In tropical environments seasonality is largely reflected in changes in water availability (Franco 2002), and temporal fluctuations in water availability are particularly important for plants growing in sandy environments, due to the low water retention capacity of sandy soils (Noy-Meir 1972). Although several studies of plant responses to drought...
in tropical dry forests and savannas have been published (Bucci et al. 2005, Franco 2002, Choat et al. 2006, Méndez-Alonzo et al. 2012), it is still unknown how tropical plants in other tropical drought-prone habitats (e.g. coastal sandy plains) will respond to drought, and which are the best traits for predicting this response.

Plants growing on sandy soils show higher water potential than those growing on clay soils, since sandy soils hold water at high water potential (Sperry et al. 2002). One important process that facilitates highly conservative water use is homeostasis of leaf water potential (Ψ_L) at midday across seasons (Bucci et al. 2005, Maseda and Fernández 2006). The ability to maintain constant Ψ_L might be achieved by mechanisms such as greater stomatal control and hydraulic adjustments, which reduce transpiration (Maseda and Fernández 2006). Species with less stomatal control may exhibit greater decreases in Ψ_L (Franks et al. 2007, Maseda and Fernández 2006). An important aspect of species that are able to maintain high Ψ_L is related to the avoidance of embolism (Sperry et al. 2002, Maseda and Fernández 2006). However, species with lower Ψ_L can maintain CO₂ gas exchange for longer periods during drought, under low soil water availability and/or high air vapor pressure deficit (VPD). On the other hand, such species might be more susceptible to water loss (Maseda and Fernández 2006). Importantly, the greater the degree of stomatal closure (associated with high Ψ_L), the dissipation of excitation energy by photosynthetic carbon assimilation will be more reduced (Maseda and Fernández 2006). In addition, greater stomatal closure may result in an increase in carbon starvation due to a decrease in photosynthesis (McDowell et al. 2008, van der Molen et al. 2011).

A further drawback of stomatal closure is that it may lead to light stress, i.e., photoinhibition caused by excess light that is not used in photosynthesis. Changes in the ratio of the yield of variable (Fᵥ) and maximum (Fₘ) chlorophyll fluorescence in dark-acclimated leaves can be used to characterize the degree of photoinhibition in plants subjected to environmental stress (Baker 2008). A decrease in the Fᵥ/Fₘ ratio indicates increased photoprotection, in which excess excitation energy is dissipated as heat, and to direct photodamage to photosystem II (PS II) centers (Krause 1988, Walker 1992). In addition, an increase in thermal dissipation in PS II is associated with a decrease in Fₘ and minimum chlorophyll fluorescence (F₀), whereas damage to the reaction centers increases only F₀ (Krause 1988, Porcar-Castell et al. 2008).

Rosado and de Mattos (2010) observed variations between wet and dry seasons in the pressure-volume relationship in species on the Brazilian coastal sandy plains, a species assemblage known as restingsas. As expected for plants growing on dry sandy soils, they observed high values of Ψ_L at the turgor loss point (Ψ_TLP), less negative osmotic pressure, and a low bulk modulus of elasticity in restinga species (Rosado and de Mattos 2010). However, most species have an average predawn Fᵥ/Fₘ of less than 0.83 during the dry season, which indicates chronic photoinhibition. To date, however, no information is available about the physiological responses of restinga plants to seasonal variation in water availability, or the possible relationship between such physiological responses and leaf phenology.

In this study we chose the two dominant species, which are able to maintain a high minimum Ψ_L during the dry season (Rosado and de Mattos 2010), and four less dominant restinga species. These six species occur in a restinga in southeastern Brazil and exhibit a range of leaf succulence, from 147 to 1105 g m⁻², and leaf phenology characteristics, from successive to concentrated leaf production in wet seasons (Rosado and de Mattos 2007, 2010). Leaf succulence reflects the amount of water stored in leaves and may act as a buffer for water loss (Lamont and Lamont 2000),
and variations in leaf phenology are commonly associated with the ability to deal with water deficits (Reich and Borchert 1982, Borchert 1994). We thus addressed the following hypotheses: 1) the six species have different strategies to maintain high $\Psi_L$ across seasons based on distinct arrays of leaf phenology and leaf morpho-physiological traits; 2) seasonal responses of chlorophyll fluorescence are dependent on seasonal variation in $\Psi_L$ and both $\Psi_L$ and chlorophyll fluorescence are redundant in the description of species’ seasonal responses to water availability. Although soil moisture availability is a function of input minus output of water and rooting depth, the number of rainless days (NRD) might be a useful surrogate for soil moisture availability, especially in sandy soils whose water retention capacity is low (Sperry et al. 2002). Given the low water retention capacity of sandy soils and the occurrence of dry spells in both wet and dry seasons (Cavalin and de Mattos 2007), coastal sandy plants may be differentially predisposed to water shortage during rainless days with varying VPD during wet and dry seasons. Our third hypothesis is therefore as follows: $\Psi_L$ responds to higher VPD only when NRD is low, but responds to an increase in NRD, regardless of VPD.

MATERIALS AND METHODS

STUDY AREA AND PLANT SPECIES

Our study was conducted in the Restinga of Jurubatiba National Park, (14 140 ha), located in the northern part of the state of Rio de Janeiro, Brazil (extending north-south between 22°00’S and 22°23’S and east-west between 41°15’W and 41°35’W). The maximum temperature (around 30 °C) occurs in January and the minimum (around 20 °C) in July (Henriques et al. 1986). The mean annual temperature is 22.6 °C. The sandy soil is characterized by low water retention capacity, low nutrient content and low organic matter content. Average annual rainfall is 1100–1300 mm, with a monthly minimum of 41 mm in dry seasons and a monthly maximum of 189 mm in wet seasons (Araujo et al. 1998). The soil water deficit is highest during the months of July and August (Henriques et al. 1986). Open Clusia scrub is the most common vegetation type, characterized by vegetation patches composed of shrubs and trees 0.5–8.0 m in height, surrounded by white bare sand that can reach 70 °C on typical summer days. Clusia scrub covers 40% of the park and is dominated by two species: Clusia hilariana Schltdl. (Clusiaceae) and Protium icicariba (DC.) Marchand (Burseraceae) (Araujo et al. 2004). The annual rainfall in 2004 and 2005 was 1319 mm and 1388 mm, respectively. In the months studied, cumulative rainfall was 246 mm in 2004 and 770 mm in 2005, with September 2004 and August 2005 receiving the lowest rainfall (Fig. 1). The rainless period in September 2004 was 30 days long, whereas in August 2005 it lasted 15 days.

![Figure 1 - Daily rainfall (mm) during the period of study. The dashed lines indicated by black circles represent the months in which $\Psi_L$ and chlorophyll a fluorescence were measured.](image)

We selected six shrub species based on variation in leaf succulence and phenology (Rosado and de Mattos 2007) in an area 300 m from the ocean: Clusia hilariana (average leaf succulence: 1105 g m$^{-2}$) and Byrsonima sericea DC. (Malpighiaceae).
(239 g m$^{-2}$) are classified as successive leaf producers because they produce leaves throughout the year, while Protium icicariba (147 g m$^{-2}$), Ocotea notata (Nees) Mez (Lauraceae) (169 g m$^{-2}$), Eugenia umbelliflora Berg (Myrtaceae) (358 g m$^{-2}$), and Erythroxylum ovalifolium Peyr. (Erythroxylaceae) (194 g m$^{-2}$) concentrate their leaf production during the wet season. Erythroxylum is the only species that shows brevi-deciduous behavior. For simplicity, we will refer to each species by its genus name. Clusia is an obligatory CAM plant (Franco et al. 1996), whereas all other species are C$_3$ plants.

**LEAF WATER POTENTIAL AND CHLOROPHYLL FLUORESCENCE**

Leaf water potential was measured using a pressure chamber (Model 1000, PMS Instruments, USA) at predawn ($\Psi_{pd}$) and midday ($\Psi_{md}$). The measurements were made using two completely exposed mature leaves from the north-facing side of the canopy of the same three individuals of each species throughout the study. For Erythroxylum measurements, samples were taken from terminal shoots because the leaves and petioles were too small to be used in the pressure chamber. When the difference in $\Psi_L$ between two leaves of each individual was greater than 0.5 MPa, we measured a third leaf to estimate the average. From April 2004 to August 2005, measurements of $\Psi_L$ were made during eight months chosen to represent the variation between the wet and the dry seasons. Twelve measurements were made at predawn and 18 at midday for each individual. To represent these data graphically we chose the days with the minimum values for each individual per month. The minimum values are good descriptors of species’ responses to water deficits (Sperry et al. 2002) because they indicate the maximum xylem tension that a given species is able to tolerate while being able to maintain physiological activity (Bhaskar and Ackerly 2006). At the same time, measurements of chlorophyll a fluorescence were performed using a pulse-amplitude modulated fluorometer with a fiber optic (FMS-2, Hansatech, UK), on the same two leaves from each individual. The fiber optic was kept at a constant distance and angle to the leaf surface using a leaf clip. The potential quantum yield of PSII, which is dependent on minimal ($F_0$) and maximal fluorescence yield ($F_m$), was measured by applying a pulse of light strong enough to saturate all the PSII reaction centers after the leaf was dark-adapted for 30 min (de Mattos and Luttge 2001). The $F/F_m$ ratio was determined just before dawn ($F/F_{mpd}$) and at midday ($F/F_{mmd}$) by dark-adapting leaves for 30 min. Despite the usual high variability between leaves and individuals, we found it useful to use the absolute values of $F_0$ and $F_m$ to indicate temporal variation in photoprotection or photodamage associated with stress (Porcar-Castell et al. 2008). The averages were calculated in the same way as for $\Psi_L$. On each sampling occasion we measured relative humidity and air temperature at 30 min intervals, starting at predawn and ending at midday.

**STATISTICAL ANALYSES**

To test hypothesis 1, we evaluated the differences in $\Psi_{pd}$, $\Psi_{md}$ and $F/F_{mpd}$, $F/F_{mmd}$ and $F_0$ and $F_m$ between seasons and among species using a two-way repeated-measures ANOVA ($P < 0.05$). Additionally, to evaluate changes in the response of species (ecophysiological performance) between seasons, we constructed a correlation matrix using the mean values of each variable for each species in the wet and dry season, respectively. The wettest period comprised the months between November 2004 and February 2005. We analyzed the correlation matrix with a Principal Component Analysis (PCA) using program MULTIV (Pillar 1997). We also used PCA to describe the association between $\Psi_L$ and the chlorophyll variables (hypothesis 2).
To test hypothesis 3, we ran a multiple linear regression to determine the contribution of VPD and NRD to variation in $\Psi_{md}$ and $\Psi_{pd}$. We chose the highest VPD on each day when $\Psi_L$ and $F_v/F_m$ were measured at both predawn and midday, and NRD during the one, two, three or four weeks before the measurements were taken. We performed a separate multiple regression for each week because we used cumulative NRD. Two-way ANOVAs and multiple linear regressions were carried out in R 2.11.1 (http://www.R-project.org).

RESULTS

LEAF WATER POTENTIAL

The physiological measurements from September 2004 were taken after 30 days of drought, and those from August 2005, after six days of drought (Fig. 1). The highest VPD values were observed during the dry seasons, i.e. September 2004 (46.1 Pa KPa $^{-1}$) and August 2005 (36.5 Pa KPa $^{-1}$) (Table I). The lowest VPD was observed on a cloudy day in July 2005 (12.3 Pa KPa $^{-1}$).

TABLE I

| Month and year | VPD (Pa KPa$^{-1}$) |
|---------------|---------------------|
| Apr-04        | 28.9                |
| June-04       | 16.1                |
| Sept-04       | 46.1                |
| Nov-04        | 27.8                |
| Jan-05        | 33.9                |
| Feb-05        | 21.2                |
| July-05       | 12.3                |
| Aug-05        | 36.5                |

The $\Psi_{pd}$ and $\Psi_{md}$ values ranged from almost zero to around -1.70 MPa and from almost zero to -4.23 MPa, respectively. All species showed the greatest decline in $\Psi_{pd}$ in September 2004 and August 2005 (Figs. 2a, 2b). In contrast to other species, Protium and Clusia showed constant values of $\Psi_{md}$ throughout the year, which were greater than those of other species (Tables II and III). Clusia showed a slightly lower $\Psi_{md}$ than Protium, but the difference was not significant. Clusia and Erythroxylum showed significantly lower values of $\Psi_{pd}$ in the dry season in comparison to the wet season, but Erythroxylum was the only species with a significantly lower $\Psi_{md}$ in the dry season (Tables II and III). In July 2005, when measurements were made on a cloudy day, all species showed increases in $\Psi_{md}$.

CHLOROPHYLL A FLUORESCENCE

In all species, predawn and midday $F_v/F_m$ varied in the ranges of 0.60-0.90 and 0.55-0.85, respectively. Byrsonima, Eugenia and Clusia showed a decline in $F_v/F_{nmd}$ in September 2004. Eugenia had the lowest $F_v/F_{nmd}$ value of all species (Table II, Fig. 2c). With the exception of Protium, all species showed a decline in $F_v/F_{nmd}$ in the dry season (Tables II and III). A decline in $F_v/F_{nmd}$ in all species was also observed in September 2004. Clusia and Protium showed the highest $F_v/F_{nmd}$ in the wet season (0.80 and 0.79, respectively), while Eugenia showed the lowest (0.66) (Table II, Fig. 2d). The highest $F_v/F_{nmd}$ values were observed for all species during July 2005. There were no significant differences between seasons for any species (Tables II and III).

The $F_{0pd}$ values varied from 180 to 450, with the lowest values observed in August 2005 (Fig. 3). Byrsonima, Clusia and Eugenia showed significant differences between seasons, with the highest values observed in September 2004 (Tables II and III). Eugenia showed the greatest variation in $F_{0pd}$ throughout the year (Fig. 3a). $F_{nmd}$ varied from 800 to 2000 (Fig. 3b). With $F_{nmd}$, Eugenia showed the greatest variation between seasons. Most species displayed a simultaneous marked decline in $F_{nmd}$ in September 2004 and August 2005. Only Eugenia showed significant differences between seasons, with the lowest values of $F_{nmd}$ measured in the dry season (Table II).
Figure 2 - Seasonal variation at predawn, midday in leaf water potential (panels a and b) and chlorophyll a fluorescence (panels c and d, respectively). Species are represented as follows: closed squares – *Byrsonima* (■); closed triangles – *Clusia* (▲); closed circles – *Erythroxylum* (●); open squares – *Eugenia* (□); open diamonds – *Ocotea* (◊) and open circles – *Protium* (○).

**Figure 2** - Seasonal variation at predawn, midday in leaf water potential (panels a and b) and chlorophyll a fluorescence (panels c and d, respectively). Species are represented as follows: closed squares – *Byrsonima* (■); closed triangles – *Clusia* (▲); closed circles – *Erythroxylum* (●); open squares – *Eugenia* (□); open diamonds – *Ocotea* (◊) and open circles – *Protium* (○).

### TABLE II

| Species   | Season | $\Psi_{pd}$ (MPa) | $\Psi_{md}$ (MPa) | $F_{v}/F_{m}$ | $F_{v}/F_{m}$ | $F_{0}$ | $F_{m}$ |
|-----------|--------|-------------------|-------------------|----------------|----------------|--------|--------|
| **Byrsonima** | Dry    | -1.78bc           | -0.28c            | 0.74ab         | 0.77b           | 342.50c | 1501.50bcd |
|           | Wet    | -1.37cd           | -0.15c            | 0.77 ab        | 0.83d           | 263.67a | 1577.00de |
| **Clusia**   | Dry    | -0.85de           | -0.92b            | 0.71ab         | 0.77b           | 335.00bc | 1482.50bcd |
|           | Wet    | -0.82de           | -0.43c            | 0.80b          | 0.84d           | 251.67a | 1617.50de |
| **Erythroxylum** | Dry  | -4.23a            | -1.70a            | 0.76 ab        | 0.79bc          | 286.50abc | 1399.25bcde |
|          | Wet    | -1.92bc           | -0.35e            | 0.78 ab        | 0.83d           | 282.50abc | 1642.50de |
| **Eugenia**  | Dry    | -1.90bc           | -0.46c            | 0.74 ab        | 0.60a           | 459.00d  | 1176.50a  |
|          | Wet    | -1.72bc           | -0.19c            | 0.66a          | 0.81cd          | 349.33c  | 1859.83e  |
| **Ocotea**   | Dry    | -2.00b            | -0.46c            | 0.72 ab        | 0.76b           | 291.50abc | 1230.00abc |
|           | Wet    | -1.68bc           | -0.22c            | 0.76 ab        | 0.82cd          | 309.83abc | 1675.17ede |
| **Protium**  | Dry    | -0.36e            | -0.20c            | 0.73 ab        | 0.79bc          | 263.50a  | 1257.50ab |
|           | Wet    | -0.32e            | -0.15c            | 0.79b          | 0.82cd          | 270.33ab | 1484.50bcd |

Midday leaf water potential ($\Psi_{md}$), predawn leaf water potential ($\Psi_{pd}$), potential quantum yield of photosystem II at midday ($F_{v}/F_{m}$), potential quantum yield of photosystem II at predawn ($F_{v}/F_{m}$), minimal fluorescence yield ($F_{0}$) and maximal fluorescence yield ($F_{m}$) during dry and wet seasons. In each column, different letters represent significant differences (two-way repeated-measures ANOVA, Tukey’s test, $P < 0.05$).
Multiple linear regressions indicated that only NRD showed a negative relationship with $\Psi_{pd}$ and $F_{v}/F_{m}$ in Clusia, Erythroxylum, and Eugenia. Both the first and second axes of the PCA explained 41.89% and 26.90% of the variation observed in the six traits, respectively (Table VI). The first axis was most strongly associated with $F_{v}/F_{m}$, $F_{m}$, and $F_{0}$, while the second axis was most strongly associated with $\Psi_{pd}$, $\Psi_{md}$, and VPD. The second axis was most strongly associated with $\Psi_{pd}$, $\Psi_{md}$, and VPD. Species symbols as for Figure 2.

### Table III

Results of two-way ANOVAs conducted to test the effects of species and seasons on the change in midday leaf water potential ($\Psi_{md}$), predawn leaf water potential ($\Psi_{pd}$), potential quantum yield of photosystem II at midday ($F_{v}/F_{m}$), potential quantum yield of photosystem II at predawn ($F_{v}/F_{m}$), minimal fluorescence yield ($F_{0}$), and maximal fluorescence yield ($F_{m}$). Significance levels are indicated as follows: *** – $P < 0.0001$; ** – $P < 0.001$; and * – $P < 0.01$. Season is abbreviated to “Se” and species to “Sp”.

| Effects | df | SS | MS | $F_{v}/F_{m}$ | MS | $F_{v}/F_{m}$ | MS | $F_{m}$ | MS | $F_{0}$ | MS | $F_{m}$ | MS | $F$ |
|---------|----|----|----|----------------|----|----------------|----|---------|----|---------|----|---------|----|-----|
| $Se$    | 1  | 2.72 | 2.71 | 23.04*** | 1.37 | 28.39*** | 0.006 | 0.006 | 270.8*** | 15708 | 15708 | 20.97*** | 81834 | 81834 | 47.1** |
| $Sp$    | 5  | 26.68 | 5.33 | 91.60*** | 3.24 | 22.86*** | 0.018 | 0.0036 | 30.42*** | 70665 | 70665 | 14133 | 13985 | 27972 | 1.10 |
| $Sp:Se$ | 5  | 5.77 | 1.15 | 9.80*** | 1.85 | 7.70*** | 0.023 | 0.004 | 34.06*** | 22668 | 22668 | 4533 | 38126 | 76254 | 4.38* |
| Residuals | 12 | 1.41 | 0.11 | 0.58 | 0.05 | 0.014 | 0.0012 | 0.002 | 0.0001 | 8988 | 749 | 20844 | 17371 |

Figure 3: Seasonal variation in predawn basal ($a$) and maximum fluorescence yield ($b$) ($F_{m}$ and $F_{m}$, respectively).
From the wet to the dry season, all species showed responses associated with the first axis of variation, being positively associated with $F_{\text{vpd}}$ and negatively with $F_{\text{vpd}} / F_{\text{mnd}}$, $F_{\text{vfm}} / F_{\text{mnd}}$ and $F_{\text{vpd}}$. For Erythroxylum, however, the response was more closely associated with the second axis, with the response from wet to dry season being negatively related to $\Psi_{\text{pd}}$ and $\Psi_{\text{md}}$ (Fig. 4).

### TABLE IV

Coefficient of multiple regression between predawn leaf water potential ($\Psi_{\text{pd}}$, MPa), $F_{\text{vpd}} / F_{\text{mnd}}$, daily maximum air vapor pressure deficit (VPD, Pa KPa$^{-1}$) and the number of rainless days (NRD). We selected the greatest VPD for each day on which measurements were taken and the number of rainless days during the one, two, three or four weeks before that. Asterisks and bold values indicate $P < 0.05$.

| Weeks | Species | Trait | Factor | 1    | 2    | 3    | 4    |
|-------|---------|-------|--------|------|------|------|------|
|       | Byrsonima | $\Psi_{\text{pd}}$ | VPD    | -0.06 | 0.04 | -0.01 | 0.00 |
|       |         |       | NRD    | -0.08 | -0.02 | -0.02 | -0.02 |
|       | Clusia  | $\Psi_{\text{pd}}$ | VPD    | 0.12  | -0.08 | -0.01 | -0.02 |
|       |         |       | NRD    | -0.05 | -0.09 | -0.04 | -0.04 |
|       | Protium | $\Psi_{\text{pd}}$ | VPD    | -0.07 | -0.03 | -0.06 | -0.05 |
|       |         |       | NRD    | -0.03 | -0.01 | -0.01 | -0.01 |
|       | Erythroxylum | $\Psi_{\text{pd}}$ | VPD    | -0.46 | -0.27 | -0.56 | -0.45 |
|       |         |       | NRD    | -0.36 | -0.21 | -0.24* | -0.12 |

### TABLE V

Coefficient of multiple regression between temporal variation in midday leaf water potential ($\Psi_{\text{md}}$, MPa), $F_{\text{vpd}} / F_{\text{mnd}}$, daily maximum air vapor pressure deficit (VPD) and the number of rainless days (NRD). We selected the greatest VPD on each day on which measurements were taken and the number of rainless days during the one, two, three or four weeks before that. Asterisks and bold values indicate $P < 0.05$.

| Weeks | Species | Trait | Factor | 1    | 2    | 3    | 4    |
|-------|---------|-------|--------|------|------|------|------|
|       | Byrsonima | $\Psi_{\text{md}}$ | VPD    | -0.64 | -0.31 | -0.29 | -0.35 |
|       |         |       | NRD    | 0.18  | 0.00 | 0.03 | 0.02 |
|       | Erythroxylum | $\Psi_{\text{md}}$ | VPD    | -0.04 | -0.03 | -0.03 | -0.03 |
|       |         |       | NRD    | 0.00  | 0.00 | 0.00 | 0.00 |
### TABLE V (continuation)

| Species | Trait | Factor | Weeks |
|---------|-------|--------|-------|
|         |       |        | 1     | 2     | 3     | 4     |
| Clusia  | Ψ<sub>md</sub> | VPD    | -0.34 | -0.3  | -0.31 | -0.28 |
|         | NRD   |        | 0.02  | -0.04 | -0.01 | -0.01 |
|         | F<sub>v</sub>/F<sub>md</sub> | VPD    | -0.05 | -0.05 | -0.07 | -0.04 |
| Protium | Ψ<sub>md</sub> | VPD    | 0.03  | 0.04  | 0.04  | 0.04  |
|         | NRD   |        | -0.01 | 0     | 0     | 0     |
|         | F<sub>v</sub>/F<sub>md</sub> | VPD    | -0.02 | -0.02 | -0.02 | -0.02 |
| Erythroxylum | Ψ<sub>md</sub> | VPD    | -0.87 | -0.45 | -1.14 | -0.54 |
|         | NRD   |        | -0.41 | -0.44 | -0.27 | -0.19 |
|         | F<sub>v</sub>/F<sub>md</sub> | VPD    | 0.01  | 0.02  | 0     | 0.01  |
| Eugenia | Ψ<sub>md</sub> | VPD    | -0.78 | -0.84 | -0.77 | -0.82 |
|         | NRD   |        | 0     | -0.04 | 0     | -0.01 |
|         | F<sub>v</sub>/F<sub>md</sub> | VPD    | -0.78 | -0.84 | -0.77 | -0.05 |
| Ocotea  | Ψ<sub>md</sub> | VPD    | -0.05 | -0.13 | 0.06  | -0.14 |
|         | NRD   |        | 0.23  | 0.12  | 0.1   | 0.05  |
|         | F<sub>v</sub>/F<sub>md</sub> | VPD    | -0.12 | -0.12*| -0.12 | -0.12*|

### TABLE VI

Eigen vector scores from a PCA of three axes obtained from a matrix of six traits and six species in two seasons. The six traits measured were midday leaf water potential (Ψ<sub>md</sub>), predawn leaf water potential (Ψ<sub>pd</sub>), potential quantum yield of photosystem II at midday (F<sub>v</sub>/F<sub>md</sub>), potential quantum yield of photosystem II at predawn (F<sub>v</sub>/F<sub>pd</sub>), minimal fluorescence yield (F<sub>0</sub>) and maximal fluorescence yield (F<sub>m</sub>). Percentage values indicate the percentage of variation accounted for by each axis. Bold values indicate the highest values for each axis.

| Physiological Traits | Axis 1 | Axis 2 | Axis 3 |
|----------------------|--------|--------|--------|
| F<sub>v</sub>/F<sub>apd</sub> | 0.928  | -0.270 | 0.218  |
| F<sub>v</sub>/F<sub>pd</sub>  | -0.838 | 0.347  | 0.224  |
| F<sub>v</sub>/F<sub>md</sub> | 0.618  | 0.013  | 0.705  |
| F<sub>v</sub>/F<sub>apd</sub> | 0.462  | -0.321 | -0.677 |
| Ψ<sub>md</sub>          | 0.440  | 0.776  | -0.287 |
| Ψ<sub>pd</sub>         | 0.402  | 0.846  | -0.026 |
DISCUSSION

Most of the six species did not differ across seasons in their ability to maintain $\Psi_{md}$. Although the dominant species, *Clusia* and *Protium* had higher values of $\Psi_{md}$ throughout, the other species, except for *Erythroxylum*, maintained similar values throughout the year, which were consistently lower than the dominant species. These values were maintained despite differences in leaf morphology and phenology. This agrees with reports indicating that the maintenance of $\Psi_{md}$ occurs in a series of ways, in contrast with the maintenance of constant hydraulic conductance, which occurs through changes in architectural traits such as leaf area per sapwood area (Meinzer et al. 1999, Bucci et al. 2005, Maseda and Fernández 2006, Franks et al. 2007). In *Protium*, for instance, the ability to maintain a higher $\Psi_{md}$ throughout the year might be mediated by a substantial reduction in leaf number during the dry season (Rosado and de Mattos 2010). Regardless of the mechanisms involved, it is noteworthy that only the two dominant species exhibited higher and constant $\Psi_{md}$, which suggests a higher safety margin for embolism (Sperry et al. 2002). Our findings support that in drought-prone habitats most species are able to maintain similar $\Psi_{md}$ between dry and wet seasons. However, only a small number of species are able to maintain a relatively high $\Psi_{md}$, which may be an important trait related to the ability of these species to achieve dominance in sandy, drought-prone environments.

**ARE THE SEASONAL RESPONSES OF CHLOROPHYLL FLUORESCENCE VARIABLES RELATED TO THE SEASONAL VARIATION IN $\Psi_L$?**

$F_v/F_m$ was lower in the restinga plants than the optimal value for most species, which indicates photoinhibition (Demmig-Adams and Adams 2006). However, this was less pronounced than
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in plants from other harsh environments, where $F_v/F_m$ may reach values below 0.5 (Bellot et al. 2004, Zunzunegui et al. 2005, Ogaya et al. 2011). Nevertheless, there is a positive linear relationship between $F_v/F_m$ and the leaf turnover rate for restinga species, which suggests that there are ecophysiological implications of even small decreases in $F_v/F_{md}$ (Rosado and de Mattos 2010). The greatest decreases in $F_v/F_{npd}$ and $F_v/F_{numd}$ occurred after a rainless period of 30 days in September 2004. These decreases were probably caused by low soil water potential and high radiation load, as observed for woody species in semi-arid Mediterranean shrubland in Spain (Bellot et al. 2004).

The reversible decline in $F_v/F_m$ reflects the protective down-regulation of PS II, which may prevent damage to the reaction centers when there is excess excitation energy (Walker 1992, Demmig-Adams and Adams 2006). However, a comparison of changes in $F_0$ and $F_m$ indicates whether species are exhibiting increased thermal dissipation of excitation energy or deactivation of the PSII reaction centers (Krause 1988, Porcar-Castell et al. 2008). Thermal dissipation is associated with decreases in $F_{o pd}$ and $F_{npd}$ as observed when the dry season of 2005 was approaching, whereas photodamage and deactivation only increase $F_o pd$ as observed in September 2004 in most species. For Clusia, Byrsonima and Eugenia, $F_o pd$ increased to various degrees, yet always significantly during the dry season, with the highest values recorded for Eugenia. This indicates that these species were subjected to photodamage (Krause 1988), as could be expected for plants exhibiting constant $Ψ_{md}$ due to strong stomatal closure (McDowell et al. 2008). Photoinhibition may have been alleviated during the cloudy day in July 2005, when all species showed the highest $F_v/F_{numd}$. Cloudy days may also have decreased the strength of the general declining trend in $F_{o pd}$ throughout 2005, because the higher accumulated rainfall in 2005 (770 mm) in comparison with 2004 (246 mm) suggests that there were more cloudy days, which would reduce average light intensity.

Irrespective of leaf succulence and leaf phenology, with the exception of Erythroxylum, physiological changes of species across seasons were more strongly captured by chlorophyll fluorescence variables than to $Ψ_l$. The multivariate analysis showed that the responses of all species across seasons were positively associated with $F_o pd$, which suggests the occurrence of photodamage during the dry season. However, the differential displacement of each species between seasons in the multivariate space indicates different degrees of susceptibility to photoinhibition, with Eugenia showing the strongest changes between seasons. The only species for which changes in fluorescence between seasons was also paralleled by changes in $Ψ_{md}$ and $Ψ_{pd}$ was Erythroxylum. For Erythroxylum, the negative relationship between $Ψ_{md}$ and $Ψ_{pd}$ was expected because of this species' higher wood density, which may decrease water transport to leaves (Rosado and de Mattos 2010).

HOW DOES VARIATION IN $Ψ_l$ DIFFER IN RESPONSE TO NRD AND VPD THROUGHOUT THE YEAR?

The less responsive behavior of Byrsonima, Clusia and Protium to NRD and VPD indicates that these species have the ability to decouple from the main factors that drive daily and seasonal variation in water availability. In fact, Byrsonima and Protium show a strong midday depression of stomatal conductance in both wet and dry seasons (Lignani 2008), and the CAM photosynthesis in Clusia (Franco et al. 1996) may explain the lack of a midday response to NRD and VPD. In Clusia, the negative relationship between $Ψ_{pd}$ and NRD might reflect nocturnal transpiration, typical in CAM species, which may lead to more negative $Ψ_{pd}$ as NRD increases. In addition, the unresponsiveness of $Ψ_{md}$ and $Ψ_{pd}$ to NRD and/or VPD in Byrsonima may be related to this species' higher leaf death rate.
and $\Psi_{TLP}$ (Rosado and de Mattos 2010). The less negative $\Psi_{TLP}$ associated with a higher rate of leaf shedding may be an escape strategy for Byrsonima, allowing it to avoid the possible adverse effects of NRD and/or VPD on $\Psi_{md}$ and/or $\Psi_{pd}$ (Rosado and de Mattos 2010).

On the other hand, Eugenia, Erythroxylum and Ocotea responded strongly to NRD and/or VPD (Table IV). The negative relationship between NRD and $\Psi_{pd}$ for Erythroxylum may indicate a lower capacity for xylem refilling during prolonged rainless periods because of the species’ high wood density (Donovan et al. 2001, Rosado and de Mattos 2010). Interestingly, predawn and midday $F_v/F_m$ showed significant negative relationships with VPD and NRD, indicating that chlorophyll fluorescence may be more descriptive of the response of a species to water availability than $\Psi_L$.

Plants growing on sandy soils have a higher $\Psi_{pd}$ than those growing on clay soils (Sperry et al. 2002, Sperry and Hacke 2002). However, this is true only when the former have access to deeper soil water profiles and/or soil moisture is frequently replenished by rainfall (Fravolini et al. 2005, Hultine et al. 2005). The $\Psi_{pd}$ of restinga species growing where mean annual rainfall was 1100–1300 mm was higher than for other species occurring in sandy environments where the mean annual rainfall was around 450 mm (Zunzunegui et al. 2005, Fravolini et al. 2005, Hultine et al. 2005). In comparison with other seasonal environments, the variation in $\Psi_{pd}$ observed in restinga plants, -0.1 to -0.43 MPa in the wet season and -0.19 to -1.70 MPa during the dry season, was not as marked. In Mediterranean plants, $\Psi_{pd}$ varies from -0.1 to -0.86 MPa during spring and from -1.45 to -2.28 MPa during summer (Mediavilla and Escudero 2004, David et al. 2007). In the Brazilian savannas, woody species show a significant decline in $\Psi_{pd}$ from values close to 0 MPa in the wet season to around -0.5 to -1.0 MPa during the dry season (Bucci et al. 2005). It must be remembered, however, that it is not only soil properties, root access to the water table and/or rainfall patterns that affect $\Psi_{pd}$ (Sperry et al. 2002, Fravolini et al. 2005). Differences in $\Psi_{pd}$ among species may also be related to processes such as hydraulic conductance and nocturnal transpiration (Donovan et al. 2001, Dawson et al. 2007). For Clusia, the CAM behavior of nocturnal stomatal opening, as also observed in C_3 plants that show nocturnal transpiration, could explain the low $\Psi_{pd}$ (Donovan et al. 2001). On the other hand, in Erythroxylum, the only brevi-deciduous species in this study, a decrease in the rate of water delivery to the leaves because of the species’ high wood density (~0.77 g cm$^{-3}$; Rosado and de Mattos 2010) could promote more negative $\Psi_{pd}$ and $\Psi_{md}$, as described in other studies (Donovan et al. 2001, Meinzer 2003).

In conclusion, temporal variation in $\Psi_L$ and chlorophyll fluorescence was found useful for differentiating between groups of sandy soil species that are responsive or unresponsive to water availability. However, chlorophyll fluorescence appeared to be a more sensitive descriptor of their seasonal and short-term responses to NRD. The use of plant traits as proxies for the responses of communities to environmental change is gaining importance (Sutherland et al. 2013). In this study we have demonstrated that a better comprehension of how tropical species from drought-prone habitats cope with changes in water availability can be based on measurements of seasonal variation in $\Psi_L$ and chlorophyll fluorescence. These findings reinforce the importance of ecophysiological knowledge when selecting functional traits for predicting responses to climate change (Rosado et al. 2013), and open important avenues for models specifically concerning species from dry environments. In addition, because we focused on species from the Atlantic rain forest complex that are able to occupy coastal sandy plains environments, our results contribute to the understanding of the environ-
mental filtering that affects community assembly in resource-poor habitats.

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RESUMO

Entre os efeitos da mudança ambiental, a intensificação dos eventos de seca é notável e a vegetação tropical é apontada como altamente vulnerável a ela. No entanto, não está claro como plantas tropicais em habitats propensos à seca responderão a essa mudança. Em um ambiente de planície costeira arenosa, avaliamos a resposta de seis espécies vegetais à dist  de água ao longo das estações, a relação entre as suas características morfo-fisiológicas, e quais características seriam as melhores descritoras de resposta das plantas à seca. Independente de suculência foliar e fenologia, respostas entre as estações foram mais fortemente relacionadas à fluorescência da clorofila. Neste estudo demonstramos que a melhor compreensão de como espécies tropicais de habitats propensos a seca lidam com mudanças na disponibilidade de água pode ser baseada na variação sazonal de potencial hídrico e fluorescência da clorofila. Esta variação temporal de potencial hídrico e fluorescência da clorofila foram úteis para diferenciar grupos de espécies que são responsivas ou não responsivas à disponibilidade de água. Ressalta-se que a fluorescência da clorofila foi o descritor mais sensível de respostas das espécies tanto em termos sazonais quanto em curto prazo.

Palavras-chave: seca, característica funcional, relações hídricas em plantas, fotoinibição, habitats sazonais.

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