Phylogenetic and Biogeographic Controls of Plant Nighttime Stomatal Conductance

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Phylogenetic and biogeographic controls of plant nighttime stomatal conductance

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Summary

- The widely documented phenomenon of nighttime stomatal conductance (g_sn) could lead to substantial water loss with no carbon gain, and thus it remains unclear whether nighttime stomatal conductance confers a functional advantage. Given that studies of g_sn have focused on controlled environments or small numbers of species in natural environments, a broad phylogenetic and biogeographic context could provide insights into potential adaptive benefits of g_sn.
- We measured g_sn on a diverse suite of species (n = 73) across various functional groups and climates-of-origin in a common garden to study the phylogenetic and biogeographic/climatic controls on g_sn and further assessed the degree to which g_sn co-varied with leaf functional traits and daytime gas exchange rates.
- Closely related species were more similar in g_sn than expected by chance. Herbaceous species had higher g_sn than woody species. Species that typically grow in climates with lower mean annual precipitation – where the fitness cost of water loss should be the highest – generally had higher g_sn.
- Our results reveal the highest g_sn rates in species from environments where neighboring plants compete most strongly for water, suggesting a possible role for the competitive advantage of g_sn.

Key Words
Stomata, gas exchange, transpiration, ecosystem flux, phylogenetic, biogeographic, adaption.

INTRODUCTION

Plants capture atmospheric CO₂ for photosynthesis and lose water vapor through stomatal pores on leaves. Thus, stomata strongly influence the carbon and water fluxes of terrestrial ecosystems and modeling stomatal behavior is essential for projections of climate impacts on ecosystems, land-atmosphere interactions, and carbon cycle feedbacks (Berry et al. 2010; Jasechko et al. 2013; Franks et al. 2017; Anderegg et al. 2018). A broad body of research has examined how stomatal conductance during the day responds to genetic controls and/or environmental conditions (Buckley, 2005; McAdam & Brodribb, 2015; Brodribb & McAdam, 2017). However, it is well established that many plants continue to lose water at night. In particular, there is extensive evidence of significant nighttime stomatal conductance (g_sn) and transpiration (E_n) in a diverse range of C₃ and C₄ plants across various habits and in multiple climate zones (Snyder et al. 2003; Daley & Phillips, 2006; Caird et al. 2007; Dawson et al. 2007; Phillips et al. 2010; Ogle et al. 2012; Hoshika et al.).
These observations are at odds with optimal stomata theories, which suggest that C₃ and C₄ plants should close stomata completely to avoid water loss when there is no carbon gain and little need to cool leaves during the night (Snyder et al. 2003; Caird et al. 2007).

\( E_n \) typically ranges from 10% to 15% of daytime rates, and can reach up to 20-30% of daytime rates (Bucci et al. 2004; Caird et al. 2007; Sellin & Lubenets, 2010; Ogle et al. 2012). This is a substantial water flux, especially for plants in dry regions where growth is often water limited (Yu et al., 2016; Chen et al., 2018). Previous studies have mainly used sap flow sensors to measure \( E_n \) at tree individual scales (Daley & Phillips, 2006; Caird et al. 2007; Dawson et al. 2007). This method, however, does not allow for the direct measurements of \( g_{sn} \). Sapflow also does not generally partition recharge of plant water storage and \( E_n \) and therefore could overestimate the magnitude of \( E_n \). Quantification of \( E_n \) at large scales remains difficult because of the technical obstacles in using eddy flux tower and remote sensing data during the nighttime (De Dios et al., 2015). However, in the few instances where quantification has been attempted, \( E_n \) has been found to be significant (8-9% of daytime transpiration) (Novick et al. 2009). Most large-scale ecosystem models either ignore nighttime water loss or set a low and constant value of \( g_{sn} \) or \( E_n \), which can lead to large biases in the estimates of plant water-use efficiency, ecosystem transpiration, and ecosystem carbon/water cycling in a changing climate (Novick et al. 2009; Zeppel et al. 2014; De Dios et al. 2015; Lombardozzi et al. 2017; Hoshika et al. 2018).

Why would plants not completely close their stomata at night? The primary non-adaptive hypothesis that has been proposed is the “leaky stomata” hypothesis, where fully closing stomata could have an energetic cost that plants only pay during very dry conditions and thus stomata remain partially open at night. This hypothesis appears to be at odds with the significant documentation of \( g_{sn} \) in dry environments, where the costs of water loss should be high.

The primary adaptive hypotheses that have been proposed center on plant functional response to environmental conditions including water stress, nutrient availability, and oxygen availability. There have been a number of studies suggested that some species can actively reduce or close stomata during the night in response to water stress and/or ABA (Caird et al. 2007; Cirelli et al. 2015), although several shrub species in North American deserts exhibited differential daytime and nighttime stomatal behavior in response to environmental conditions (Ogle et al., 2012; Zeppel et al., 2012). This could lead to refilling capacitance and removing embolism in stems (Zeppel et al. 2014), which would lead to improved early morning photosynthesis (Caird et al. 2007). However, experiments manipulating water/nutrient conditions or vapor pressure deficit (VPD), which can also affect photosynthesis, showed a divergent response of \( g_{sn} \) (Caird et al. 2007; Zeppel et al. 2014).
Studies have also proposed that $g_{sn}$ could benefit plants through increasing nutrient availability for root uptake (Scholz et al., 2007) and oxygen delivery for sapwood parenchyma cells (Daley & Phillips, 2006). The hypothesis of improved nutrient availability has been extensively studied (Caird et al., 2007; Zeppel et al., 2014). Both $g_{sn}$ and $E_n$ result in increased access by roots surfaces to soil nutrients through mass flow and diffusion (Oliveira et al. 2010; Kupper et al. 2012) and thus likely improves nutrient availability to plants. The causality between $g_{sn}$ and nutrient uptake, however, has garnered weak-to-mixed evidence (Howard & Donovan, 2007; Christman et al. 2009; Kupper et al. 2012).

An additional adaptive ecological mechanism that has been proposed suggests that $g_{sn}$ could be a strategy to reduce hydraulic redistribution in the soil, thereby keeping water resources close to an individual plant rather than moving that water towards neighboring competitors (Huang et al. 2017); this hypothesis has shown some initial empirical support (Neumann et al. 2014; Yu et al. 2018). Ultimately, the factors or mechanisms affecting $g_{sn}$ remain rather unclear and studies on this hypothesis to date have been largely carried out in a controlled (e.g. greenhouse or growth chamber) environment (Caird et al. 2007; Zeppel et al. 2014).

Some studies have indicated that there may be genetic controls on $g_{sn}$ (Caird et al. 2007; Costa et al. 2015; Reuning et al. 2015) and that $g_{sn}$ may have a biogeographic signal (Caird et al. 2007; Zeppel et al. 2014). However, it remains unclear whether or not there is a strong phylogenetic signal in functional traits of $g_{sn}$ (i.e., the tendency for trait relatedness among related species) and whether or not $g_{sn}$ has evolved as an adaptation to climate or soil nutrient conditions in a species’ native range. A strong phylogenetic or biogeographic signal could inform the estimates of $g_{sn}$ at large scales (Moles et al., 2005; Swenson et al., 2017), which are currently limited by technical obstacles (De Dios et al. 2015) but crucial for Earth system models (Lombardozzi et al. 2017). The genetic controls on $g_{sn}$ could also offer new horizons for breeding programs to cultivate crops that have lower $g_{sn}$ and the same growth, thus increasing water-use efficiency (Costa et al. 2015; Coupel-Ledru et al. 2016). The studies to date only leveraged a narrow spatial range of native habitats with a small number of co-occurring species to examine $g_{sn}$ in native climate conditions (Snyder et al. 2003; Ogle et al. 2012). While Lombardozzi et al. (2017) compiled a large dataset ($n=204$) to better represent $g_{sn}$ in land surface models and allow the comparison of $g_{sn}$ among different functional groups, these observed $g_{sn}$ values were estimated by a variety of methods and measurement conditions, which limits rigorous comparison across functional groups, and had very limited information on boreal climate zones. Hoshika et al. (2018) compiled a large dataset to show the correlation between daytime maximum $g_s$ in woody plants and mean annual precipitation, but the information of climatic (biogeographic) controls on $g_{sn}$ is limited.
Thus, there is a critical need for a large common garden study in which the $g_{sn}$ of species from a broad diversity of clades can be sampled in the same climate/growth environment to provide rigorous quantification of cross-species patterns and insights into potential adaptive drivers of $g_{sn}$.

Here, we quantify $g_{sn}$ on 73 species among a number of different clades growing in a common garden in a dry climate (e.g. substantial VPD) to examine the phylogenetic and biogeographic controls on $g_{sn}$.

The species represent various life forms (i.e., trees, shrubs, grasses and forbs) originating from diverse climate zones (i.e., boreal, n = 32; temperate dry, n = 15; and temperate wet climate, n = 26). We ask:

1) is there evidence for a phylogenetic signal in $g_{sn}$? (2) does $g_{sn}$ vary among species as a function of their native climate, and soil nutrient conditions and, if so, to which climate or soil nutrient variables? 3) are there consistent differences in $g_{sn}$ among different life forms and climate zones? 4) does $g_{sn}$ correlate with leaf anatomy and physiology among species?

MATERIAL AND METHODS

Study site, species identification, and methods of observations

We carried out this study in the Red Butte Garden in Salt Lake City, Utah, USA (40.7655° N, 111.8238° W), the largest botanical garden in the Intermountain West. The climate is characterized by a semi-desert steppe with hot, dry summers and long, cold winters. Mean annual precipitation is about 400-500 mm with most precipitation occurring in winter and spring. At the beginning of May 2017, we identified 93 species representative of various life forms (i.e., trees, shrubs, grasses, and forbs) (Engemann et al., 2016), leaf forms, and leaf shapes. Species names were confirmed with the Taxonomic Name Resolution Service (TNRS; Boyle et al. 2013). Three individuals with similar size and proximity in distance were identified in each species. In each individual, one recently mature and fully-expanded leaf was marked for the measurements of gas exchange and traits. To control for differences in microclimate, leaves on woody plants were located at similar canopy heights and were generally sun-exposed and south-facing.

Nighttime gas exchange was measured for each individual using a LI-6800 (Li-Cor, Inc., Lincoln, NE, USA) with the 6 cm$^2$ leaf chamber (circle; radius = 1.38 cm) in a closed system mode. These measurements were conducted on several continuous clear nights (4-5) to ensure similar climate for each sampling event. Sampling was carried out once a month from May to August. For all of the analyses in this study, we used the measurements from one sampling event during June because it best captured the $g_{sn}$ max of all species. Volumetric soil water content at soil depth of 20 cm was measured (n = 3 for each species) at locations near to (<1 m from plant base) where gas exchange measurements were made; the measurements were conducted in the late afternoon before nighttime gas exchange using decagon GS3 soil moisture sensor. A circadian rhythm in $g_{sn}$ has been observed in some species (Caird et al. 2007; Ogle et al. 2012; Resco de Dios & Gessler, 2018), with a gradual
increase of $g_{sn}$ after midnight and maximum values during predawn hours. Thus, to approximately estimate the maximum $g_{sn}$ across a large sample size of species, nighttime gas exchange measurements were made 2-3 h before dawn. We present the maximum $g_{sn}$ observed the magnitude of which is quite low relative to daytime $g_s$, but provides a useful species-level trait similar to daytime maximum stomatal conductance (e.g. Oren et al. 1999). We took the maximum $g_{sn}$ for each of the individuals of a species and then averaged. Thus, the mean of maximum $g_{sn}$ over individuals across species was used for analyses. This allowed for less uncertainty in measurements of $g_{sn}$ over a large sample size of species. During the measurements, reference CO$_2$ was set to 400 µmol mol$^{-1}$, while VPD and temperature tracked ambient. To reduce the bias of data recording (logging), the same standard of judging the stability of gas exchange data was used. We monitored the $g_{sn}$ and took the measurement when the slope of $g_{sn}$ vs. time was smaller than 0.0015 mol m$^{-2}$ s$^{-2}$. For some species with small or (semi)cylinder shaped leaves that do not completely cover the leaf chamber area, the net gas exchange rate (stomatal conductance and respiration) was determined as $G = G_r \times 6/S$, where $G_r$ is the recorded value of the net gas exchange rate by LI-6800 and $S$ is the surface area (cm$^2$) of the leaf (Table S1).

Day time gas exchanges were also measured between 09:30 h and 12:00 h on the days before nighttime measurements; the 'one-point method' was then used to estimate maximum carboxylation capacity ($V_{cmax}$) for each species (De Kauwe et al., 2016). This method requires estimating leaf respiration during the day. Assuming that leaf respiration during the day is 1.5% of $V_{cmax}$, this method was found to estimate $V_{cmax}$ with an $r^2$ of 0.95, as compared to the traditional A–Ci curve fitting (De Kauwe et al., 2016). In the middle of June, a subset (n = 20) of species across the range of $g_{sn}$ and life forms (n = 8 for trees; n = 3 for shrubs; n = 4 for grasses; n = 5 for forbs) were selected for estimates of stomatal density. Stomata peels from adaxial surfaces of the leaves used for gas exchange were sampled using clear nail polish and tape. Stomatal density was counted independently by two trained observers using light microscope images (n = 10 per leaf) on each individual leaf peel. At the end of June 2017, a subset (n = 54) of species across the range of $g_{sn}$ and life forms were also sampled for estimating specific leaf area (SLA).

**Phylogenetic tree, species native ranges and climate**

The phylogenetic tree was constructed using phylomatic in PHYLOCOM 4.2 (Webb et al. 2008) with the ‘R20100701’ megatree. Approximate crown ages for each clade were calculated using bladj. Internal node constraints were from Bell et al. (2010) and subsequently corrected for file transcription errors (Gastauer & Meira-Neto, 2013). To construct a binary tree, we used the multi2di function in the ‘ape’ package.

Global Biodiversity Information Facility (GBIF; http://www.gbif.org) was used to determine native distribution of the species in May 2018. To extract species’ geo-referenced locations (latitude and longitude), we first used the ‘dismo’ R package. We then used the following criteria (Zohner et
al., 2016) to filter reliable records (locations) of species: 1) only records from a species’ native continent (North America, Europe, and Asia) were included; 2) coordinate duplicates within a species were removed; 3) records based on fossil specimen were removed; 4) spatially clustered records within 10 km were removed to correspond with the spatial resolution (bio 2.5m; 10 km) of WorldClim (http://www.worldclim.org/bioclim) used to determine the climate of native species ranges.; 5) hybrid species and species without records in GBIF were excluded. This led to a total sample size of 73 species with adequate distribution data to calculate climate-of-origin (Table S1).

Species-specific climate ranges were derived from WorldClim variables based on species’ georeferenced locations. WorldClim variables included annual mean temperature (BIO1), mean temperature of driest quarter (BIO9), mean temperature of warmest quarter (BIO10), annual precipitation (BIO12), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), annual mean vapor pressure deficit (VPD), vapor pressure deficit of driest quarter (VDQ), and vapor pressure deficit of warmest quarter (VWQ) and were chosen based on previous research on $g_{sn}$ (Daley & Phillips, 2006; Caird et al. 2007; Dawson et al. 2007; Zeppel et al. 2014). Temperature and precipitation variables were downloaded from the standard WorldClim Bioclimatic variables for WorldClim v2 (http://worldclim.org/version2), while VPD, VDQ, VWQ were not available as WorldClim outputs. Thus, we quantified VPD, VDQ, VWQ following the same protocol as the outputs of standard WorldClim Bioclimatic variables (http://worldclim.org/bioclim). With respect to the impacts of soil nutrients, we used a global soil nitrogen (SN) database with > 3500 soil profiles (Zinke et al. 1998) and inverse distance interpolation approach to generate a global raster of soil nitrogen. We also used a global raster of soil organic carbon (SOC) at 2 m soil depth with 10 km spatial resolution, which plays an important role in nutrient cycling (Schmidt et al. 2011) and thus was represented here as a coarse proxy for soil nutrients; the data was downloaded from https://www.soilgrids.org.

To investigate the difference of $g_{sn}$ among climate zones, the species’ georeferenced locations were assigned to sub-climate zones using the Koeppen–Geiger system (Peel et al., 2007). When species were located in multiple sub-climate zones, the sub-climate zone where the species has the maximum number of records was used (Table S1). To evaluate the $g_{sn}$ on a larger (climate zone) scale, we then joined sub-climate zones into broader climate zones - temperate dry (TeD), temperate wet (TeW), and boreal (Bo) climate zones, following the criteria: TeD (BSk, n = 10; Csa, n = 4; Csb, n = 1); TeW (Cwa, n = 1; Cfa, n = 14; Cfb, n = 11); Bo (Dwb, n = 1; Dfa, n = 4; Dfb, n = 26; Dfc, n = 1) (Peel et al. 2007).
Data analysis

Two complementary approaches were used to determine how species’ phylogenetic relationships and native climate and soil nutrient ranges (biogeography) affects $g_{sn}$.

The first approach was to determine the phylogenetic and biogeographic signals of $g_{sn}$ separately. To this end, we tested for phylogenetic signal in mean $g_{sn}$ among the three individuals in each species using Pagel’s lambda ($\lambda$) following Münkemüller et al. (2012). Values of $\lambda$ approaching 1 indicate the fit of a Brownian motion model of evolution and suggest that mean values of $g_{sn}$ are more closely related among relatives than expected by chance, while values approaching 0 indicate phylogenetic independence. Pagel’s lambda ($\lambda$) was determined using the phylosig function in the ‘phytools’ package; 1000 simulations of Pagel’s lambda ($\lambda$) were performed and the significance was assessed using a likelihood ratio test (Revell 2012).

For the biogeographic signal of $g_{sn}$, we determined the biogeographic signal of $g_{sn}$ by testing which species’ native climate and soil nutrient variables (BIO1, BIO9, BIO10, BIO12, BIO17, BIO18, VPD, VDQ, VWQ, SN, SOC) were important in affecting $g_{sn}$ using multivariate linear regression. To avoid multicollinearity in our models, we used a pairwise correlation matrix and removed any variable that had high correlations (R>0.7) with other predictor variables following previous studies (Anderegg et al. 2013). The variables (BIO1, BIO10, BIO12, BIO17, VDQ, SOC) that gave the best prediction of species-level variation in $g_{sn}$ were retained in the model. SOC was used as the sole soil nutrient variable because SOC and SN were highly correlated.

After standardizing all independent and dependent variables to z-scores, we then used two methods of investigating variable importance of this reduced set of predictor variables in affecting $g_{sn}$. In the first approach, both forward and backward stepwise model selection via Akaike Information Criterion were used to determine the most parsimonious model and the coefficients of the predictor variables that remained in the model (Burnham & Anderson, 2004). In the second approach, machine learning algorithm Random Forests were used to determine variable importance for each variable (Breiman, 2001). Higher values of the mean decrease in accuracy ($\%$IncMSE) indicate the increased importance of the variables. We ran 1000 simulations of machine learning algorithm Random Forest and calculated mean $\pm$ standard deviation values of $\%$IncMSE.

In the second approach, we used hierarchical Bayesian models to investigate the influence of native climate and soil nutrient ranges (biogeography) on $g_{sn}$ by accounting for possible effects of shared evolutionary history (phylogenetics). To this end, using the Bayesian phylogenetic regression method (Villemereuil et al., 2012), we incorporated the phylogenetic structure of the data into the hierarchical Bayesian models by converting the 73-species ultrametric phylogeny into a scaled (0–1) variance–covariance matrix (Zohner et al. 2016). The resulting posterior distributions are a direct statement of the effect magnitude of species native climate and soil nutrient (represented by mean and median values of climate and soil nutrient ranges) on mean $g_{sn}$.

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Using the hierarchical Bayesian models accounting for possible effects of shared evolutionary history, we also separately investigated the difference of $g_{sn}$ among different life forms (trees, shrubs, grasses, and forbs) and among different climate zones (Bo, TeD, and TeW). In these analyses, different life forms (trees, shrubs, grasses, and forbs) or different climate zones were treated as binary (independent) variables. We also incorporated plant functional types (trees, shrubs, grasses, and forbs) as random effects into the hierarchical Bayesian models because $g_{sn}$ was significantly different among life forms and then examined the influence of native climate and soil nutrient ranges (biogeography) on $g_{sn}$. To investigate the sensitivity of results to number of observations (records) in each species in GBIF, we excluded species with less than 30 geo-referenced records within their native continent. Most of the species with limited observations were from Asia (Table S1). All of the analysis using hierarchical Bayesian models used standardised data and the results can be interpreted as relative effect sizes. Thus, to better visualize the variations of unstandardized $g_{sn}$ among different life forms and climate zones, we 1) used box and whisker plots to display the distribution of $g_{sn}$ and 2) analyzed/plotted the mean and 95% confidence interval.

Finally, we used univariate linear regression analysis to examine the relationships between $g_{sn}$ and a number of environmental, physiological and morphological variables including volumetric soil water content, day time $g_{s}$, photosynthetic rates, maximum carboxylation capacity of photosynthesis ($V_{cmax}$), nighttime respiration, stomatal density, and specific leaf area (SLA). In addition, due to the strong relationship between $V_{cmax}$ and $g_{sn}$, we included $V_{cmax}$ as a fixed effect factor and plant functional group as a random effect factor in the hierarchical Bayesian models to investigate the influence of biogeographic climate on $g_{sn}$.

**Data availability**

The data supporting the results are archived on the Hive, the University of Utah’s Open Access Institutional Data Repository and the data DOI is https://doi:10.7278/S50D-E9J1-NYG0.

**RESULTS**

We observed a wide range of $g_{sn}$ across the diverse set of species, with mean $g_{sn}$ ranging from 0.002 mol m$^{-2}$ s$^{-1}$ to 0.05 mol m$^{-2}$ s$^{-1}$ across species and generally smaller values in conifers (Fig. 1). Pagel’s lambda ($\lambda$) in our species was 0.37 (n = 73) and the p-value of the likelihood ratio test was 0.047. This indicates weak evidence that mean $g_{sn}$ is more closely related among relatives than would be expected by chance alone under a model of Brownian motion.

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With respect to the influence of biogeography examined via model selection, we found that mean annual precipitation (MAP) of species’ native climate ranges was the single best predictor of $g_{sn}$ ($P = 0.008$ for mean and $P = 0.007$ for median) and plants from locations with lower precipitation had higher $g_{sn}$ (Figs 2a, S1a). Precipitation of the driest quarter (PDQ) was retained in the model selection, but its relationships with $g_{sn}$ were not significant ($P = 0.122$ for mean and $P = 0.086$ for median). In contrast, both soil nitrogen (SN) and soil organic carbon (SOC), used as a coarse proxy for soil nutrients, were not good predictors of $g_{sn}$ and were not retained in the model. We observed similar patterns using the machine learning algorithm Random Forests, which showed highest values of the mean decrease in accuracy (%IncMSE) and thus the importance of MAP in native climate in influencing mean $g_{sn}$ (Figs 2b, S1b).

Using hierarchical Bayesian models to investigate the influence of biogeographic control on $g_{sn}$, while accounting for possible effects of shared evolutionary history, we found that Pagel’s $\lambda$ values were significantly greater than 0.2 for the scenarios of using mean and median values of native climate and soil nutrient condition ranges (AMT, MTW, MAP, PDQ, VDQ, SOC or SN), respectively (Figs. 3a, S2). Similar to the results above, this provides some evidence for $g_{sn}$ values being more closely related among relatives than would be expected by chance alone. After accounting for the plant phylogenetic differences, MAP still showed the significant and negative relationship with $g_{sn}$ ($n = 73$) both using mean and median values of native climate and soil nutrient condition ranges (Figs. 3b, c, S2). This pattern was robust after excluding species ($n = 9$) with less than 30 georeferenced records in GBIF (Table S1; Fig. S3).

The results, without accounting for possible effects of shared evolutionary history, demonstrated a higher $g_{sn}$ in herbaceous species (grasses and herbs) than trees and/or shrubs (Figs 4a, S4a) and a higher $g_{sn}$ in boreal climate zone than temperate climate zones (Figs 4b, S4b). Using the hierarchical Bayesian models to account for possible effects of shared evolutionary history, the results demonstrated similar patterns of higher $g_{sn}$ in herbaceous species and in the boreal climate zone (Fig. 4c, d), but the differences in $g_{sn}$ across species between the boreal and temperate climate zones were large (Figs 4d, S4b). Since $g_{sn}$ largely varied across life forms, we accounted for the influence of life forms by incorporating them as random (binary) factors into the hierarchical Bayesian models to examine the biogeographic control on $g_{sn}$. The results still showed the significant negative relationship between $g_{sn}$ ($n = 73$) and MAP, in contrast to PDQ and SOC (Fig. S5).

The univariate regression analysis showed a non-significant correlation between $g_{sn}$ and the local soil water conditions in the common garden ($P = 0.35$, Fig. S6a), suggesting minimal influence of local soil conditions on $g_{sn}$. The $g_{sn}$ was significantly related to daytime stomatal conductance ($P < 0.0001$, Fig. S6b), photosynthetic rate ($P < 0.0001$, Fig. S6c) and higher night
respiration (P < 0.0001, Fig. S6d) across species. The results also showed that $g_{sn}$ significantly increased with maximum carboxylation capacity ($V_{cmax}$; n = 73) (P = 0.0006, Fig. S7a), while the relationships with stomatal density (n =20; P = 0.17) (Fig. S7b) and specific leaf area (SLA) (n =54; P = 0.98) (Fig. S7c) were not significant. Finally, to account for the effects of $V_{cmax}$ in biogeographic signals, we also incorporated it as a fixed effect factor plus plant functional group as a random effect factor into the hierarchical Bayesian models. Accounting for these effects did not change the results (Fig. S8). Overall, the analyses robustly suggested that MAP was the best predictor of $g_{sn}$; plants in locations with lower rainfall conditions had higher $g_{sn}$.

DISCUSSION

We analyzed the underlying phylogenetic and biogeographic influences, as well as leaf morphological and physiological traits, on plant nighttime stomatal conductance by measuring a suite of diverse species grown in a common climate. The values of $g_{sn}$ were within the lower ranges of previously reported values (Caird et al. 2007; Zeppel et al. 2014; Lombardozzi et al. 2017). We found weak evidence for a phylogenetic pattern of $g_{sn}$ wherein closely related species had more similar $g_{sn}$ than expected by chance (Figs 1 and 3). For instance, the conifers (e.g., Pinales) all had similarly low mean $g_{sn}$, while the grasses (e.g., Poaceae) all had particularly high mean $g_{sn}$. This suggests that estimates of $g_{sn}$ at large scales carried out by reconstructing $g_{sn}$ based on their phylogenetic positions may not be an appropriate approach (Moles et al. 2005; Swenson et al. 2017). We did, however, detect substantial differences in $g_{sn}$ between life forms of grasses versus woody plants, which could be valuable for quantifying and simulating the large-scale impacts of $g_{sn}$ on carbon/water cycling in Earth system models that typically simulate ecosystems with “plant functional types” (Lombardozzi et al. 2017).

Mean annual precipitation (MAP) rather than temperature has been found to affect daytime maximum $g_s$ (Hoshika et al., 2018). In this study, we found robust evidence of $g_{sn}$ adaptations to species’ native climate range instead of local soil (water) conditions. Even after accounting for weak patterns of phylogenetic conservatism, species typically found in locations with lower MAP had higher $g_{sn}$ (Fig. 3). This pattern does not change based on choice of number of species (Fig. S3), the incorporation of plant functional group as a random effect (Fig. S5), or $V_{cmax}$ as a fixed effect (Fig. S8). Our results are consistent with previous studies that found higher $E_n$ (20-30%) relative to daytime rates among species in deserts and savannas (Bucci et al. 2004; Caird et al. 2007; Ogle et al., 2012). These results are interesting because they suggest that in dry regions where water is frequently limiting plant growth (Yu et al. 2016; Chen et al. 2018), plants exhibit substantial nighttime stomatal conductance. This supports an adaptive benefit of nighttime stomatal conductance because the fitness costs of water loss in these regions are likely high. In other words, the non-adaptive “leaky stomata”
hypothesis would predict that highest $g_{\text{sn}}$ should occur in regions where the fitness cost of water loss during the night is lowest (i.e. wet regions), which is not what we observed.

Previous studies have extensively investigated the impacts of soil nutrients on $g_{\text{sn}}$ and have shown divergent response of $g_{\text{sn}}$ to soil nutrient conditions (Howard & Donovan, 2007; Christman et al. 2009; Kupper et al. 2012). Our study did not find strong evidence for a correlation between $g_{\text{sn}}$ and the native soil nitrogen (SN) or soil organic carbon (SOC) (Figs. 3, S2, S5, S8), at least within the coarse constraints of the global datasets analyzed here. While we did not measure the local soil nutrients in the common garden, it is likely that local soil nutrient conditions would have minimal influence on $g_{\text{sn}}$. In fact, a causal relationship between $g_{\text{sn}}$ and soil/plant nutrients still remains highly elusive (Kupper et al. 2012). In contrast to the hypothesis and evidence that $g_{\text{sn}}$ changes with low soil nutrient conditions (Scholz et al. 2007), extensive studies have shown the opposite pattern: higher rates of $g_{\text{sn}}$ in species with relatively high overall growth rate and leaf/soil nitrogen concentrations (Daley & Phillips, 2006; Phillips et al. 2010; Kupper et al. 2012).

Our observation that species from dry regions have higher $g_{\text{sn}}$ is consistent with hypotheses that plant night stomatal opening may benefit plants by refilling capacitance and removing embolism in stems (Zeppel et al. 2014), especially considering that plants are water stressed and in some cases may have higher risk of embolism in drylands (Tyree & Zimmermann, 2002; Brodersen & McElrone, 2013). Thus, the large variation in $g_{\text{sn}}$ in lower mean annual precipitation (i.e., MAP < 1200 mm) (Fig. 2) may reflect differences in plant functional strategies with respect to capacitance and vulnerability to embolism (Brodersen & McElrone, 2013; Mcculloh et al. 2014; Zeppel et al. 2014). Alternatively, we suggest that our biogeographic patterns are more consistent with the hypothesis that nighttime water loss may provide a competitive advantage by curtailing passive water flow or hydraulic redistribution (Neumann et al. 2014; Yu et al. 2018), thereby improving plant fitness where plants compete belowground for water. Maintaining stomata slightly open would create a water potential gradient in the plant-soil hydraulic continuum that would favor keeping soil water close to an individual plant’s rooting system and prevent water from diffusing along passive water potential gradients in the soil to neighboring plants (Huang et al. 2017). Plant competition for water is likely most intense in dry regions and these scenarios are exactly where we observed the highest $g_{\text{sn}}$ (Figs 2, 3). This hypothesis of hydraulic redistribution may be more important for woody plants, which usually have deep roots and thus higher rates of hydraulic redistribution than herbaceous species (Neumann & Cardon, 2012; Yu & D’Odorico, 2015).

We observed the shifted and higher rates of $g_{\text{sn}}$ in forbs than grasses after accounting for plant phylogenetics, thus suggesting the role of plant phylogenetics in affecting $g_{\text{sn}}$ across life forms (Figs 4a, S4). However, regardless of influence of plant phylogenetics, higher rates of $g_{\text{sn}}$ were found in herbaceous species (grasses and forbs) than woody species (Figs 4a, S4), similar to other studies.
(Lombardozzi et al. 2017; O’Keefe & Nippert, 2018). The high rate of $g_{sn}$ in herbaceous species may result from relatively high overall growth rate (Chen et al. 2018) and thus plant photosynthesis and starch accumulation, which have been found to affect guard cell osmoregulation and increase $g_{sn}$ (Lascève et al. 1997; Easlon & Richards, 2009), consistent with our observed positive relationship between daytime stomatal conductance and photosynthesis and $g_{sn}$ (Fig. S6). The high rate of $g_{sn}$ in herbaceous species may support the hypothesis of the competitive advantage of soil moisture uptake by herbaceous species in shallow soil due to the high density or surface areas of fine roots (Steudle, 2000; Lombardozzi et al. 2017; O’Keefe & Nippert, 2018). In drylands, precipitation events more often wet soil shallow layers (Yu et al. 2016; Chen et al. 2018) and it could be advantageous for herbaceous species to preferentially use the shallow soil moisture through daytime and nighttime water uptake/transpiration. This reduces the diffusion of water along passive water potential gradients in the soil to its competitors (i.e., woody plants) and can even lead to the bottleneck effect of restricting the tree recruitment (Bond 2008).

Previous studies have found high values of $g_{sn}$ in tropical deciduous trees (Caird et al. 2007), as well as high values of $E_n$ estimated by sap flow in Mediterranean ecosystems (Barbeta et al., 2012) and tropical rainforests (Wallace & McJannet, 2010). All of these studies, however, were limited to a few species and lacking information from boreal climate zone. This study found higher $g_{sn}$ in species from boreal as compared to temperate climate zones regardless of plant phylogenetics (Fig. 4b, d). After accounting for plant phylogenetics, the higher $g_{sn}$ in the boreal climate zone was even more striking (Figs 4d, S4b), in which the conifers dominate and had similarly low $g_{sn}$. However, we note that our species mainly capture the drier regions of boreal climate zone (Fig. S9), which have likely led to the high observed $g_{sn}$, consistent with the effects of MAP on $g_{sn}$. The substantial role of climate-of-origin in influencing cross-species patterns of nighttime stomatal conductance sheds light on potential adaptive drivers of nighttime water loss. Thus, more studies evaluating $g_{sn}$ and its role of native climate ranges are greatly needed in a diversity of vegetation types and across climates.

Our findings that species $g_{sn}$ are coupled to, and thus potentially adaptations to, native climate indicates that reducing $g_{sn}$ through breeding or genetic modifications may impair plant growth. This was demonstrated by an earlier study in Arabidopsis thaliana mutant (Christman et al. 2008). However, two recent studies on mutants of Arabidopsis thaliana and Vitis vinifera found that growth was unaffected by manipulated reductions in $g_{sn}$ and transpiration (Costa et al. 2015; Coupel-Ledru et al. 2016). The divergent results of these studies may be due to different environmental conditions (i.e., water availability) during experimental treatments. In fact, growth was not affected by reduced $g_{sn}$ or $E_n$ on Arabidopsis only in well-water conditions where the fitness cost of water loss is low (Christman et al. 2009).
Our study has multiple implications for evaluating current ecosystem carbon and water fluxes and their dynamics under future climate change scenarios. First, our finding of higher gs rates in plants from dry regions (Fig. 2) (Bucci et al. 2004; Caird et al. 2007; Ogle et al. 2012) highlights the need to incorporate representations of gs in estimating ecosystem carbon/water fluxes, particularly in drylands (Dios et al. 2015; Lombardozzi et al. 2017). Second, in increasingly changing climate (Easterling 2000), nighttime water loss is likely to alter future ecosystem carbon/water fluxes, while quantification of its impacts remains challenging because of uncertainty of the precipitation projections (Woldemeskel et al. 2015). Thus, critical consideration of the phylogenetic and biogeographic patterns of gs can help shed insight into the adaptive benefits of gs in plants and inform modeling efforts to predict ecosystem carbon/water dynamics under climate change scenarios. Third, high values of gs correspond with higher values of Vcmax, which typically leads to higher plant night respiration. This further suggests the higher fitness cost of water loss in dry regions, whereby the higher gs should confer a functional advantage.

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Author contributions
KY and WRLA designed the study with inputs from GRG; KY, YW and WRLA collected the data; KY performed data analysis with inputs from GRG; KY wrote the manuscript with revisions from all coauthors.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and median of annual precipitation (MAP).

Fig. S2 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and its native climate and soil nutrients (soil nitrogen, SN) estimated from hierarchical Bayesian models.

Fig. S3 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and its native climate and soil nutrients (soil organic matter, SOC) estimated from hierarchical Bayesian models after excluding species with less than 30 georeferenced records in GBIF.

Fig. S4 Means and 95% CIs of g_{sn} among different life forms and different climate zones without accounting for possible effects of shared evolutionary history (phylogenetics).

Fig. S5 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and its native climate and soil nutrients (soil organic matter, SOC) estimated from hierarchical Bayesian models which also account for plant life forms as a random effect.

Fig. S6 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and local volumetric soil water content estimated by univariate regression analysis.

Fig. S7 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and daytime plant stomatal conductance (g_{sd}), and plant traits.

Fig. S8 Relationship between species’ maximum plant night time stomatal conductance (g_{sn}) and its native climate and soil nutrients (soil organic matter, SOC) estimated from hierarchical Bayesian models which also account for plant life forms as a random effect and maximum carboxylation capacity (V_{cmax}) as a fixed effect.

Fig. S9. Annual precipitation (MAP, mean and 95% CIs) in boreal (Bo), temperate dry (TeD), and temperate wet (TeW) biomes.

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Table S1 A summary of species information used in this study.

Figure Legends

**Figure 1** Phylogenetic tree of 73 species from across various functional groups and climates-of-origin (see details in Supporting Information Table S1) and its maximum plant night time stomatal conductance ($g_{sn}$).

**Figure 2 (a)** Relationship between species’ maximum plant night time stomatal conductance ($g_{sn}$) and mean of annual precipitation (MAP). Regression lines represent univariate relationships rather than the output of the full model and are for visualization purposes only. (b) Mean decrease in accuracy (%IncMSE, mean and standard deviation) estimated from 1000 simulations of random forests in evaluating the importance of native climate, represented by mean, on $g_{sn}$. Native climate variables are annual mean temperature (AMT), mean temperature of warmest quarter (MTW), annual precipitation (MAP), precipitation of driest quarter (PDQ) and vapor pressure deficit of driest quarter (VDQ). Soil organic carbon (SOC) is represented as an approximation to native soil nutrient conditions.

**Figure 3** Relationship between species’ maximum plant night time stomatal conductance ($g_{sn}$) and its native climate and soil nutrients (soil organic carbon, SOC) estimated from hierarchical Bayesian models. (a) Phylogenetic signal (Pagel’s $\lambda$, mean and 95% CIs) for $g_{sn}$ ($n = 73$). (b, c) Standardized coefficient estimates (effective posterior means and 95% CIs) for the effects of native climate, represented by mean (b) and median (c), on $g_{sn}$ ($n = 73$). Values reflect standardised data and can be interpreted as relative effect sizes. Native climate variables are annual mean temperature (AMT), mean temperature of warmest quarter (MTW), annual precipitation (MAP), precipitation of driest quarter (PDQ) and vapor pressure deficit of driest quarter (VDQ). Soil organic carbon (SOC) is represented as an approximation to native soil nutrient conditions.

**Figure 4** Box and whisker plots of maximum plant night time stomatal conductance ($g_{sn}$) among different life forms (a) and climate zones (b) without accounting for possible effects of shared evolutionary history (phylogenetics). Standardized coefficient values (means and 95% CIs) for differences in $g_{sn}$ between different life forms (c) and climate zones (d) estimated from hierarchical Bayesian models. Life forms are trees, shrubs, grasses, and forbs. Climate zones are boreal (Bo), temperate dry (TeD), and temperate wet (TeW). In box and whisker plots, the horizontal lines and ranges represent median, first quartile, and third quartile of $g_{sn}$. In hierarchical Bayesian models, values reflect standardised data and can be interpreted as relative effect sizes.

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Nighttime stomatal conductance ($g_{st}$) (mol m$^{-2}$ sec$^{-1}$)

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