

Diversity in nighttime transpiration behavior of woody species of the Atlantic Rain Forest, Brazil

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ABSTRACT

Nighttime transpiration (NT) has been documented in many plant species but we do not yet have a thorough understanding of the abiotic and biotic controls of this phenomenon. In this study we examined interspecific variation in NT behaviors in plants with distinct crown exposures (CE) and occurring at lowland (100 m) and montane forests (1000 m) in the Brazilian Atlantic rainforest to answer the following questions: are there different NT behaviors in plants subjected to distinct conditions associated with degree of CE and/or altitude? Are there higher rates of NT relative to daily maximum values at the montane forest due to higher vapor pressure deficit (VPD)? Taking into account that low VPD should generally produce low relative NT fluxes, should we expect that understory species in both altitudes will have quite uniform low relative rates of NT in comparison to overstory species owing to the buffered nature of within-canopy microclimate? NT did show differences between altitude and species. Of most significance was a prominent non-linear relationship between the NT and VPD, observed at the montane site. This non-linearity is in contrast to most previously published NT kinetics and suggests stomatal and/or leaf energy balance controls on NT. Our findings raise a new perspective concerning thermodynamic contributions to non-linear NT kinetics and some possible reasons for this interesting behavior are discussed.

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1. Introduction

Transpiration by plants follows a force–flux relationship that is shaped by the ability of a plant's transport system to conduct water. Plants can modify their hydraulic conductivity throughout the entire transport system and perhaps the strongest and most dynamic control is that of stomatal aperture which affects conductance to water vapor: understanding stomatal behavior in response to environmental conditions is thus key to understanding variation on plant water fluxes (Cowan and Farquhar, 1977; Jones and Sutherland, 1991; Cruziat et al., 2002). A commonly known relationship between transpiration and vapor pressure deficit (VPD) shows a non-linear saturating response of daytime transpiration to VPD because of decreases in stomatal conductance in response to a drying atmosphere (Jones and Sutherland, 1991; Cruziat et al., 2002). While daytime transpiration dynamics are

quite well studied, nighttime transpiration (NT) has only recently become an important research subject.

NT has been described for quite a few plant species from contrasting environments (Donovan et al., 2001; Dawson et al., 2007; Snyder et al., 2008; Christman et al., 2008; Novick et al., 2009) and while generally lower in magnitude relative to daytime transpiration, it is none-the-less significant enough for potential impacts on whole-plant water relations and ecosystem hydrology (Dawson et al., 2007; Wood et al., 2008). Functional consequences of NT include pre-dawn disequilibrium, increases in oxygen supply (Daley and Phillips, 2006) and facilitation of bulk flow of water to the roots overnight which may promote nutrient uptake (Donovan et al., 2001; Snyder et al., 2003). Although little is known about the controls of NT, a strongly linear positive relationship between sapflow and VPD has been documented in several ecosystems, suggesting that VPD is the most important environmental driving force for nocturnal water loss and that the crown conductivity is constant (Fisher et al., 2007; Dawson et al., 2007).

In tropical forests, small-scale changes in abiotic factors such as VPD, air temperature and solar radiation are common due to the large heterogeneity of canopy structure, topography and wind patterns (Motzer, 2005). Thus, even in short horizontal and altitudinal distances, plant species may be subjected to high

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spatio-temporal heterogeneity (Leigh et al., 2004). In view of the large heterogeneity in tropical forests, many studies have been performed to evaluate species responses at different scales (Bruijnzeel and Veneklaas, 1998; Velázquez-Rosas et al., 2002; Graham et al., 2005; Holder, 2007; Beaumont and Burn, 2009; Poorter, 2009). Species responses along altitudinal gradients have been studied especially at a morphological level (Bruijnzeel and Veneklaas, 1998; Velázquez-Rosas et al., 2002; Holder, 2007; Rosado et al., 2010) where species from contrasting environments exhibit different leaf morphologies in response to the degree of crown exposure (CE) and the environmental regime this implies (Poorter, 2009). To date however, whole plant water relations of tropical rain forest trees have not been examined along altitudinal gradients (Santiago et al., 2000, 2004; Oliveira et al., 2005a). It is equally true that the science surrounding NT has been poorly advanced in tropical rain forests (Dawson et al., 2007). Since tropical rain forests present high environmental heterogeneity and high species diversity (Myers et al., 2000; Leigh et al., 2004), this is both a major hindrance and major research need for understanding how nighttime conditions affect plant ecophysiology and overall forest function.

The Atlantic Forest occurs along the Brazilian coast and is considered a biodiversity hot-spot (Myers et al., 2000). This biome is a mosaic of ecosystems that belongs to the Atlantic Dominion including lowland and montane forests (Joly et al., 1999). In addition, altitudinal gradients present in the Atlantic rain forest produce directional changes in abiotic factors such as increases in solar radiation and VPD (Rosado et al., 2010) and decreases atmospheric pressure (Rosado, 2011). These changes promote strong directional changes in plant traits such as increases in leaf longevity and decreases in transpiration (Grubb, 1977; Bruijnzeel and Veneklaas, 1998; Leuschner, 2000; Körner, 2007).

Considering the high environmental heterogeneity that tropical forest plants are subjected to via vertically complex canopies and altitudinal variation, we chose four woody species from Atlantic Rainforest co-occurring in Lowland (100 m) and Montane forests (1000 m) and occupying different canopy strata to answer the following questions: are there different nocturnal transpirational behaviors in plants subjected to distinct conditions associated with degree of crown exposure (CE) and/or altitude? Taking into account that low VPD should generally produce low relative NT fluxes, should we expect that understory species in both altitudes will have quite uniform low relative rates of NT in comparison to overstory species owing to the buffered nature of within-canopy microclimate? Answering these questions concerning the diversity of NT ecophysiology within Atlantic Rainforest, using sap flow measurements to trace day and nighttime transpiration, will deepen our understanding of the structural and functional complexity of these valuable forests which face threats from fragmentation and shifting environmental conditions.

2. Materials and methods

2.1. Study sites and species

Our study was conducted in lowland and montane forests in the Serra do Mar State Park, covering 315,000 ha in the Atlantic Rain Forest in the north of São Paulo state, Brazil. The Lowland forest is around 100 m above sea level – (23°31′–23°34′ S and 45°02′–45°05′ W) and has a tropical climate and a mean annual precipitation of 2200 mm. Usually, the driest months are July and August. The Montane forest is 1000 m above sea level – (23°17′–23°24′ S and 45°03′–45°11′ W), has a tropical temperate climate, with mean annual precipitation around 2000 mm and fog frequency is around 100 days per year (Rosado et al., 2010). All physiognomies are characterized as broadleaf evergreen tropical forests.

We selected plant species according to co-occurrence at both sites, contrasting canopy position (overstory, intermediary and understory) and species belonging to different families to avoid phylogenetic effects on the study of NT behavior. The species chosen were: *Hyeronima alchorneoides* Allemão (Phyllanthaceae), *Alchornea triplinervea* (Spreng.) Müll. Arg. (Euphorbiaceae), *Mollinedia schottiana* (Spreng.) Perkins (Monimiaceae); and *Rustia formosa* Klotzch (Rubiaceae). *Hyeronima* and *Alchornea* are overstory species while *Rustia* is an intermediary and *Mollinedia* an understory species. For simplicity, we will refer to each species by their generic names.

2.2. Sap-flow probe installation and measurements

We used the heat ratio method (Burgess et al., 1998, 2001) to make continuous measurements of sap flow in trunks in three individuals per species at each site. The HRM measures the increase in temperature following a heat pulse at two symmetrical points, 5 mm above and below a heater inserted 30 mm into the active sapwood. This technique allows bi-directional measurements of sap flow and also measures very slow flow rates which we might expect during the night. Sap flow sensors (HRM30 ICT International Pty Ltd., Armidale, NSW, Australia) were inserted into the xylem tissue of the trunks at breast height of three individuals per species. The heater was set up to send a pulse every 30 min and temper-

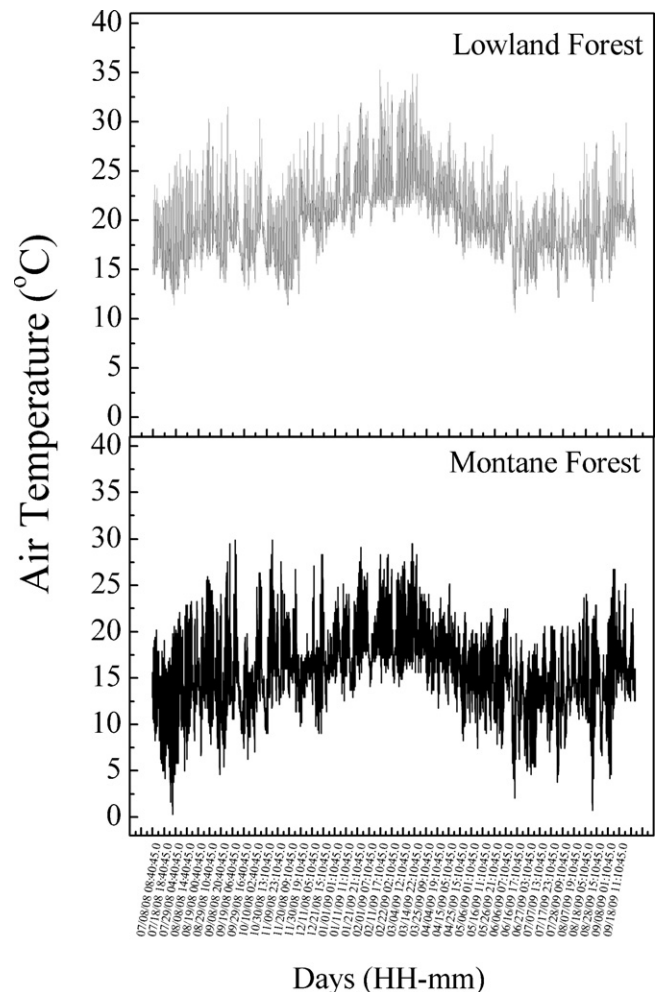


Fig. 1. Mean air temperature (°C) of three strata (overstory, intermediary and understory) at the Lowland and Montane forests along the whole period of study. Data collected every 30 min.

ature ratios were recorded continuously with a data logger (SL5 Smart Logger ICT International Pty Ltd, Armidale, NSW, Australia). We calculated the heat pulse velocity (cm h^{-1}) following Burgess et al. (1998, 2001) as:

$$V = (kX^{-1}) \times \ln(v1(v2)^{-1}) \times 3600$$

where k is the thermal diffusivity of the fresh (green) wood, X is the distance (cm) between the heater and the thermocouples, and $v1$ and $v2$ are the differences between the initial temperature ($^{\circ}\text{C}$) at the two thermocouples (downstream and upstream the flow in relation to the heater, respectively) and the temperature measured after a heat pulse. To estimate k , density of the sapwood and water content were measured on wood cores taken from the stems (Burgess et al., 2001) in September 2009. Since it was not possible to cut the xylem to establish zero flow, we selected a series of cloudy dawn, after rainfall events, whose VPD were close to zero, to establish the baseline. The measurements were performed from June 2008 to September 2009. We selected data from thirty representative nights of two dry seasons (July 2008 and June 2009) and one wet season (December 2008). The dry season of 2008 had two rainless months and was drier than 2009. NT was calculated as % of maximum daytime transpiration rate during each period per individual. Summertime maximum transpiration rate (wet season), was calculated as the mean of three half-hourly measurements made between 11:00 and 12:30 h for six consecutive sunny days during the wet season. Data collected between 19:00 and 05:00 h were considered for NT analysis. Noisy data caused by equipment failure were excluded from the analysis. To show the low extent of nocturnal xylem refilling on the species studied, we selected two damp nights (i.e. to eliminate NT) that occurred after dry days (high expected tissue water deficits) during the dry season 2008 to show how % sap velocity decays rapidly into night periods, when VPD (kPa) is correspondingly low (Fig. 2).

2.3. Environmental variables

Three air temperature and relative humidity sensors (HOBO) were placed at different heights at the approximate distance from the ground of 20, 10 and 2 m (canopy, intermediary and understory, respectively at both sites), set up to collect data every 30 min. These data were used to calculate the vapor pressure deficits (VPD, kPa).

2.4. Statistical analysis

We used regression analyzes to evaluate the relationship between NT and VPD where each data point refers to every half-hourly measurements made during the selected nights. For each individual per season, fitted linear and non-linear models (logarithmic functions) were compared by the Akaike Information Criterion (AIC). For each individual at each season (represented separated in different panels in Figs. 3 and 4), the R^2 is indicated for each regression of each season and the correspondent significance level ($P < 0.05$). Given that VPD values reached overall higher values at the Montane forest than at the Lowland site, we filtered the data of the different sites to span a common VPD range so as to examine whether the sites differed under common nighttime conditions (data not shown).

3. Results

3.1. Environmental variables

Mean air temperatures at all strata were lower (Fig. 1) while VPD and global solar radiation were higher at the Montane forest compared to the Lowland forest (Rosado et al., 2010). Nocturnal VPD increased from understory to overstory in both forests in the

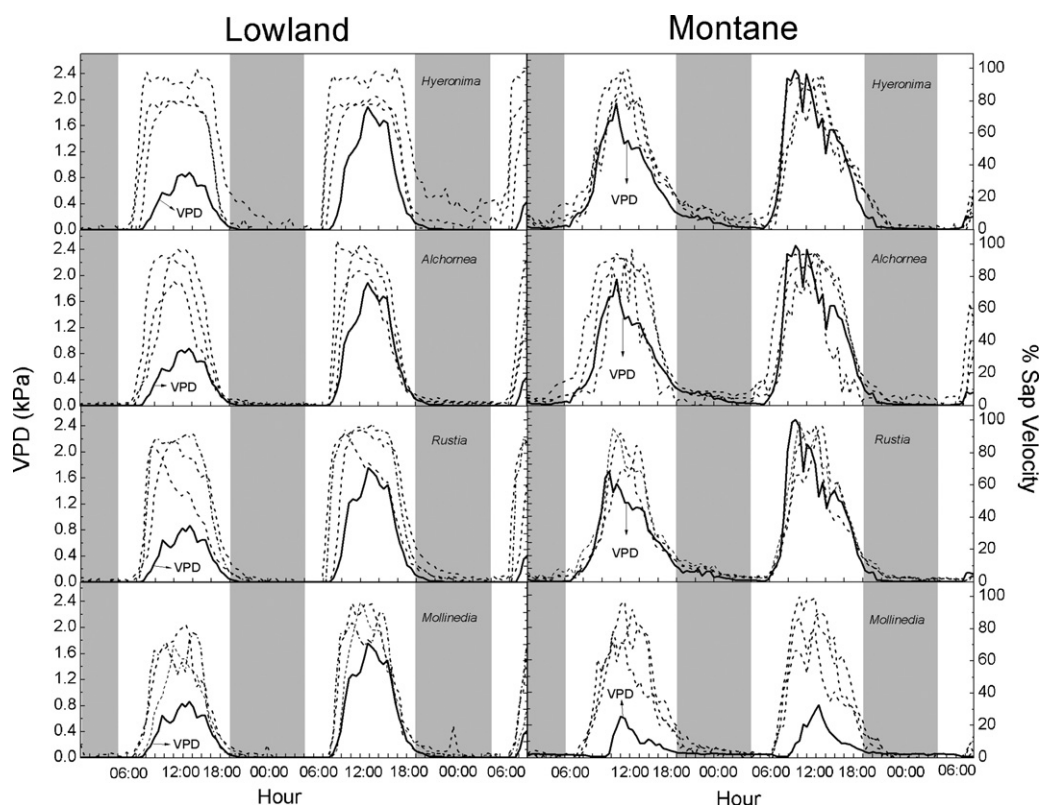


Fig. 2. Diurnal patterns of vapor pressure deficit (VPD) and % sap velocity during 48 h during the dry season 2008. In each panel, the dashed lines represent the % sap velocity of each individual of each species at both sites. VPD is represented by bold lines in each panel. Shaded bars represent periods between 19:00 and 5:00.

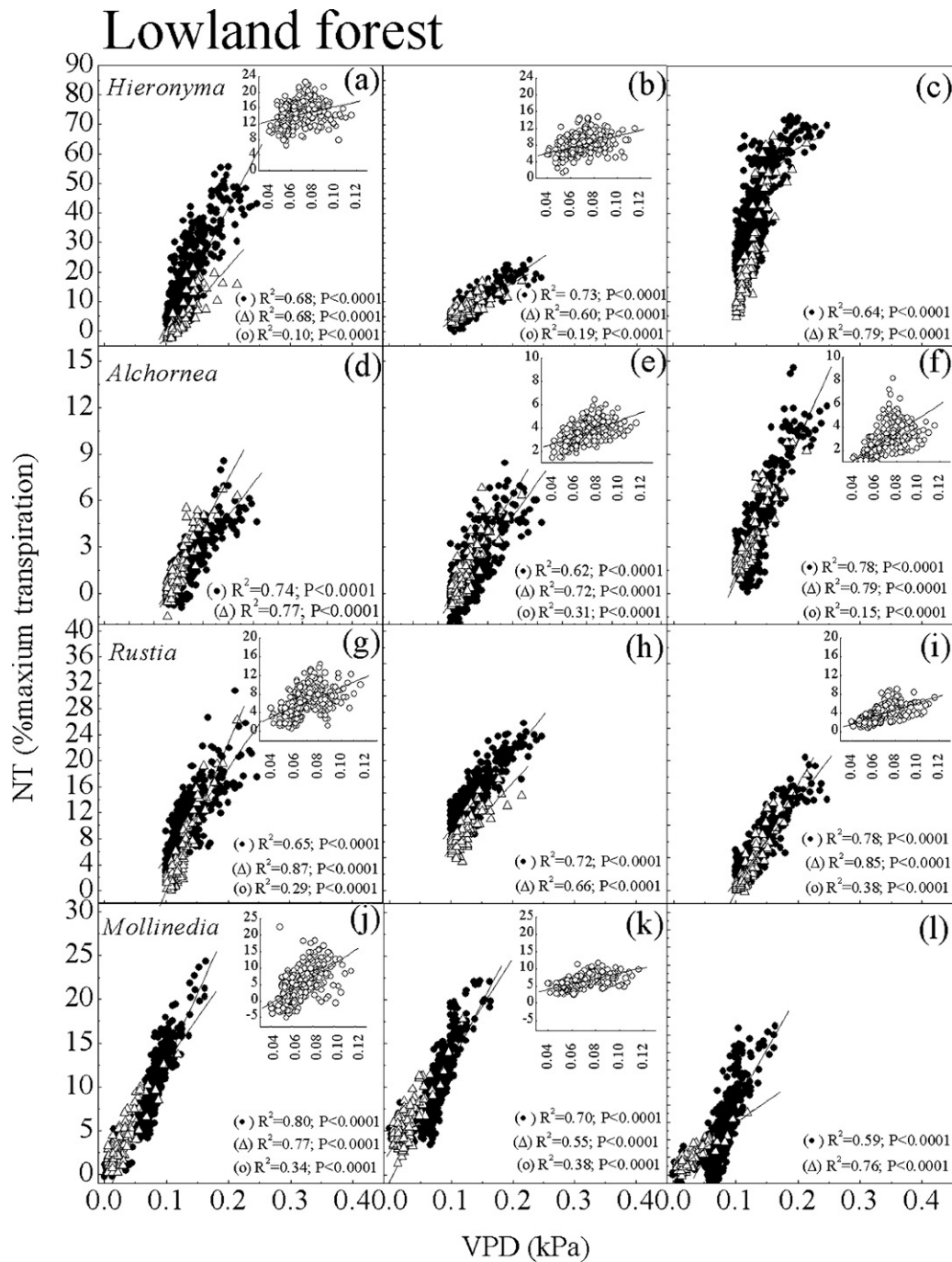


Fig. 3. Relationship between vapor pressure deficit (VPD, kPa) and nighttime transpiration (NT) in four species at the Lowland forest expressed as % maximum transpiration rate at the wet season. Each panel represented by different letters corresponds to data from one individual. Measurements were made between 19:00 and 5:00 h over 30 nights in the dry season 2008 (●), the wet season (Δ) and the dry season 2009 (○). The values for the dry season 2009 are displayed in the insets, rather than the main plots. Each point refers to every half-hourly measurements made during the selected nights. For each individual, are indicated the R^2 for each regression of each season and the correspondent significance level ($P < 0.05$).

dry and wet season of 2008. In general, nocturnal VPDs were similar between dry season of 2008 and the wet season. In the dry season of 2009, VPDs were similar among strata (Figs. 3 and 4). At night, maximum values reached 0.20 kPa in the overstory during the dry season of 2008 in the lowland forest and 0.70 kPa in the same period in the overstory of the Montane Forest. In fact, the nocturnal maximum VPD at the understory in the Montane forest (around 0.3 kPa), was higher than the overstory at the Lowland site.

3.2. Nighttime transpiration

To examine whether refilling of xylem capacitance is a substantial contributor to night time xylem water flux, we selected two damp nights (i.e. very low VPDs but not raining) during the dry season (i.e. when daytime water deficits should be highest) of 2008 (Fig. 2). Under such conditions, we observed rapid cessation in sap velocity at the commencement of nighttime thus indicating that any xylem refilling is short lived and is not a major contributor to

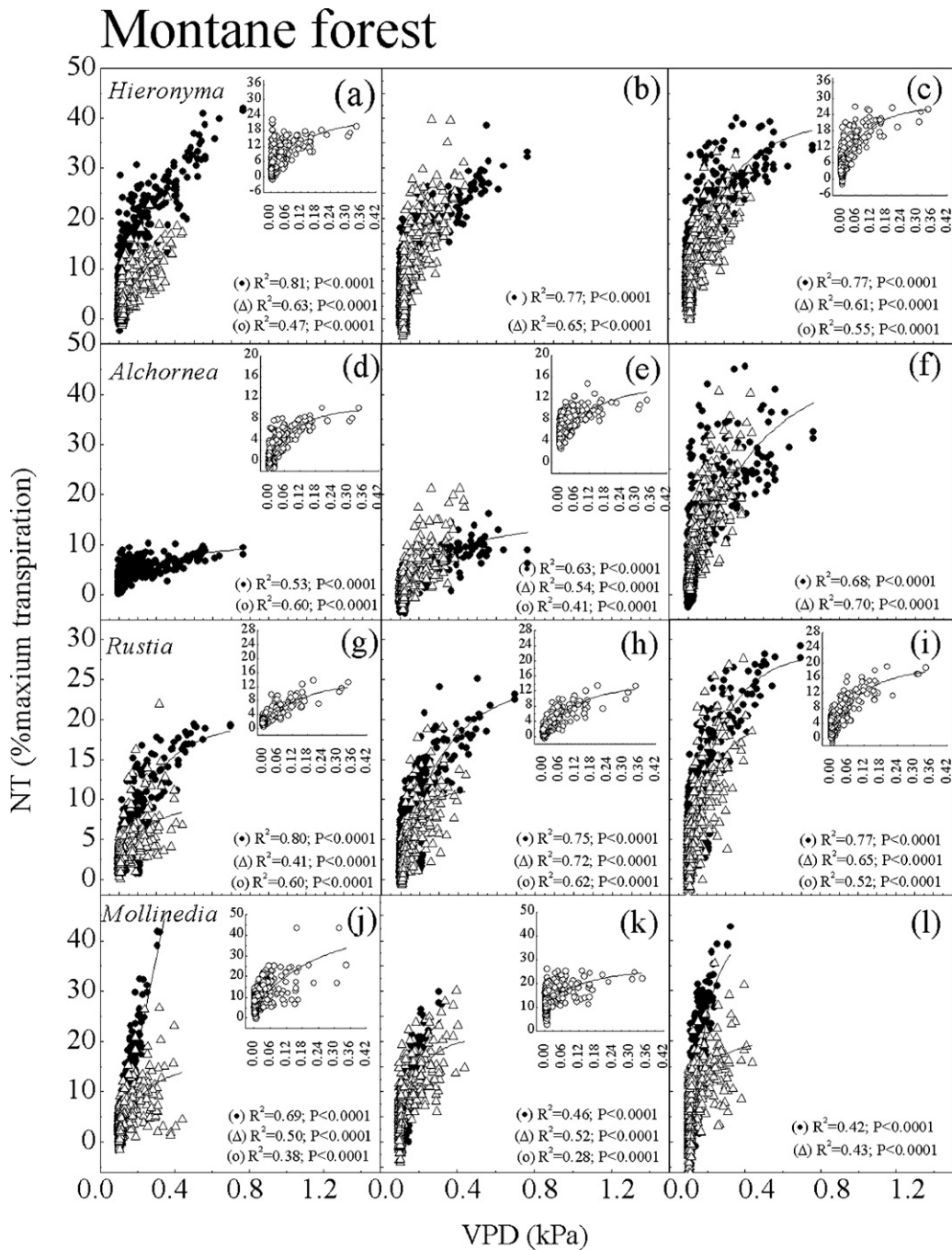


Fig. 4. Relationship between vapor pressure deficit (VPD, kPa) and nighttime transpiration (NT) in four species at the Montane forest expressed as % maximum transpiration rate at the wet season. Each panel represented by different letters corresponds to data from one individual. Measurements were made between 19:00 and 5:00 h over 30 nights in the dry season 2008 (●), the wet season (△) and the dry season 2009 (○). The values for the dry season 2009 are displayed in the insets, rather than the main plots. Each point refers to every half-hourly measurements made during the selected nights. For each individual, are indicated the R^2 for each regression of each season and the correspondent significance level ($P < 0.05$).

night-time sap flow patterns in our study system. By contrast we found strong positive relationships between nighttime sap flow and VPD in all periods, sites and species (Figs. 3 and 4) indicating that atmospheric demand for water was driving NT. However, plant responses differed considerably between sites because of differences in the magnitude of VPD found at each site. The range of VPDs at lowland forest was comparatively low (0–0.20 kPa) and under this regime plants responded linearly to VPD (Table 1; Fig. 3). At the Montane forest (where VPD reached higher values, up to 0.70 kPa), for most of individuals and both seasons NT showed

an overall non-linear response to the range of VPD conditions (Table 1; Fig. 4). A site comparison using filtered data (not shown) to cover a common VPD range (0–0.2 kPa) indicated that when VPDs were lower at the Montane forest then NT showed the same linear response as seen at the Lowland site.

We tested to see whether the non-linear NT–VPD relationship at the Montane site was related at all to the time of night. Non-linear responses could be observed at any time from the beginning (~19:00) to the end (~5:00) of the nighttime period suggesting that for both seasons, the factors causing NT–VPD to become non-linear

Table 1
Equations from regressions between NT and VPD for each individual (Ind; represented by letters as indicated in Figs. 3 and 4) of each species in different seasons at the Lowland and Montane forest. Non-linear relationships are represented in bold.

Site	Species	Ind	Dry season 2008	Wet season	Dry season 2009
Lowland	<i>Hyeronima</i>	a	NT = -38.02 + 398.89VPD	NT = -25.72 + 237.20VPD	NT = 10.17 + 61.34VPD
		b	NT = -11.21 + 145.72VPD	NT = -6.73 + 115.88VPD	NT = 3.17 + 69.24VPD
		c	NT = 172.6 + 62.16 ln(VPD - 0.01)	NT = 193.77 + 69.76 ln(VPD - 0.03)	-
	<i>Alchornea</i>	d	NT = -5.15 + 50.99VPD	NT = -7.69 + 75.40VPD	-
		e	NT = -6.12 + 55.35VPD	NT = -6.77 + 65.64VPD	NT = 1.28 + 33.49VPD
		f	NT = -8.12 + 89.68VPD	NT = -7.47 + 87.05VPD	NT = -1.39 + 61.09VPD
	<i>Rustia</i>	g	NT = 40.89 + 11.76 ln(VPD - 0.05)	NT = -23.00 + 228.02VPD	NT = -1.30 + 107.48VPD
		h	NT = -2.37 + 117.25VPD	NT = -3.88 + 102.20VPD	-
		i	NT = -11.21 + 125.91VPD	NT = -16.91 + 166.26VPD	NT = -1.27 + 73.88VPD
	<i>Mollinedia</i>	j	NT = -2.94 + 156.71VPD	NT = 0.45 + 119.92VPD	NT = -8.97 + 202.73VPD
		k	NT = -0.74 + 135.88.71VPD	NT = 3.38 + 101.11VPD	NT = 0.82 + 0.41VPD
		l	NT = -5.35 + 124.40VPD	NT = 0.21 + 55.53VPD	-
Montane	<i>Hyeronima</i>	a	NT = 41.99 + 13.22 ln(VPD - 0.04)	NT = 24.62 + 10.05 ln(VPD - 0.01)	NT = 25.49 + 6.43 ln(VPD + 0.02)
		b	NT = 33.19 + 8.54 ln(VPD - 0.06)	NT = 43.58 + 16.04 ln(VPD - 0.03)	-
		c	NT = 40.73 + 9.81 ln(VPD - 0.06)	NT = 39.37 + 15.9 ln(VPD + 0.008)	NT = 37.52 + 9.79 ln(VPD + 0.02)
	<i>Alchornea</i>	d	NT = 8.68 + 1.59 ln(VPD - 0.08)	-	NT = 14.92 + 4.45 ln(VPD + 0.02)
		e	NT = 12.37 + 3.84 ln(VPD - 0.05)	NT = 21.47 + 14.01 ln(VPD + 0.11)	NT = 13.74 + 1.98 ln(VPD + 0.01)
		f	NT = 37.3 + 10.65 ln(VPD - 0.06)	NT = 44.26 + 17.8 ln(VPD - 0.008)	-
	<i>Rustia</i>	g	NT = 22.78 + 7.97 ln(VPD - 0.02)	NT = 12.15 + 4.00 ln(VPD - 0.01)	NT = 17.32 + 6.17 ln(VPD + 0.07)
		h	NT = 24.73 + 7.43 ln(VPD - 0.05)	NT = 17.59 + 4.94 ln(VPD - 0.06)	NT = 16.44 + 4.78 ln(VPD - 0.03)
		i	NT = 31.71 + 8.78 ln(VPD - 0.05)	NT = 28.09 + 9.08 ln(VPD - 0.04)	NT = 27.22 + 9.03 ln(VPD + 0.06)
	<i>Mollinedia</i>	j	NT = -17.83 + 180.87VPD	NT = 16.24 + 3.70 ln(VPD - 0.07)	NT = 31.38 + 6.39 ln(VPD + 0.01)
		k	NT = -10.40 + 132.72VPD	NT = 25.82 + 5.86 ln(VPD - 0.07)	NT = 26.51 + 3.68 ln(VPD + 0.01)
		l	NT = -15.26 + 196.98VPD	NT = 26.03 + 6.11 ln(VPD - 0.07)	-

were independent of time since sunset. Within a given species in each forest, we observed intraspecific variation in NT: for example, depending on the season, linear and non-linear relationships between NT and VPD were found in one individual of *Hyeronima* and *Rustia* at the Lowland forest (Table 1; Fig. 3) and in three individuals of *Mollinedia* at the Montane forest (Table 1; Fig. 4).

The maximum rate of NT varied between and within species. NT for *Hyeronima* reached extremely high values, which represented as much as 80% and 45% of maximum daytime transpiration rate for the Lowland and Montane forest, respectively. The other three species at the lowland forest showed maximum NT values between 25% and 32% of maximum daytime transpiration rate. *Alchornea* and *Mollinedia* specimens growing at the Montane site showed higher maximum NT values (around 45%) than those at the Lowland forest (Figs. 3 and 4). Only *Rustia* did not show great differences in the maximum NT between Lowland and Montane forest with values around 40% and 30%, respectively.

4. Discussion

Several studies have documented the wide occurrence of nighttime transpiration and suggested its possible causes and significance, for example in promoting nutrient supply (Scholz et al., 2007) and carbohydrate export (Marks and Lechowicz, 2007). However a robust physiological basis and understanding of its significance remain to be elucidated (Dawson et al., 2007; Marks and Lechowicz, 2007; Scholz et al., 2007; Christman et al., 2008, 2009; Novick et al., 2009).

One area of considerable agreement to date is that NT is a largely passive process which shows a strongly linear response to nighttime VPD (Donovan et al., 1999; Dawson et al., 2007). Such an uncontrolled process could be viewed simply as a consequence of lack of stomatal regulation of water loss at night, without having to invoke 'reasons' for its occurrence beyond this. If leaf turgor is sufficiently high at night to permit stomata to remain open, this may hold a benefit for respiratory gas exchange (Daley and Phillips, 2006; Marks and Lechowicz, 2007; Mott and Peak, 2010). Such losses of water loss could therefore be viewed as little different to the water forfeited each day to facilitate daytime carbon dioxide uptake.

As with daytime transpiration, which involves a number of variables belonging to different hierarchical levels of control (i.e. physiological and atmospheric) (Pieruschka et al., 2010), NT controls may include considerable complexity. A novel finding of our present study is that NT-VPD can be highly non-linear: this indicates that either that the source-sink relationship cannot be characterized by VPD alone, or that leaf conductance to water vapor varies at night (or both). We observed strongly non-linear NT-VPD relationships chiefly at our Montane site where the highest VPDs were recorded. This suggests some controls that might limit NT under high nocturnal demand, including: (1) nighttime leaf water status, stomatal regulation and other hydraulic controls, (2) factors affecting sink strength (VPD, windspeed, boundary layer) or (3) factors affecting source strength (soil and leaf water status and leaf energy balance as it affects leaf vapor pressures). The fact that non-linear responses only begin at higher VPDs (as found at the Montane forest) makes the possibility of a role for leaf water status and stomatal behavior quite plausible, particularly given Mott and Peak (2010) report that the basic mechanisms of stomatal responses to humidity and temperature apply day or night (Mott and Peak, 2010). Indeed, the rapidly asymptotic nature of some of the responses we measured point to isohydric control of leaf water status by stomatal closure. It has previously been shown that nocturnal stomatal conductance decreases linearly in response to increases in VPD, especially in plants growing in dry conditions (Bucci et al., 2004; Barbour and Buckley, 2007). Also, the relative sensitivity of stomata to VPD at night is higher than during the day (Bucci et al., 2004; Barbour and Buckley, 2007). Thus even though the basic 'mechanisms' of control remain common for both day and night (Mott and Peak, 2010), leaves may be subject to a different physiological regime at night such that day and night transpiration-VPD functions do not follow the same response curve (our daytime data (not shown) indicate precisely this).

Although NT might also have a contribution of cuticular water loss, stomatal transpiration has been documented as the predominant contributor to NT (Caird et al., 2007; Howard and Donovan, 2007; Zeppel et al., 2010). Indeed recent findings that epicuticular waxes might be thicker on leaves of montane forest species compared to lowland species argues against a major cuticular pathway for NT (Rosado et al., 2010). This is one further reason to move

beyond previous views of NT as largely passive, to one where stomatal behavior, leaf water status and leaf energy balance must be considered.

Given that mechanisms of stomatal control are common across both day and night, albeit with different thresholds and sensitivities, it would be unwise to discount the possibility that the thermodynamic aspects of transpiration involving energy input, sensible and latent heat flux, which are commonly considered for daytime transpiration (Pražák et al., 1994; Williams et al., 1998; Dekker et al., 2000; Wang et al., 2007) must also be considered at night. Some of the curved (but not asymptotic) NT–VPD trajectories we recorded might be a result of progressive cooling of the leaf by evaporation (latent heat flux). Recently, Pieruschka et al. (2010) modeled the comparative contribution of the leaf-to-air vapor pressure gradient and leaf energy balance to *push* and *pull* water from leaves, respectively. In their model, NT, also mediated by epidermis, is caused by the water evaporation driven by heat from the air.

At present, neither nocturnal leaf energy-balance, nor nocturnal stomatal behaviors are well understood and it is likely that characterization of both, including their combination, will be required before NT can be fully characterized. In addition to these, a thorough understanding of nocturnal micrometeorology is also required. Important factors include: (1) wind dynamics (altering speed, temperature and VPD), (2) cloud cover (altering forest heat storage and cooling rate) (3) mists/fog and (4) seasonal/daily soil and plant water deficits. Unraveling the contributions of various drivers and controls, including where thresholds may be reached to yield non-responses will require further modeling and measurement some of which is already underway.

To our knowledge, we are aware of only three studies reporting non-linear relationship between NT (assessed by sap flow) and VPD, in contrasting cases where values reached 1.0 kPa (Sellin and Lubenets, 2010), 2.5 kPa (Zeppel et al., 2010) and 4.0 kPa (Moore et al., 2008). Moore et al. (2008) explained the non-linear relationship based on a minimum threshold required for NT under low VPD. However, their study was performed with the heat dissipation method for which accurate estimation of zero and slow flows is not always achieved (Lu et al., 2004; Dawson et al., 2007; Burgess and Dawson, 2008). Our own study used the HRM which is designed to accurately measure slow, zero and reverse sap flow rates (Burgess et al., 2001), but without cutting trees to stop flow completely, estimates of zero flow can have some error. Fortunately, any error presents as a small offset and does not affect the linearity of transpirational flows detected by the HRM (since it behaves symmetrically and linearly throughout low positive or negative flow ranges). A bigger problem arises if small reverse flow events occur due to foliar uptake of water (Burgess and Dawson, 2004; Oliveira et al., 2005b). These produce a range of small velocities at zero VPD and if zero flow determination is also slightly inaccurate, some of this range may present as spurious positive numbers. This can skew curve fitting at the origin and where further data are sparse (e.g. spanning only a certain representation of soil, plant and atmospheric conditions) the overall shape of any ‘curve’ can be hard to ascertain. In previous reports where the shape of NT–VPD relationships was not a specific focus in the study (e.g. Zeppel et al., 2010), we suggest some of the above considerations could help clarify the degree of non-linearity present.

Other than the above three studies, the non-linear relationships we describe in our paper are largely at odds with the strongly linear trends reported for a range of other biomes by Dawson et al. (2007). Most of these trends however were for relatively dry sclerophyllous forest (e.g. Benyon, 1999; Bucci et al., 2004; Dawson et al., 2007) and given the paucity of information for wetter biomes such as the Atlantic rainforest and Montane tropical forests it may well be that there is more diversity in NT behaviors than previously suspected. We suggest that our results add some novel insights into

the controls of NT beyond simple linear responses to VPD: this is one more piece in the puzzle needed to describe the impact and significance of NT on terrestrial ecosystems globally. We hope that with further measurement other indications of non-linear NT behaviors (Moore et al., 2008; Sellin and Lubenets, 2010; Zeppel et al., 2010) can be confirmed. To do this careful application of methods will be required to handle such issues as zero flow, foliar uptake and separation of refilling and transpiration processes by various means (e.g. Fisher et al., 2007; Dawson et al., 2007 and our own attempts in this study).

4.1. The role of site conditions and canopy strata

Despite limitations to our present conceptual understanding of the controls and drivers of NT, we can draw some important conclusions regarding the differences we observed in NT at different sites. Firstly, in the Montane site, the diurnal thermal regime had higher amplitude than the lowland forest due to higher incoming radiation and higher outgoing nighttime thermal radiation (Körner, 2007). Although there is of course no solar heating of leaves at night, the circulating air mass contains thermal energy and as ambient heat is advected to the leaf with air flow it may provide a low but constant heat source analogous to incident solar radiation. This however may be strongly offset by the cooling of leaves during subsequent transpiration. The balance between this heat input and loss may be a central control of NT and could, perhaps, help explain why in some case NT becomes unresponsive to increasing atmospheric VPD (insufficient energy at the leaf and thus low leaf vapor pressure). Consequently, the higher leaf-to-air vapor pressure gradients at the Montane site (see also Leuschner, 2000) and the resulting higher NT rates should reach putative stomatal and/or leaf energy balance limits more readily. This would explain why almost all individuals of all species at the Montane forest showed strongly asymptotic curves between NT and VPD in all seasons. If stomatal regulation causes asymptotic curves at the Montane site, the lower nighttime stomatal conductance could be linked to generally higher water use efficiency and lower daytime stomatal conductance as observed in plants occurring in drier habitats with high VPD (Barbour and Buckley, 2007; Christman et al., 2008). In contrast to the observed differences between altitudes, canopy strata position does not seem to be an important factor affecting leaf energy balance or stomatal controls of maximum NT nor the degree of linearity/non-linearity. This is perhaps due to comparatively homogenous conditions within the canopies at our study sites when compared to the larger site differences.

In conclusion, the curved relationships between NT and VPD indicate that transpiration models still require improvement for better understanding of the functional importance of NT. At broad scales, where NT has been advocated as an important inclusion in ecohydrological models (Fisher et al., 2007; Novick et al., 2009), we suggest that relying only on the linear NT–VPD relationships commonly reported to date will lead to overly simplistic and inaccurate models. Ultimately, further work is needed to bring our understanding of the nocturnal half of forest functioning in line with our knowledge of daytime behaviors: the diversity of behaviors we have identified from field studies should now be followed by further careful characterization under controlled laboratory conditions.

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