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Research

Optimization theory explains nighttime stomatal responses

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Summary

- Nocturnal transpiration is widely observed across species and biomes, and may significantly impact global water, carbon, and energy budgets. However, it remains inclusive why plants lose water at night and how to model it at large scales.
- We hypothesized that plants optimize nighttime leaf diffusive conductance (g_{wn}) to balance potential daytime photosynthetic benefits and nocturnal transpiration benefits. We quantified nighttime benefits from respiratory reductions due to evaporative leaf cooling. We described nighttime costs in terms of a reduced carbon gain during the day because of water use at night. We measured nighttime stomatal responses and tested our model with water birch (*Betula occidentalis*) saplings grown in a greenhouse.
- The g_{wn} of water birch decreased with drier soil, higher atmospheric CO_2, wetter air, lower leaf temperature, and lower leaf respiration rate. Our model predicted all these responses correctly, except for the response of g_{wn} to air humidity. Our results also suggested that the slow decrease in g_{wn} after sunset could be associated with decreasing leaf respiration.
- The optimality-based nocturnal transpiration model smoothly integrates with daytime stomatal optimization approaches, and thus has the potential to quantitatively predict nocturnal transpiration across space and time.

Introduction

Nocturnal transpiration in land plants has been observed globally across all functional types, biomes, and climates (Resco de Dios *et al.,* 2019; Yu *et al.,* 2019), and typically ranges from 5% to 30% of the daytime transpiration (Caird *et al.,* 2007). However, nocturnal transpiration is often considered to be at odds with existing optimal stomatal behavior theories, which predict no stomatal opening at night due to the inability to photosynthesize (Cirelli *et al.,* 2016; Yu *et al.,* 2019). Despite numerous studies in which observations of nocturnal transpiration have been presented (Fisher *et al.,* 2007; Novick *et al.,* 2009; Zeppel *et al.,* 2011; Resco de Dios *et al.,* 2015), no theory satisfactorily explains why stomata open at night or why they respond to the environment. As a result, nocturnal transpiration is often omitted in gas exchange simulations (e.g. Mackay *et al.,* 2015; Venturas *et al.,* 2018; Love *et al.,* 2019) and, when present at all, is not mechanistically represented in land surface models (e.g. it is simply treated as a constant empirical minimal stomatal conductance term; Barnard & Bauerle, 2013; Lombardozzi *et al.,* 2017).

If land surface models do not mechanistically and dynamically simulate nocturnal transpiration, this may lead to biases in predictions of energy and water fluxes during both day and night. Because nocturnal transpiration leads to additional total water use over the course of a whole day and thus to more rapid soil water depletion, the subsequent photosynthetic carbon gain becomes more limited. Faster soil drying also threatens plants with increased water stress, further impacting the simulation of carbon and water fluxes. Therefore, inaccurately accounting for nocturnal transpiration has the potential to introduce substantial errors in simulated water, carbon, and energy fluxes, the extent of which has not been fully quantified.

Plant nighttime transpiration rates (E_n) and nighttime leaf diffusive conductances ($g_{wn} = E_n/D$, where D represents leaf-to-air vapor pressure deficit) do not stay constant throughout the night due to the varying environmental conditions and short-term changes in plant physiology during the night. Typically, g_{wn} decreases gradually after sunset, reaches a minimal value at midnight, and then increases before sunrise (Ogle *et al.,* 2012; Resco de Dios *et al.,* 2016). While predawn stomatal opening may be explained as a result of circadian rhythm for increasing early morning photosynthesis, little is known about why stomata close slowly after sunset. Thus, there must be an unknown benefit for the slow stomatal closure after sunset. Further, g_{wn} typically decreases when soil gets drier (Cavender-Bares *et al.,* 2007; Cirelli *et al.,* 2016), is higher for plants grown at elevated CO_2 (Zeppel *et al.,* 2012), and increases with higher leaf respiration rate (Marks & Lechowicz, 2007; Coupel-Ledru *et al.,* 2016).

However, g_{wn} is reported to stay unchanged or decrease with drier air in some studies (Cirelli *et al.,* 2016) but increase in others (Barbour *et al.,* 2005; Dawson *et al.,* 2007; Zeppel *et al.,* 2012; Yu *et al.,* 2019). Identifying the physiological and ecological drivers for all these observed nighttime stomatal behaviors requires further investigation.

Observations that nocturnal transpiration varies with environment and time suggest the following: first, plants are actively controlling stomatal aperture at night; and second, plants are balancing the upsides for using water at night ('benefits' of nocturnal transpiration) and the downsides of not being able to use the water in the day ('costs' of nocturnal transpiration). The upsides and downsides of nocturnal transpiration fit well into a trade-off framework. However, similar to daytime stomatal optimization theories (Mencuccini *et al.,* 2019; Wang *et al.,* 2020), the difficulty is how to quantify and weigh the benefits and costs in various forms and measures using the same units.

A number of theories and hypotheses centered on the causes and consequences of nocturnal transpiration have been proposed to explain why plants lose water at night (Zeppel *et al.,* 2014). Common hypotheses include evaporative cooling, leaky stomata, nutrient uptake, oxygen delivery, CO_2 flush-out, suppression of hydraulic redistribution driven by competition, embolism removal, capacitance refilling, genetic control, and circadian rhythm for early morning stomatal opening (Table 1). These physiological and ecological causes and consequences may b plausible reasons for stomatal opening at night, and some do partially explain the observed nighttime stomatal behaviors (Table 1). However, there is not yet a theory that is able to explain all the observed patterns. In particular, the contrasting nighttime stomatal responses to air humidity cannot be explained by any of the proposed upsides of nocturnal transpiration.

The present study asks whether optimality theory that plants balance the benefits and costs of nocturnal transpiration can explain the observed nocturnal transpiration patterns. To answer the question, we proposed a new optimization model following the trade-off framework to explain why plants regulate stomata at night. We then tested the model predictions with water birch (*Betula occidentalis*) saplings grown in a glasshou

Materials and Methods

The theory

Water use at night cou l res lt in negative consequences for plants as the water used d rin e nighttime is unavailable for daytime photo ynt esis. A s oil water content declines, soil water potential b ecom more negative, and thus the plant hydraulic system is n e stress d a d photosynthetic gain decreases. Therefore, nocturna transpiration inherently results in a carbon cost separa d time costs occur in the subsequent daytime whereas bene ts occu in the nighttime). The benefits associated with nightt e w er loss may include nutrient uptake, competition, vaporative cooling (a detailed summary in Table 1). Among these physiological and ecological consequences of nocturnal tra piration, we posit that leaf cooling is a particularly

Table 1 A summary of the causes and consequences of noctu al t ns iration.

	Causes and consequences
Theory	*Leaky stomata*. Fully closing the stomata ould be energetically expensive, so plants pay the price only when soil is dry.
References	Barbour *et al*. (2005), Cavender- s *et a* 07), Dawson *et al*. (2007), Cirelli *et al*. (2016)
Theory	*Nutrient uptake*. Transpiration tream elps d ver nutrients to the leaves.
References	Caird *et al*. (2007), Dawson e l. (2007 Zeppe *et al*. (2012)
Theory	*Oxygen delivery*. Oxyg dissol d in e transpiration stream helps provide oxygen to the living cells.
References	Dawson *et al*. (2007) Zeppe t *al*. 012)
Theory	*Competition*. A plan ought to achieve igher fitness when suppressing hydraulic redistribution, thereby keeping water in its own root-zone and incr sing bsequent c bon uptake, along with potentially having its competitors perform relatively worse.
References	Caird e al. (2007), eppe *et al*. (2012), Huang *et al*. (2017)
Theory	*Refillin capacitanc* Plants can store water for daily use, especially in an arid environment. Nighttime flow might help this capacitance refilling. Ho ever, it sh ld be noted that nocturnal transpiration differs from water flow out of the soil, as the latter consists of both nocturnal transpirat n a capacitance refilling. As nocturnal transpiration makes fluid pressure more negative, the pressure gradient to refill the capacitance will be lower. Thus, in theory, refilling progress will be inhibited rather than promoted by nocturnal transpiration.
References	Zeppel *et al*. (2012), Huang *et al*. (2017)
Theory	*Embolism removal*. Cavitated xylem conduits may be refilled at night, and nocturnal transpiration might promote embolism removal. Similar to the capacitance refilling, nocturnal water uptake may help with embolism, but nocturnal transpiration is unlikely to promote embolism removal. The more negative fluid pressure in the xylem will slow down the refilling, if it occurs, rather than speed it up.
References	Zeppel *et al*. (2012)
Theory	*Circadian rhythm*. Stomatal opening before sunrise facilitates photosynthesis earlier in the day.
References	Caird *et al*. (2007), Dawson *et al*. (2007), Resco de Dios *et al*. (2016, 2019)
Theory	*Preventing CO_2 build-up in leaf*. CO_2 build-up in the leaf might be toxic for leaf metabolism. Thus, nocturnal transpiration benefits the plant by removing the accumulated CO_2.
References	Marks & Lechowicz (2007)
Theory	*Leaf cooling*. The transpiration rate (E_n) ought to be sufficient to lower leaf temperature and hence leaf respiration.
References	Coupel-Ledru *et al*. (2016)
Theory	*Marginal respiratory cost relative to marginal carbon gain*. Plants could save more photosynthate by nocturnal-transpiration-induced leaf cooling. The higher this saving compared to daytime photosynthesis, the more stomata open (this study).

promising and readily quantifiable benefit that matches the unit of daytime photosynthetic gain, but it has often been neglected in previous nocturnal transpiration studies.

If leaf cooling is considered a main benefit (B) of nighttime stomatal opening, when B exceeds the potential carbon cost of the nocturnal transpiration (Θ), the plant will use water at night. For instance, when the marginal reduction in respiration resulting from leaf cooling ($\partial B/\partial E_n = -\partial R_{leaf}/\partial E_n$, where R_{leaf} is the respiration rate at night; marginal gain of nocturnal transpiration) exceeds the marginal cost of nocturnal transpiration ($\partial\Theta/\partial E_n$), plants will open stomata more at night until the marginal nighttime cost exceeds the marginal nighttime gain. Otherwise, plants will close their stomata more to avoid overusing the soil water at night. Note that our hypothesis differs from a previous theory that highlighted the benefit of leaf cooling (e.g. Coupel-Ledru *et al.*, 2016) as our model accounts for the cost as well as the benefit.

We hypothesized that plants optimize nocturnal transpiration to balance associated benefit and cost. As the nocturnal transpiration cost is quantified using the daytime carbon gain, it ought to resemble the format of the cost function of daytime transpiration (e.g. a carbon risk in plant hydraulic integrity). Therefore, we posited that nighttime transpiration rate is optimal when

$$\frac{\partial B}{\partial E_n} - \frac{\partial \Theta}{\partial E_n} = -\frac{\partial R_{leaf}}{\partial E_n} - f_f \cdot \frac{A_d(E_n)}{E_{crit} - E_n} = 0 \qquad \text{Eqn 1}$$

where $A_d(E_n)$ is the potential daytime leaf photosynthetic rate at a given transpiration rate E_n (assuming the same transpiration rate during the day and at night), f_f is a fitness factor, and E_{crit} the maximal leaf transpiration rate, beyond which the leaf desiccates because of hydraulic failure. The formulation $\partial\Theta/\partial E_n = f_f \cdot A_d/(E_{crit} - E_n)$ is modified from the cost function proposed by Wang *et al.* (2020) (see Fig. 1 for a detailed example of nocturnal transpiration optimization).

The fitness factor f_f describes the importance of daytime carbon cost relative to nighttime benefits, as leaf cooling may only be one of several potential benefits of nocturnal transpiration. For example, for a plant with sufficient water supply (e.g. with a fixed water table in the root zone), the plant may benefit more from other upsides of nocturnal transpiration (such as nutrient uptake and competition), and f_f ought to be lower. Therefore, f_f ought to be variable depending on the environment. We use $f_f < 1$ to account for other benefits of nocturnal transpiration (f_f decreases when the other benefits increase).

Note that $\partial R_{leaf}/\partial E_n$ can be computed analytically (see Supporting Information Notes S1 for the steps of the derivation) as follows:

$$\frac{\partial R_{leaf}}{\partial E_n} = \frac{-\lambda}{2c_p g_{be} + 4f_{view} T_{leaf}^3} \cdot R_{leaf} \frac{\Delta H}{R T_{leaf}^2} \qquad \text{Eqn 2}$$

where λ is the latent heat of vaporization, c_p is the specific heat of dry air at a constant pressure, g_{be} is the boundary layer conductance for sensible energy flux, f_{view} is the mean view factor of the leaves from the air (the proportion of radiated energy that escapes from the canopy; we assume $f_{view} = 1/LAI$, where LAI is the leaf area index), ε is the leaf emissivity (Campbell & Norman, 1998), σ is the Stefan–Boltzmann constant, T_{leaf} is leaf temperature in K, ΔH is the activation energy for the temperature dependence of R_{leaf} (Bernacchi *et al.*, 2001), and R is the ideal gas constant.

Model prediction

Our model predicts that if nighttime water use will result in a higher carbon cost for a given plant (i.e. if duting the daytime on the subsequent day the value of $A_d/(E_{crit}-E_n)$ is higher, e.g. when soil is drier), it tends to use less water at night. On the other hand, if the plant can save more carbon through reduced respiration at night (higher $-\partial R_{leaf}/\partial E_n$, e.g. when R_{leaf} is higher), it tends to use more water at night. Further, if f_f decreases (e.g. as a result of

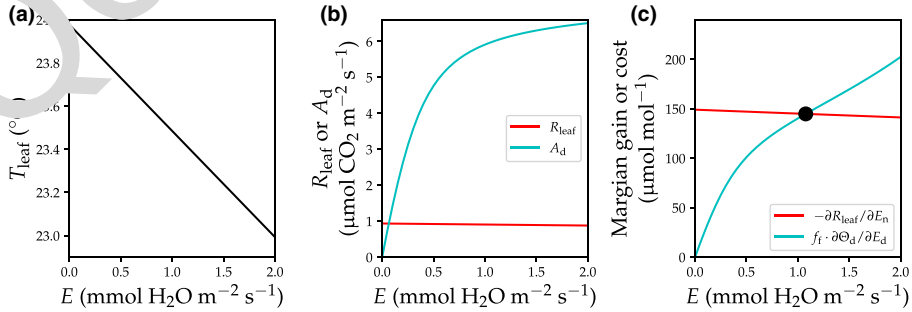

Fig. 1 Optimal nocturnal transpiration model framework. (a) When nighttime transpiration rate (E) increases, leaf temperature (T_{leaf}) decreases due to the increasing latent heat flux out of the leaf. (b) Decreasing T_{leaf} results in decreasing leaf respiration rate (R_{leaf}, red line), which is of carbon benefit to the plant. At the same T_{leaf}, if the nighttime transpiration rate is used in the day, it benefits the plants with a higher photosynthetic rate in the daytime (A_d, cyan curve). The faster soil water depletion could also result in drier soil, leading to a higher risk of failure of the plant's hydraulic system. Nocturnal transpiration therefore results in a carbon cost separated in time. (c) We use marginal respiratory reduction caused by evaporative cooling to describe the marginal carbon benefit of nocturnal transpiration ($-\partial R_{leaf}/\partial E_n$, red line). We used $f_f \cdot A_d/(E_{crit} - E_n)$ to describe the marginal carbon cost of nocturnal transpiration (see our description of Eqn 1 for more details about the formulation). When the marginal carbon gain and cost curves intersect, nighttime transpiration rate is optimized (black circle).

increased benefit from other upsides), the plant will use more water at night.

The following paragraphs analyze model predictions for nighttime stomatal responses to the environment, leaf respiration, and fitness factor. We evaluated the model by predicting the nighttime stomatal responses of a leaf or an individual tree to a new environment (e.g. drier soil, elevated atmospheric CO_2 C_a, drier air, and higher temperature). Note that the predicted responses apply to a scenario in which trees have not acclimated their traits to the new environment.

Response to soil drought When the soil gets drier while other environmental conditions stay unchanged, at the same transpiration rate, the plant's risk of hydraulic failure increases ($A_d/(E_{crit}-E_n)$ increases because E_{crit} decreases, solid cyan line to dashed cyan line in Fig. 2a). The nighttime benefit as a function of E_n, however, is not impacted by changes in soil moisture (solid red curve in Fig. 2a). The optimal E_n (intersection of $\partial\Theta/\partial E_n$ and $-\partial R_{leaf}/\partial E_n$) and g_{wn} should decrease with drier soil (from gray dot to black dot in Fig. 2a).

Response to C_a When atmospheric CO_2 increases while other environmental conditions stay unchanged, at the same transpiration rate, plant hydraulic risk stays unchanged whereas photosynthetic gain increases and $A_d/(E_{crit}-E_n)$ increases due to higher potential daytime carbon gain (solid cyan line to dashed cyan line in Fig. 2b). $-\partial R_{leaf}/\partial E_n$ as a function of E_n is not impacted by C_a (solid red curve in Fig. 2). Thus, the optimal E_n and g_{wn} ought to decrease at a higher C_a (from gray dot to black dot in Fig. 2b).

Response to atmospheric vapor pressure deficit (VPD) When only VPD increases (i.e. the air gets drier, daytime and nighttime) at the same transpiration rate, the risk of hydraulic system failure stays unchanged but photosynthetic rate increases; $A_d/(E_{crit}-E_n)$ ought therefore to decrease with drier air (solid cyan line to dashed cyan line in Fig. 2c). VPD, however, does not impact the leaf cooling for a given E_n (solid red curve in Fig. 2c). Thus, the optimal E_n increases with higher VPD. However, g_{wn} may increase, stay constant or decrease with higher VPD, and the trend depends on whether D or leaf-to-air vapor pressure deficit D increases more (E_n increases with higher VPD when E_n increases more).

Response to R_{leaf} Holding environmental conditions constant, when R_{leaf} increases, $A_d/(E_{crit}-E_n)$ slightly decreases because of

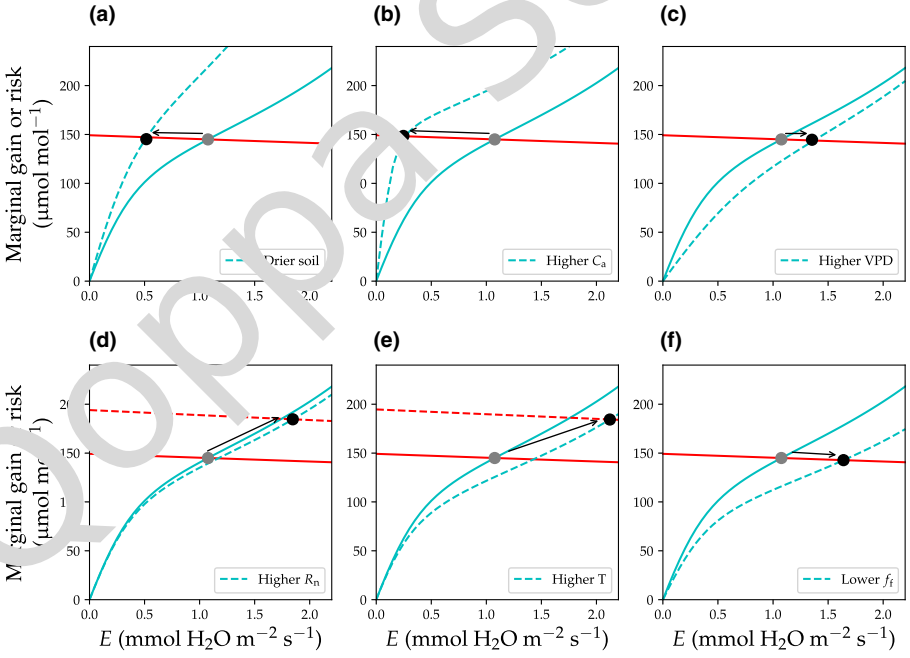

Fig. 2 Mechanism of nighttime stomatal response to the environment and dark respiration (R_{leaf}). The *x*-axis plots the nighttime transpiration rate (E_n). The *y*-axis plots the marginal benefit at night ($-\partial R_{leaf}/\partial E_n$, red lines) and the marginal cost as calculated in the daytime ($f_f \cdot A_d/(E_{crit}-E_n)$, cyan lines, f_f is the fitness factor multiplier). The optimal solution is represented by the intersection of the marginal benefit and marginal cost lines. (a) When soil gets drier, $A_d/(E_{crit}-E_n)$ increases (from solid cyan line to dashed cyan line) whereas $-\partial R_{leaf}/\partial E_n$ line stays unchanged. The optimal E_n and nighttime leaf diffusive conductance (g_{wn}) decrease with drier soil (shift from the gray dot to black dot). (b) When atmospheric CO_2 (C_a) increases, $A_d/(E_{crit}-E_n)$ increases (from solid cyan line to dashed cyan line) whereas the $-\partial R_{leaf}/\partial E_n$ line stays unchanged. The optimal E_n and g_{wn} decrease with higher C_a. (c) When the air gets drier, $A_d/(E_{crit}-E_n)$ decreases (from solid cyan line to dashed cyan line) whereas the $-\partial R_{leaf}/\partial E_n$ line stays unchanged. The optimal E_n increases with drier air, but the g_{wn} may increase or decrease to provide the optimal E_n. (d) When R_{leaf} increases, $A_d/(E_{crit}-E_n)$ decreased slightly in the tested case, whereas $-\partial R_{leaf}/\partial E_n$ increases (from solid red curve to dashed red line). The optimal E_n and g_{wn} increase with higher R_{leaf}. (e) When temperature increases, $A_d/(E_{crit}-E_n)$ decreases (from solid cyan line to dashed cyan line) whereas $-\partial R_{leaf}/\partial E_n$ increases (from solid red curve to dashed red line). The optimal E_n increases with higher temperature, but g_{wn} may increase or decrease. (f) When the fitness factor decreases, both $A_d/(E_{crit}-E_n)$ and $\partial R_{leaf}/\partial E_n$ stay unchanged, but $\partial\Theta/\partial E_n$ decreases. The optimal E_n and g_{wn} increase with lower f_f.

the subtraction of respiration rate in the calculation of the net photosynthetic rate (solid cyan line to dashed cyan line), whereas $-\partial R_{leaf}/\partial E_n$ increases proportionally (solid red curve to dashed red line in Fig. 2d). As a result, stomata should open more at night due to the higher R_{leaf}.

Response to temperature A warmer temperature (both daytime and nighttime) results in both a higher VPD in the air (drier air) and hence lower $A_d/(E_{crit}-E_n)$ (solid cyan line to dashed cyan line in Fig. 2e), and an exponentially increasing $-\partial R_{leaf}/\partial E_n$ with higher temperature (solid red curve to dashed red line in Fig. 2e). Thus, the optimal E_n ought to increase at a higher temperature. However, how g_{wn} responds to higher temperature depends on whether E_n or leaf-to-air vapor pressure deficit D increases more.

Response to fitness factor Holding environmental conditions and respiration rate constant, when f_f decreases, $\partial R_{leaf}/\partial E_n$ and $A_d/(E_{crit}-E_n)$ are not impacted by f_f. However, $\partial\Theta/\partial E_n$ decreased proportionally with declining f_f (solid cyan line to dashed cyan line, Fig. 2f). As a result, stomata should open more at night with lower f_f.

In sum, our model predicts that E_n and g_{wn} decrease with drier soil, higher C_a, lower R_{leaf}, and higher f_f. Our model also predicts that E_n increases with drier air and higher temperature, but g_{wn} may either increase or decrease with drier air and higher temperature (assuming constant f_f).

Glasshouse measurements

In this study, we used 2-yr-old water birch (*Betula occidentalis* Hook.) seedlings grown in the glasshouse at the School of Biological Sciences, University of Utah (Salt Lake City, USA). Each sapling was grown in a 5-gallon (*c.* 22.7 l) pot with local sandy clay loam soil starting in October 2016 (Wang *et al.*, 2019). Trees were well watered every day at the end of the day except during drought treatment. From November 2016 to April 2017, trees were under a supplemental light (Luminox LU1000; GE Lighting, East Cleveland, Ohio, USA) from 08:00 h to 18:00 h. Air temperature was *c.* 25°C and relative humidity was *c.* 50% during this period. The plants were then exposed to natural temperature, light, and air humidity variations from May 2017. From July to September 2017, trees (*c.* 1.5 m tall) were used to test how nocturnal transpiration responds to the environment and leaf respiration.

Responses to the environment

Twelve water birch saplings were used for this experiment. Six saplings were used to study how stomata respond to soil drought (subjected to drought treatment), and six saplings were used to test the C_a, VPD, and temperature responses (well watered every day). To ensure stable nighttime gas exchange measurements, after sunset, trees to be measured were moved from the glasshouse to the laboratory, where room temperature was controlled at *c.* 25°C. Leaf gas exchange measurements were conducted between 23:00 h and 04:00 h. After finishing the measurements, the trees were moved back to the glasshouse.

Drought response Six water birch trees were used to test how stomata respond to soil drought at night using a water stress treatment, where the trees were left un-watered for four consecutive days. From 23:00 h to 12:00 h during the night before drought treatment, nighttime gas exchange rates were measured for three mature leaves from each tree with a portable photosynthesis system (Li-6800; Li-Cor Inc., Lincoln, NE, USA). The Li-6800 chamber was set to maintain the leaf temperature at 25°C and the chamber relative humidity at 50%. The trees were bagged for at least 90 min to minimize the impact of nighttime transpiration rate on the estimation of soil water potential from leaf xylem pressure. For each tree, we chose a mature leaf close to the three leaves used for gas exchange measurements, measured its leaf xylem pressure with a pressure chamber (PMS Instruments, Corvallis, OR, USA; precision ± 0.05 MPa), and used this as a proxy for soil water potential (P_{soil}). After measuring P_{soil}, the bags were removed, and drought stress was initiated for the six trees. We note that there were > 200 leaves per sapling, and thus the leaf removal (≤ 4) had minimal impact on the physiology of the remaining leaves. Nighttime gas exchange rates (measured on the same three leaves) and P_{soil} were measured for drought-stressed trees for four consecutive nights during the drought treatment.

C_a response Six well-watered trees (a different set of trees from the drought-stressed ones) were used to test how stomata respond to CO_2 at night. Only one mature leaf from each tree was used for the CO_2 response curve. At the beginning of a CO_2 response curve, the Li-6800, chamber was set to a C_a of 0 ppm and relative humidity of 50%. Leaf temperature was maintained at 25°C for the whole the CO_2 response curve. Then the chamber C_a was set to 200, 400, 600 and 800 ppm in steps, while the chamber relative humidity was maintained at 50%. At each step (from 0 to 800 ppm), gas exchange rates were recorded after E_n reached an equilibrium (stabilization typically takes > 1 h when we change CO_2 concentration). A total of six CO_2 response curves were constructed from six trees. For three out of the six CO_2 response curves, an additional 100 ppm step was added between the 0 ppm and 200 ppm steps. The g_{wn} at equilibrium was used as the nighttime leaf diffusive conductance at each C_a setting for each tree. The CO_2 response curves of g_{wd} were also constructed for the same six trees (leaf temperature controlled at 25°C, chamber relative humidity at 50%, and photosynthesis-active radiation at 1000 μmol m^{-2} s^{-1}) to compare with g_{wn}.

VPD response One mature leaf from each well-watered tree was used for the VPD response curve (the same trees used for the CO_2 response curves, but different leaves). For each VPD response curve, the Li-6800 chamber VPD ranged from low to high (0.5–3.0 kPa) while maintaining leaf temperature at 25°C throughout the measurements (see Fig. S1 for an example of the stable leaf temperature). At each VPD setting, leaf-level gas exchange was monitored until an equilibrium was reached, and

g_{wn} at equilibrium was used as the nighttime leaf diffusive conductance at that VPD setting.

Temperature response One mature leaf from each of the six well-watered trees was used to test the nighttime stomatal response to leaf temperature (the same trees used for the CO_2 and VPD response curves, but different leaves). For each leaf in the Li-6800 chamber, leaf temperature ranged from low to high (19–35°C), and the leaf-to-air VPD was maintained at 2.2 kPa throughout the measurements. At each leaf temperature setting, leaf gas exchange was monitored until an equilibrium was reached, and the g_{wn} at equilibrium was used for that temperature setting. As the diffusive coefficient of water vapor increases with higher air temperature, g_{wn} increases with higher temperature if stomatal pore aperture stays unchanged. Thus, we further normalized g_{wn} to a reference temperature of 25°C ($g_{wn,25}$) to examine whether stomatal pore aperture changed during the temperature response curve. The temperature correction was made using $g_{wn} = g_{wn,25} \cdot \left(\frac{T_{leaf}}{298.15}\right)^{1.8}$, where T_{leaf} is the leaf temperature in K (Nobel, 2009).

Response to leaf respiration

Variation among leaves Leaf gas exchange rates were measured on different leaves to test how g_{wn} varies with leaf respiration. For six consecutive nights, each of the six well-watered trees (the same trees used for the CO_2 response curves) was moved to the laboratory. From 23:00 h to 01:00 h, R_{leaf} and g_{wn} were measured for all the mature and healthy leaves of each tree using the portable photosynthesis Li-6800 system. The Li-6800 chamber was set to maintain its relative humidity at 50% and air temperature at 25°C. The R_{leaf} and g_{wn} were recorded when the leaf gas exchange was stable (usually within 2 min).

Variation with time Leaf gas exchange was monitored on one single leaf per tree continuously to test how nighttime leaf gas exchange changes after sunset. For six consecutive days, each of the six well-watered trees was moved to the laboratory early in the morning. In the lab, supplemental light (Lucalox LU1000; GE Lighting, East Cleveland, Ohio, USA) was applied to the tree, and the photosynthetically active radiation was > 1200 μmol m^{-2} s^{-1} on the sunlit leaves. At the time of sunset, the supplemental light was turned off, and a sunlit leaf was attached to the Li-6800. The Li-6800 chamber was set to maintain a relative humidity of 50% and an air temperature of 25°C. Leaf gas exchange was monitored continuously for 4–5 h to test whether R_{leaf} and g_{wn} covary after sunset.

Model simulation

We compared our model predictions quantitatively with experimental observations. To run the model, we used the hydraulic and photosynthetic traits measured on plants of the same cohort of trees used in this study (data from Wang *et al.*, 2019). The traits we used are as follows: a mean leaf area index of 4.76 (f_{view} = 0.21), leaf width of 0.1 m, root Weibull B = 1.879 MPa, root Weibull C = 2.396, root maximal hydraulic conductance of 34.383 mol H_2O m^{-2} s^{-1} (per basal area), stem Weibull B = 2.238 MPa, stem Weibull C = 9.380, stem maximal hydraulic conductance of 76.029 mol H_2O m^{-2} s^{-1} (per basal area), leaf Weibull B = 1.897 MPa, leaf Weibull C = 2.203, leaf maximal hydraulic conductance of 0.0176 mol H_2O m^{-2} s^{-1} (per leaf area), leaf maximal carboxylation rate at 25°C of 61.74 μmol CO_2 m^{-2} s^{-1}, leaf maximal electron transport rate at 25°C of 111.13 μmol CO_2 m^{-2} s^{-1}, and leaf dark respiration rate at 25°C of 1.5 μmol CO_2 m^{-2} s^{-1}. We used the temperature dependency parameter of respiration rate from Bernacchi *et al.* (2001) (ΔH_a = 46 390 J mol^{-1}). Code for the simulations is publicly available for download at https://github.com/Yujie-WANG/Published-Codes-Yujie-WANG.

Briefly, for any combination of environmental conditions (soil moisture, VPD, and C_a) and leaf respiration rate, we solved the unique E_n, where $-\partial R_{leaf}/\partial E_n - f_f \cdot A_d/(E_{crit} - E_n) = 0$ (Fig. 1) using the following steps. (1) For a given nighttime transpiration rate E we calculated leaf temperature using known air temperature and a prescribed wind speed of 0.1 m s^{-1}. (2) We numerically computed E_{crit}, at which leaf hydraulic conductance reached 0.5% of the maximum. (3) We used the same transpiration rate E and leaf temperature for the daytime photosynthesis. With the known leaf temperature and transpiration rate, we were able to calculate leaf stomatal conductance assuming a boundary layer conductance for water vapor of 3 mol m^{-2} s^{-1}, and thus photosynthetic rate using the classic photosynthesis model (Farquhar *et al.*, 1980). (4) We computed the $\partial\Theta/\partial E_n$ using an assumed f_f. (5) With the calculated leaf temperature, we computed $-\partial R_{leaf}/\partial E_n$ analytically using Eqn 2. (6) By tuning E (repeating steps 1–5), we were able to find the optimal E_n and g_{wn}. We note that daytime leaf temperature is typically higher than nighttime leaf temperature (and thus VPD is higher), so in practice it is better to use mean daytime leaf conditions (including temperature and solar radiation) to calculate the daytime photosynthesis in the cost function. However, to reduce the uncertainty in the model, we used nighttime leaf temperature and VPD to calculate photosynthetic rate.

We varied soil water potential from 0 to −1.5 MPa in 0.1 MPa incremental steps, while holding atmospheric VPD constant at 1.67 kPa and atmospheric CO_2 constant at 400 ppm. At each soil water potential, we calculated optimal g_{wn} using a constant prescribed f_f = 0.15 and air temperature of 25°C, and plotted the predicted curve against experimental observations. We varied C_a from 50 to 800 ppm in 50 ppm steps, while holding soil water content at saturation point (i.e. soil water potential of 0) and atmospheric VPD constant at 1.67 kPa. At each C_a, we computed optimal g_{wn} using a constant f_f = 0.15 and air temperature of 25°C. We varied VPD from 0.5 to 3.0 kPa in 0.05 kPa steps, while holding soil water content at saturation point and C_a constant at 400 ppm. At each VPD, we calculated optimal g_{wn} using a constant f_f = 0.15 and air temperature of 25°C. Air temperature ranged between 19 and 35°C in 1°C steps, holding soil water content at saturation point, VPD = 1.67 kPa, and C_a = 400 ppm. At each air temperature, we calculated optimal

g_{wn} using a constant $f_f = 0.15$. We varied leaf dark respiration (normalized to 25°C) from 0.1 to 2.0 μmol CO_2 m^{-2} s^{-1} in 0.1 μmol CO_2 m^{-2} s^{-1} steps, while holding soil water saturated, VPD = 1.67 kPa, and C_a = 400 ppm. For each leaf respiration rate setup, we calculated optimal g_{wn} using a constant $f_f = 0.15$ and air temperature of 25°C.

Fitness factor The f_f may be variable as the importance of respiratory reduction may change with the environmental conditions. To test how a variable f_f improves the model predicted g_{wn}, we implemented an arbitrarily decreasing f_f with increasing VPD: $f_f = 0.16 - 0.03 \cdot \text{VPD}$ along the VPD steps. We also tested an arbitrarily increasing f_f with increasing temperature (T): $f_f = 0.021 \cdot \exp(0.075 \cdot T)$. As f_f represents the other upsides of nocturnal transpiration that are hard to quantify, the estimation of f_f has to rely on curve fitting existing data. Yet f_f is not solely an empirical fitting parameter because of its ecological significance, and the f_f responses to the environment may indicate how a plant weighs the benefits of nocturnal transpiration when environmental conditions change.

Results

Responses to the environment

Nighttime transpiration (i.e. E_n) and nighttime leaf diffusive conductance (i.e. g_{wn}) decreased with drier soil (Fig. 3a) and higher CO_2 (Fig. 3b), and increased with higher VPD (Fig. 3c). The g_{wn} ranged from 0.014 to 0.08 mol m^{-2} s^{-1} for well-watered water birch trees and decreased to between 0.002 and 0.02 mol m^{-2} s^{-1} when the soil dried down (Fig. 3a, each color represented an individual tree). At very low leaf level C_a (< 0 ppm), g_{wn} ranged from 0.09 to 0.37 mol m^{-2} s^{-1} and averaged 0.20 mol m^{-2} s^{-1}, showing no significant difference from the mean g_{wd} value of 0.15 mol m^{-2} s^{-1} in the daytime (leaf-level C_a = 0 ppm; t-test, $n = 6$, $P = 0.5$). The maximal stomatal opening at night for C_a = 0 ppm agreed with our model prediction, as the plants should reduce nighttime respiration rate as much as possible if there is no photosynthetic gain in the day. For all six leaves from the six well-watered trees, g_{wn} increased with atmospheric VPD (Fig. 3c); this is the opposite of what is typically observed in the daytime (i.e. decreasing g_{wd} with higher VPD).

The g_{wn} increased when leaf temperature increased from 19 to 35°C while holding leaf-to-air D constant (Fig. 4a). The increase in g_{wn} was a result of the increased diffusive coefficient of H_2O at higher temperatures and the more open stomatal aperture (as $g_{wn,25}$ also increased, Fig. 4b). All the observed nighttime stomatal behaviors were qualitatively predicted by the model using a constant fitness multiplier, except for VPD response (Figs 2–4). In particular, our model quantitatively predicted the trends for soil moisture and C_a responses. The disagreement in modeled and observed g_{wn} responses to VPD and temperature changes may be a result of the variable fitness multiplier f_f.

Response to leaf respiration

The g_{wn} increased with higher nighttime leaf respiration rate for every well-watered tree (Fig. 5). The R_{leaf} and its correlation with g_{wn} differed among trees (Fig. 5). However, g_{wn} always showed a positive correlation with R_{leaf} ($P < 0.05$, solid regression lines in Fig. 5). Using a constant $f_f = 0.15$, our model was able to quantitatively track the nighttime stomatal responses to higher respiration rates (gray curve in Fig. 5).

After 'sunset' (when the light was turned off in the laboratory), g_{wn} and R_{leaf} covaried for all six monitored leaves (Fig. 6a). Taking one leaf as an example (red circles in Fig. 6a), R_{leaf} declined after 'sunset' (red circles in Fig. 6b), and g_{wn} decreased accordingly (gray line in Fig. 6b). This covariation suggested that the slow decline of g_{wn} after sunset was likely a result of slowly decreasing R_{leaf}.

We emphasized here that the leaf temperature varied by < 1.3°C for the leaves whose data is shown in Fig. 5 and < 0.8°C for those depicted in Fig. 6. The 1.3°C and 0.8°C temperature variations resulted in respiration rate changes for an individual leaf at 25°C of *c.* 8.2% and 5.0%, respectively (See Notes S1 for the computation of $\partial R_{leaf} / \partial T_{leaf}$). Thus, the variation in respiration rate among leaves depicted in Figs 5 and 6 was not the result of leaf cooling, but was likely the driving force behind nocturnal transpiration.

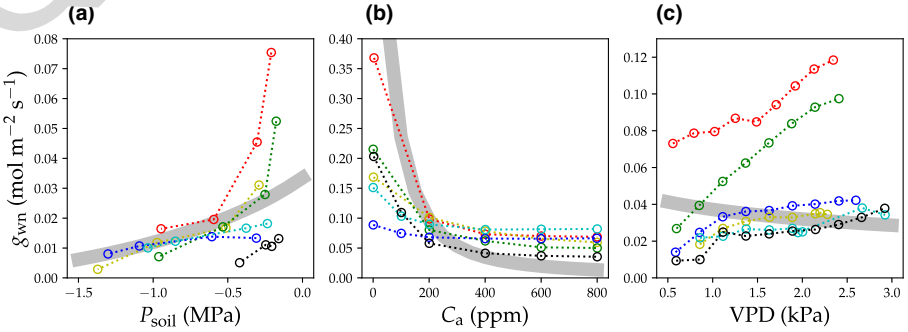

Fig. 3 Nighttime leaf diffusive conductance (g_{wn}) responses to the environmental cues. Each color represents a mature leaf from a tree. (a) The g_{wn} response to soil water potential (P_{soil}) in six drought-stressed trees. The light gray curve plots our model predicted g_{wn} using a constant fitness multiplier. (b) The g_{wn} response to atmospheric CO_2 (C_a) in six well-watered trees. (c) The g_{wn} response to atmospheric vapor pressure deficit (VPD) for the same six well-watered trees used to measure the CO_2 response.

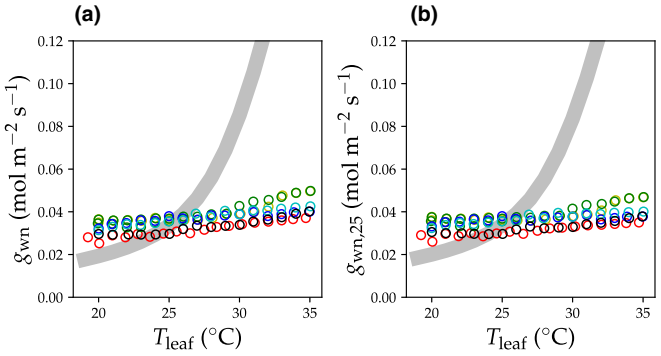

Fig. 4 Nighttime leaf diffusive conductance (g_{wn}) response to changes in leaf temperature (T_{leaf}). Each color represents data from a mature leaf from a well-watered tree. (a) The g_{wn} is not corrected by temperature. The light gray curve plots our model predicted g_{wn} using a constant fitness multiplier. (b) The g_{wn} is normalized to 25°C ($g_{wn,25}$).

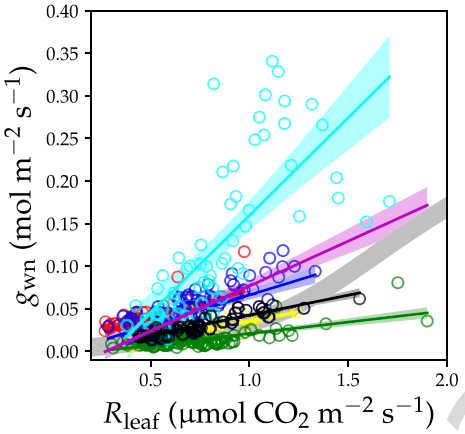

Fig. 5 Nighttime leaf diffusive conductance (g_{wn}) and leaf respiration (R_{leaf}) covary for mature leaves. Each symbol represents a leaf, and each corresponding color represents a well-watered tree. Each colored solid line plots the linear regression of $g_{wn} \sim R_{leaf}$ from each tree, and each shaded region indicates the confidence interval ($P < 0.05$ for all fittings). The light gray curve plots our model predicted g_{wn} using a constant fitness multiplier. The purple line plots the linear regression of all leaves.

Fitness factor

A constant fitness factor was able to explain quantitative nighttime stomatal responses to soil moisture, CO_2 concentration, and respiration, and qualitative responses to temperature (Figs 3–5). However, the qualitative disagreement in nighttime stomatal responses to VPD (the g_{wn} trend differed, though the E_n trend agreed) and quantitative disagreement in response to temperature suggest a varying fitness factor in these scenarios. When we adopted a linearly decreasing f_f with higher VPD and a exponentially increasing f_f with higher temperature, the model predicted g_{wn} was able to track the observations quantitatively (Fig. 7).

Discussion

We proposed a new model that explains several puzzling plant responses associated with stomatal opening at night and stomatal responses to nocturnal environment cues. The model uses leaf respiration rate to quantify the benefits of using water at night, as the transpiration-induced cooling reduces leaf respiration rate. The water lost at night, if it were used in the day, would benefit the plant with increased photosynthetic gain. However, nocturnal transpiration can be beneficial to plants if the marginal nocturnal benefit exceeds the marginal daytime benefit, and plants ought to optimize nighttime stomatal behavior to balance the benefits and costs of nocturnal transpiration.

Our new framework predicts that plants decrease their nighttime transpiration rate when soil is drier, atmospheric CO_2 is higher, atmospheric vapor pressure deficit is lower, leaf temperature is lower, and dark respiration is lower. In terms of nighttime leaf diffusive conductance, the model predicts decreased conductance under conditions of drier soil, higher CO_2, lower temperature, and lower respiration. The nighttime leaf diffusive conductance at higher VPD, however, can be higher or lower depending on the environmental conditions (see section 'g_{wn} and the environment'). Model prediction of g_{wn} responses to the environment and respiration were validated using glasshouse-based measurements of water birch physiological responses. The covariation of nighttime respiration and transpiration also suggests that the decreasing nighttime leaf diffusive conductance after sunrise may be the result of decreasing respiration during the night. Future research on how the circadian rhythm relates to dynamic regulations of respiration rate will help understand the dynamics of nighttime leaf diffusive conductance.

Built on previous theories of the upsides and downsides of nocturnal transpiration, we proposed an optimization theory in which plants regulate nighttime stomatal aperture to balance the upsides and downsides. Our model significantly advances on previous nocturnal transpiration theories in a number of ways: first, it quantifies nighttime transpiration rate by optimizing the trade-off between nighttime benefits and daytime benefits (nighttime costs); second, it predicts observed nighttime stomatal responses to the environmental variables; third, it can be merged seamlessly with existing daytime stomatal optimization models; and fourth, it allows for mechanistic incorporation of nocturnal transpiration in larger-scale vegetation models via an analysis of how the fitness factor varies with the environment.

g_{wn} and the environment

Our model predicts that nighttime leaf diffusive conductance decreases with increasing CO_2 (Fig. 2b), and it is experimentally validated with water birch sapling. However, trees grown at elevated CO_2 showed the opposite stomatal behavior – the g_{wn} was found to be higher for trees which were acclimated to elevated CO_2 (Zeppel *et al.*, 2012; Resco de Dios *et al.*, 2016). This contrasting response is probably due to the difference in physiological traits of the acclimated trees. Typically, when C_a increases, leaf area increases (Ainsworth *et al.*, 2002; Ainsworth & Long, 2005; Sperry *et al.*, 2019). Thus, average view factor decreases due to higher leaf area when plants are acclimated to elevated C_a, and $\partial R_{leaf}/\partial E_n$ increases according to Eqn 2. Another reason could be the decreasing fitness factor f_f at elevated CO_2 because the plants grow faster and nutrient demand therefore increases.

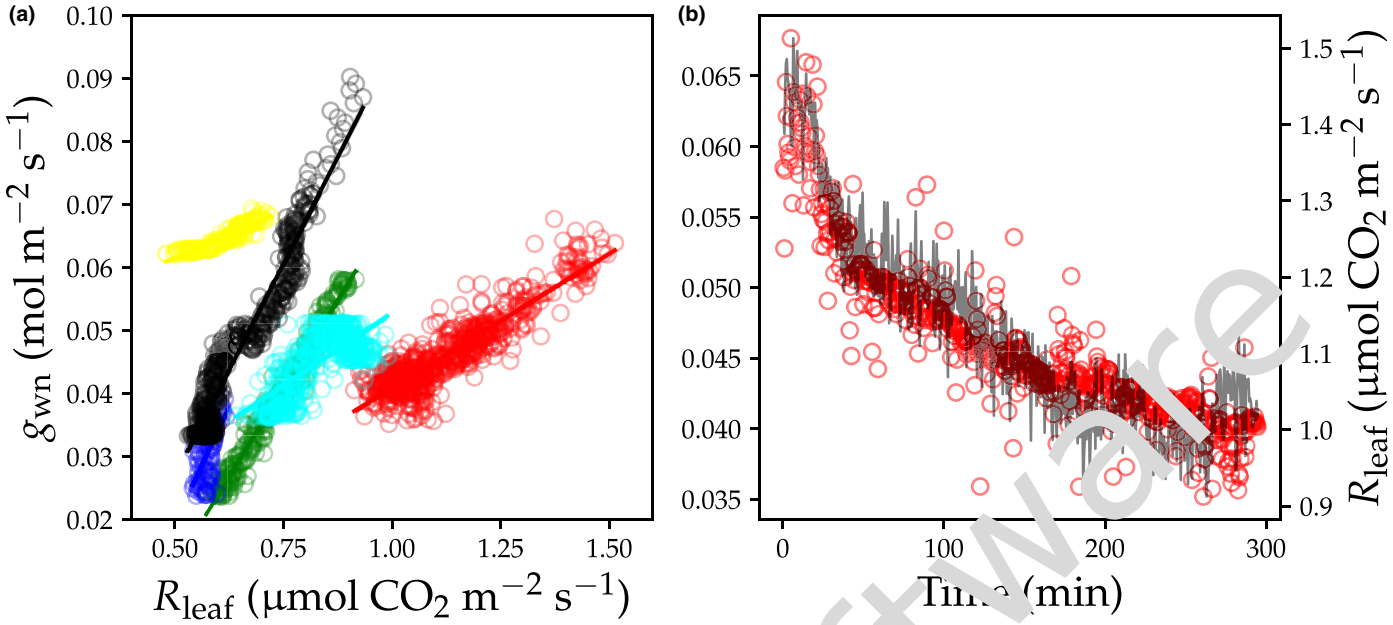

Fig. 6 Nighttime leaf diffusive conductance (g_{wn}) and leaf respiration rate (R_{leaf}) covary with time after sunset. Each color represents a well-watered tree, and each symbol plots the gas exchange of a mature leaf every 30 s. (a) Correlation between g_{wn} and R_{leaf}. The solid lines plot linear regression of $g_{wn} \sim R_{leaf}$ in a time series ($P < 0.05$). (b) An example of how g_{wn} and R_{leaf} change with time (same data as the red circles in (a)). The red circles plot g_{wn}, and the gray curve plots R_{leaf}.

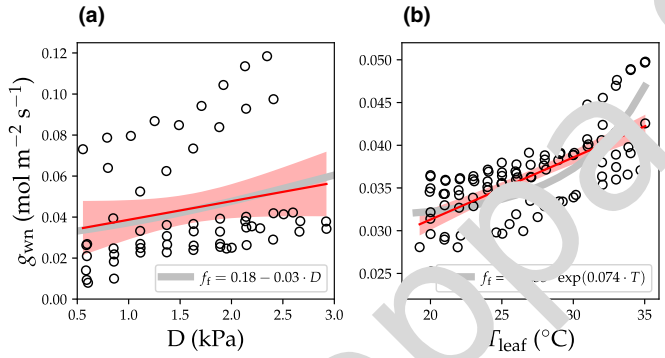

Fig. 7 Comparison of nighttime leaf diffusive conductance (g_{wn}) at different leaf-to-air vapor pressure deficit (D) and temperature vs model predictions using a variable stress multiplier. (a) The g_{wn} response to leaf-to-air vapor pressure deficit from six well-watered trees (the data are the same as those plotted in Fig. 3c). The light gray curve plots our model predicted g_{wn} using a variable fitness multiplier depending on D. The red line plots the linear regression of all data, and the shaded region plots the confidence interval. (b) The g_{wn} response to leaf temperature (T_{leaf}) at a constant D (the data are the same as those plotted in Fig. 4a). The light gray curve plots our model predicted g_{wn} using a variable fitness multiplier depending on T_{leaf}.

As a result, using water at night may aid in plant nutrient uptake. The increased benefits of nocturnal transpiration translate to a decreased f_f and increased nocturnal transpiration rate. Similarly, plants grown under different environmental conditions (e.g. different soil moisture and air humidity) ought to have different traits, and a comparison of nighttime stomatal responses should be made cautiously with respect to such differences in plant traits. We note that our proposed model is process- and trait-based, and that the impacts from changing traits can be accounted for by our model, making it a promising tool for use with vegetation models.

Various studies have described g_{wn} responses to VPD that are in contrast to those presented here (Barbour *et al.*, 2005; Dawson *et al.*, 2007; Zeppel *et al.*, 2012; Cirelli *et al.*, 2016; Yu *et al.*, 2019). Importantly, these differences can be explained using our model framework. In our simulation of a fixed fitness multiplier, the model predicted slightly decreasing nighttime leaf diffusive conductance under drier air conditions, which explains some of the accounts of decreasing g_{wn} (Cirelli *et al.*, 2016). However, the model predicted increasing g_{wn} when we used a linearly decreasing f_f with drier air, in agreement with the increasing g_{wn} observed under drier air conditions (present study; Barbour *et al.*, 2005; Dawson *et al.*, 2007; Zeppel *et al.*, 2012; Yu *et al.*, 2019). Therefore, it is very likely that f_f decreases with higher VPD (an environmental stress), which means that the vaule of other nocturnal transpiration upsides increases. Similarly, an increasing f_f with higher temperature better explains the increasing g_{wn} with higher temperature (VPD was held constant in our research, and thus the stress to the plant is a result of the increasing respiration rate), suggesting that the value of respiratory reduction increases when leaf temperature increases.

A potential deficit of the model is that it uses nighttime leaf temperature to estimate the potential photosynthesis during the day. As we mentioned in the Materials and Methods section, the use of mean daytime environmental conditions would be more appropriate. We therefore verified the argument by using the exact model parameterization but with leaf and air temperature elevated by 10°C. Note that daytime VPD also increased because of the higher air temperature (we set atmospheric vapor pressure

constant). We re-plotted the model predictions shown in Figs 3–5, and the results are shown in Figs S2–S4. In general, the g_{wn} response to changes in soil moisture and VPD showed little improvement (Fig. S2a,c); the g_{wn} response to changes in C_a was less steep and agreed better with experimental observations (Fig. S2b); the g_{wn} response to changes in temperature was less steep and agreed better with observations (Fig. S3); and the responses to changes in respiration rate became more linear and agreed better with observations. Therefore, experimentally modeling and measuring both the daytime and nighttime leaf gas exchanges will better serve the purpose of modeling nighttime stomatal conductance.

g_{wn} and the fitness factor

Though nocturnal transpiration results in faster soil water consumption and less daytime carbon gain, plants may actually benefit from losing water at night in some circumstances. For example, plants with sufficient water supply (e.g. riparian trees and rainforests) will accumulate more photosynthate if transpiration-induced leaf cooling occurs at night, and accumulate more nutrients through transpiration flow (Zeppel *et al.,* 2014; Siddiq & Cao, 2018). Understory plants (such as grasses and shrubs) may restrict their competitors' growth by quickly draining the soil, and thus potentially increasing the competitive edge of the understory under scarce canopy light conditions (Caird *et al.,* 2007). Deciduous trees and annual plants tend to use water more aggressively to compete with evergreen plants because they cannot use soil water for photosynthesis after they shed the leaves, and using more water (both during the day and at night) results in less soil water for their competitors (Zeppel *et al.,* 2014). Moreover, drought tolerant plants may out-compete more vulnerable competitors by draining the soil, and the vulnerable competitors suffer due to the hydraulic impairment (Yu *et al.,* 2019). Plants with shallow roots (such as grasses and herbs) may transpire more at night to suppress soil water redistribution to deep soil and keep the water in their own root zone (Howard *et al.,* 2009; Neumann *et al.,* 2014; Huang *et al.,* 2017; Yu *et al.,* 2019). Thus, it is expected that plants under higher competition stress will have higher nighttime transpiration rates (due to lower f_f). Even though the nocturnal transpiration might result in the plants suffering from future drought stress, the fitness benefits that arise could drive plants to take the risk.

Nighttime stomatal behaviors are likely the result of evolutionary adaptation to the environment. For example, stomata close more at elevated CO_2 (during both the daytime and nighttime), but plants may not experience great atmospheric CO_2 concentration change throughout their lives. In this case, stomatal response to changes in C_a may not be useful to short-lived plants. However, such a response makes plants more competitive in the long run compared to plants that have no response to changes in CO_2, as the former can use water more efficiently and maintain their hydraulic transport capability. Acclimation to local environment (through trait plasticity) would further enhance these benefits; for example, plants that evolved in arid regions tend to lose more water at night compared to plants that evolved in wet regions when growing them in the same environment (Yu *et al.,* 2019). Understanding how plants adapt and acclimate to the environment not only in the daytime but also at nighttime would help advance land surface models, particularly under conditions of unprecedented climate change (Nicotra *et al.,* 2010; IPCC, 2014; Sperry *et al.,* 2019; Trugman *et al.,* 2019).

The use of a 'fitness factor' in our model helps account for other benefits of nocturnal transpiration besides evaporative cooling, by lowering the daytime marginal water use efficiency to match the nighttime respiration benefit. Our proposed model provides a feasible way to model nocturnal transpiration and its response to the environment in vegetation models. However, mechanistically modeling nocturnal transpiration at the landscape level requires knowing not only how f_f varies spatially, but also how f_f may vary temporally. Currently, the following are still unclear: whether and how f_f varies with time; whether and how f_f responds to the environment, including the plant competitive environment; how f_f differs within and among species; how nocturnal respiration rate varies spatially and temporally; how cuticular conductance contributes to nighttime transpiration; and how nutrient dynamics can be improved by nocturnal transpiration and how this process contributes to f_f. More in-depth research into f_f will help to address these questions and make it possible to accurately model the nighttime carbon, water, and energy fluxes spatially and temporally.

Conclusion

The proposed nighttime transpiration model explains critical observed nighttime stomatal responses to the environment, and also helps to explain the observed dynamic changes with time. The model serves future research for quantitatively and accurately modeling nocturnal transpiration well. Incorporating nocturnal transpiration into larger scale models is eminently feasible by building upon daytime stomatal optimization models and leaf respiration processes, which are already simulated in most models. More thorough surveys of nocturnal transpiration with respect to dynamic respiration regulation and a variable fitness factor will help improve the modeling of global water and carbon cycles.

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Author contributions

YW, WRLA, and KY planned the research. YW and KY performed experiments. YW analyzed the data, designed the model, and led the writing. YW, WRLA, MDV, ATT, KY and CF wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Example of stable leaf temperature in stomatal response to vapor pressure deficit.

Fig. S2 Fig. 3 with higher daytime temperature.

Fig. S3 Fig. 4 with higher daytime temperature.

Fig. S4 Fig. 5 with higher daytime temperature.

Notes S1 Derivation of nighttime marginal respiratory saving.

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