

Hydraulic Determination of Leaf Nutrient Concentrations in Cycads

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Abstract

Cycads are the most ancient living seed plants. Extant cycads exhibit high morphological diversity and grow in diverse habitats, but little is known of their physiology. We tested for a novel linkage of leaf water transport with nutrient concentrations in cycads, which could contribute to variation in photosynthesis and growth rate. We measured leaf hydraulic conductance, stomatal conductance, and nutrient concentrations of 11 cycad species grown in a tropical botanical garden. The leaf concentrations of chlorophyll, nitrogen, phosphorus, iron, and sulfur were positively associated with leaf hydraulic and

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stomatal conductance across the cycad species, suggesting that high leaf water transport and transpiration may promote greater leaf nutrient assimilation. The facilitation of nutrient accumulation by transpiratory flow is a long-standing hypothesis, here supported by our data for 11 cycad species, indicating an adaptive function of transpiration, especially for plants with low leaf water transport and nutrient use efficiency such as cycads.

Introduction

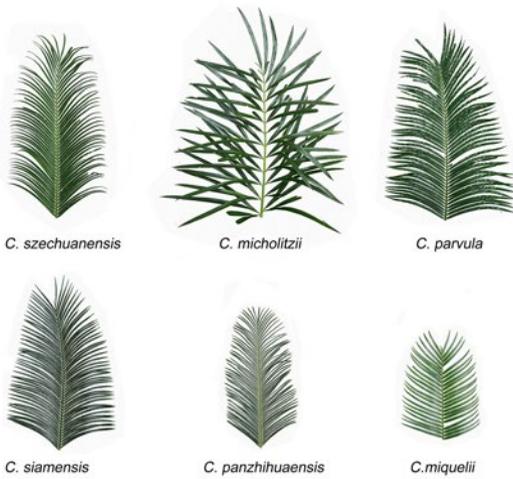
Cycads are the most ancient living lineage of the gymnosperms, and thus of all seed plants (Brenner et al., 2003). The oldest known cycad fossil dates to the Paleozoic (Mamay, 1969; Gao & Thomas, 1989), and the group reached dominance in the Mesozoic. Although there are only about 300 extant cycad species (Walters & Osborne, 2004), they exhibit substantial morphological diversity and grow successfully in a variety of habitats (Norstog & Nicholls, 1997; Whitelock, 2002). Cycads also vary strongly in photosynthetic performance (Marler & Willis, 1997; Zhang, 2011; Wei, 2012; Zhang et al., 2015), which could be related to differences in leaf water supply and leaf nutrient concentrations. On average, gymnosperms have lower leaf hydraulic conductance (K_{leaf}) than angiosperms (Brodribb et al., 2007; Simonin et al., 2012). Thus, leaf water supply could be a limiting factor in nutrient accumulation, photosynthesis and growth rate in some gymnosperms, such as cycads. In addition, cycads may have lower photosynthetic nutrient use efficiencies than angiosperms because of their high concentrations of lignin, mucilage conduct, and secondary compounds (Norstog & Nicholls, 1997; Zhang et al., 2015), which require substantial investment of nitrogen and other nutrients. Since nutrient uptake and transport could potentially be facilitated by high water mass flow driven by transpiration (Barber, 1962; Epstein, 1972; Wright et al., 2003; Cramer et al., 2008; Cramer et al., 2009), leaf nutrient concentrations may be associated with plant water transport and transpiration in this plant lineage, with low nutrient use efficiencies and associated high nutrient demands.

The mechanistic linkage between water transport and nutrient accumulation remain poorly understood. Nutrient absorption can be facilitated by transpiration-driven water mass flow from the bulk of the soil to the rhizosphere and nutrient translocation depends on xylem sap flow (Barber, 1962; Epstein, 1972; Wright et al., 2003; Cramer et al., 2008; Cramer et al., 2009), and thus the rate of nutrient assimilation in leaves may be linked to long distance water transport. Further, xylem water transport efficiency can

respond to increased nutrient availability. The addition of nutrients by fertilization (Bucci et al., 2006) and increasing ion concentration in the sap (Zwieniecki et al., 2001; Nardini et al., 2011) can enhance xylem water transport efficiency by increasing the conductance of pit membranes of the vascular conduits. Among angiosperms, both high water transport efficiency and high leaf nutrient concentrations are related to high relative growth rates (Ishida et al., 2008; Zhang & Cao, 2009). However, the potential relationship of foliar nutrient concentrations to leaf hydraulics has not yet been determined.

We studied leaf hydraulics and the concentrations of three macronutrients and one micronutrient in 11 cycad species grown in a common garden. The objectives of this study were to determine the variation in K_{leaf} , stomatal conductance, and nutrient concentrations of cycads, and to test whether leaf nutrient concentrations are associated with leaf water transport in this ancient plant lineage, characterized by low leaf water transport rates and low nutrient use efficiencies.

Cycadaceae



Zamiaceae

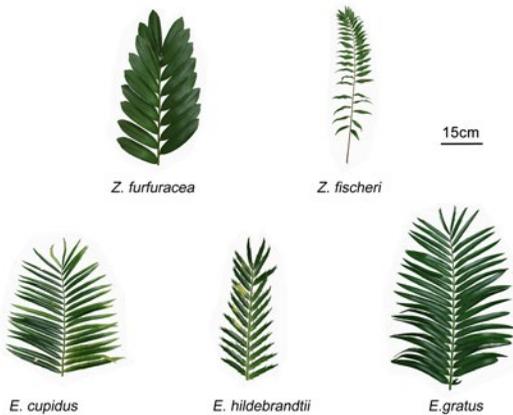


Figure 14-1. Leaf form for the 11 cycad species studied (genera *Cycas*, *Encephalartos*, and *Zamia*).

Materials and Methods

STUDY SITE AND PLANT MATERIAL

This research was carried out in the Cycad Garden in the Xishuangbanna Tropical Botanical Garden (21°41'N, 101°25'E; elevation 570 m) in southern Yunnan Province, China. Mean annual temperature is 21.7°C, and mean

Table 14-1 Cycad species studied and their original distribution.

Species	Original distribution
Cycadaceae	
<i>Cycas micholitzii</i> Dyer	China, Vietnam, Laos
<i>Cycas miquelii</i> Warb.	China, Vietnam
<i>Cycas panzihuaensis</i> L.Zhou & S.Y.Yang	China
<i>Cycas parvula</i> S.L.Yang ex D.Yue Wang	China
<i>Cycas siamensis</i> Miq.	China, Vietnam, Thailand, Burma
<i>Cycas szechuanensis</i> W.C.Cheng & L.K.Fu	China
Zamiaceae	
<i>Encephalartos cupidus</i> R.A.Dyer	South Africa
<i>Encephalartos gratus</i> Prain	Mozambique, Malawi
<i>Encephalartos hildebrandtii</i> A.Braun & C.D.Bouché	Kenya, Tanzania
<i>Zamia fischeri</i> Miq.	Mexico
<i>Zamia furfuracea</i> L.f. ex Aiton	Mexico

annual precipitation is 1,560 mm. The soil of the cycad garden is sandy alluvium; its composition, determined from 4 samples using standard methods (State Forest Administration, 1999), was 1.12 mg/g N, 0.51 mg/g P, 8.49 mg/g K, 8.33 mg/g Ca, 2.89 mg/g Mg, 23.0 mg/g Fe, 0.56 mg/g Mn, 73.25 mg/kg Zn, and 22.0 mg/kg Cu (0–20 cm depth).

For 11 cycad species, healthy individuals with mature, sun-exposed leaves were selected. Six species were in the genus *Cycas* (family Cycadaceae), and five in the genera *Encephalartos* and *Zamia* (family Zamiaceae) (Table 14-1, Fig. 14-1). *Cycas* is the most ancient cycad genus, a sister lineage to all other cycads, while *Zamia* is among the most recently derived cycad genera (Treutlein & Wink, 2004; Chaw et al., 2005; Zgurski et al., 2008). Leaflets were chosen as the fundamental unit for measurement, because they are developmentally and functionally analogous to angiosperm simple leaves or leaflets, typically measured in studies of functional traits. Values of hydraulic and stomatal conductance, and nutrient concentrations from a paper (Zhang et al., 2015) were analyzed here to address a clearly different question.

LEAF HYDRAULIC CONDUCTANCE

Leaf hydraulic conductance (K_{leaf}) was measured using the evaporative flux method (Sack et al., 2002; Brodribb & Holbrook, 2006; Brodribb et al., 2007; Sack & Scoffoni, 2012).

Leaves were cut from the plants in the late afternoon, recut immediately under water and placed with cut ends under water and covered with black plastic bags to rehydrate overnight. Measurements were made on segments from fronds, including rachis with 4–18 leaflets, excised underwater. The leaf section was held horizontally in a frame strung with fishing line, above a fan, and under full sunlight to drive transpiration, with the cut end of the rachis connected to a pressure-drop flow meter with two calibrated transducers (Brodribb et al., 2007; Melcher et al., 2012) logging to a computer, and the transpiration rate (E) was monitored. When a steady state E was reached (i.e., almost constant within 3 min), the leaf was removed into a Ziploc bag that had been exhaled into, to prevent dehydration of the leaf. Leaf water potential (Ψ_L) was determined after at least 30 min equilibration, using a pressure chamber (SKPM 1400, Skye Instruments Ltd., Lladrindod Wells, UK), after which leaf area was measured (LI-3000A leaf area meter; LI-COR, Lincoln, NE). The K_{leaf} was calculated as the steady state E/Ψ_L , normalized by leaf area, and corrected for the effects of temperature on the viscosity of water by standardizing to 20°C (Yang & Tyree, 1993; Sack et al., 2002; Brodribb et al., 2007). The K_{leaf} reported is the maximum, determined by plotting K_{leaf} against Ψ_L and extrapolating to zero Ψ_L (Brodribb & Holbrook, 2006; Brodribb et al., 2007). Maximum mass-based K_{leaf} (leaf water supply per dry mass) was determined as K_{leaf} divided by leaf mass per area (LMA). For LMA determination, leaf area was measured using the LI-3000A leaf area meter, after which the leaves were oven-dried at 70°C to a constant mass before weighing.

STOMATAL CONDUCTANCE

Stomatal conductance (g_s) was measured using a portable photosynthetic system (LI-6400, LI-COR). Six sun exposed mature leaves from 3 to 6 individuals per species were measured on sunny days between 08:30 and 10:30 hours, at a photosynthetic photon flux density of 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and ambient temperature (ranged 33–35°C) and CO_2 concentration. Relative humidity ranged from 55% to 63%. Stomatal conductance per leaf dry mass (g_{s-m}) was determined as g_s divided by LMA.

NUTRIENT AND CHLOROPHYLL CONCENTRATIONS

Six sun-exposed mature leaves from three to six individuals per species were collected for determination of foliar nutrient concentrations. Leaves with rachis removed were

oven-dried at 70°C for 48 hours and analyzed for nitrogen (N), phosphorus (P), Iron (Fe), and sulfur (S) concentrations. Leaf total N concentration was determined using a Vario MAX CN auto element analyzer (Elementar Analysensysteme, Germany) after the leaf samples were digested with concentrated H₂SO₄. Leaf P, Fe, and S concentrations were measured using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER, Thermo Jarrell Ash Corporation, Franklin, MA) after the samples were digested with concentrated HNO₃-HClO₄. Leaf chlorophyll concentration (Chl) was measured with a chlorophyll meter (SPAD 502, Minolta Corp., Osaka, Japan) for another six sun-exposed mature leaves from three to six individuals per species. For each leaf, 10 measurements across the lamina were averaged (Chl_a; in SPAD units). Previous studies reported strong relationships between SPAD measurements and total extracted chlorophyll (a + b) per area, for multiple species varying strongly in nutrient composition, thickness and surface features, with the majority of species tending to have statistically similar relationships (e.g., Marquard & Tipton, 1987; Marengo et al., 2009). Chlorophyll concentration per mass (Chl_m; units SPAD·m²·g⁻¹) was determined as Chl_a divided by LMA.

DATA ANALYSIS

Linear or exponential regressions were fitted to test relationships between $K_{\text{leaf-m}}$ and leaf chlorophyll or nutrient concentrations and between $g_{\text{s-m}}$ and chlorophyll or nutrient concentrations (Sokal & Rohlf, 1995).

Results

The cycad species varied strongly in leaf morphology (Fig. 14-1), leaf hydraulic conductance (Table 14-2, Fig. 14-2), stomatal conductance (Fig. 14-3), and nutrient concentrations (Figs. 14-2, 14-3). The mean $K_{\text{leaf-a}}$ and $K_{\text{leaf-m}}$ of cycads were comparable with the mean values reported for other gymnosperms, and lower than mean values for angiosperms (Table 14-2). The $K_{\text{leaf-a}}$ varied threefold across the measured cycad species (from 3.9 to 13.7 mmol·s⁻¹·m⁻²·MPa⁻¹), while the $K_{\text{leaf-m}}$ varied 13-fold (from 12.1 to 157 mol·s⁻¹·g⁻¹·MPa⁻¹). The $g_{\text{s-m}}$ of the cycad species varied nine-fold (from 0.39 to 3.48 mmol·s⁻¹·m⁻²). The N_m , P_m , Fe_m and S_m of the cycad species varied by twofold, threefold, eightfold and fourfold, respectively (Fig. 14-2).

Table 14-2 Comparison of cycads in this study with other gymnosperms and angiosperms in leaf hydraulic conductance expressed on an area basis and on a mass basis.

		Cycad	Gymnosperm	Angiosperm
$K_{\text{leaf-a}}$	Mean	5.3	7.1	11.4
	Minimum	3.1	1.4	2.0
	Maximum	9.6	16.7	41.6
$K_{\text{leaf-m}}$	Mean	39.6	43.0	153
	Minimum	12.1	7.4	8.0
	Maximum	157	158	783

The data for angiosperms ($n=75$) and gymnosperms excluding cycads ($n=8$) were taken from a data set compiled from previously published values (Simonin et al., 2012).

Units of leaf hydraulic conductance expressed on an area basis ($K_{\text{leaf-a}}$): $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$ and mass basis ($K_{\text{leaf-m}}$): $\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}\cdot\text{MPa}^{-1}$.

The leaf N_m , Chl_m , Fe_m , and S_m were strongly and positively associated with $K_{\text{leaf-m}}$ and g_{s-m} across species (Figs. 14-2, 14-3). The relationships of $K_{\text{leaf-m}}$ and g_{s-m} with P_m and/or N/P were not significant if *Z. fisheri* was included, given its exceptionally high $K_{\text{leaf-m}}$ among the 11 species, whereas other relationships were significant with or without *Z. fisheri* (Figs. 14-2, 14-3).

Discussion

We found a large variation in leaf hydraulic conductance, stomatal conductance, and foliar concentrations of nutrients and chlorophyll in the cycad species studied, which could potentially contribute to variation in photosynthetic rate and growth rate. Assuming that the variation in these leaf physiological traits is adaptive, this diversification may contribute to the dominance of cycads across a wide range of habitats in the Mesozoic. The positive relationships between leaf hydraulic conductance, stomatal conductance, and leaf nutrient concentrations (Figs. 14-2, 14-3) are also a first suggestion that high leaf water transport and transpiration rates may promote leaf nutrient assimilation in cycads, a hypothesis to be confirmed with further experimental approaches.

A higher leaf water transport efficiency allows the leaf to maintain mesophyll hydration and therefore a higher photosynthetic rate and transpiration rate (Sack & Holbrook,

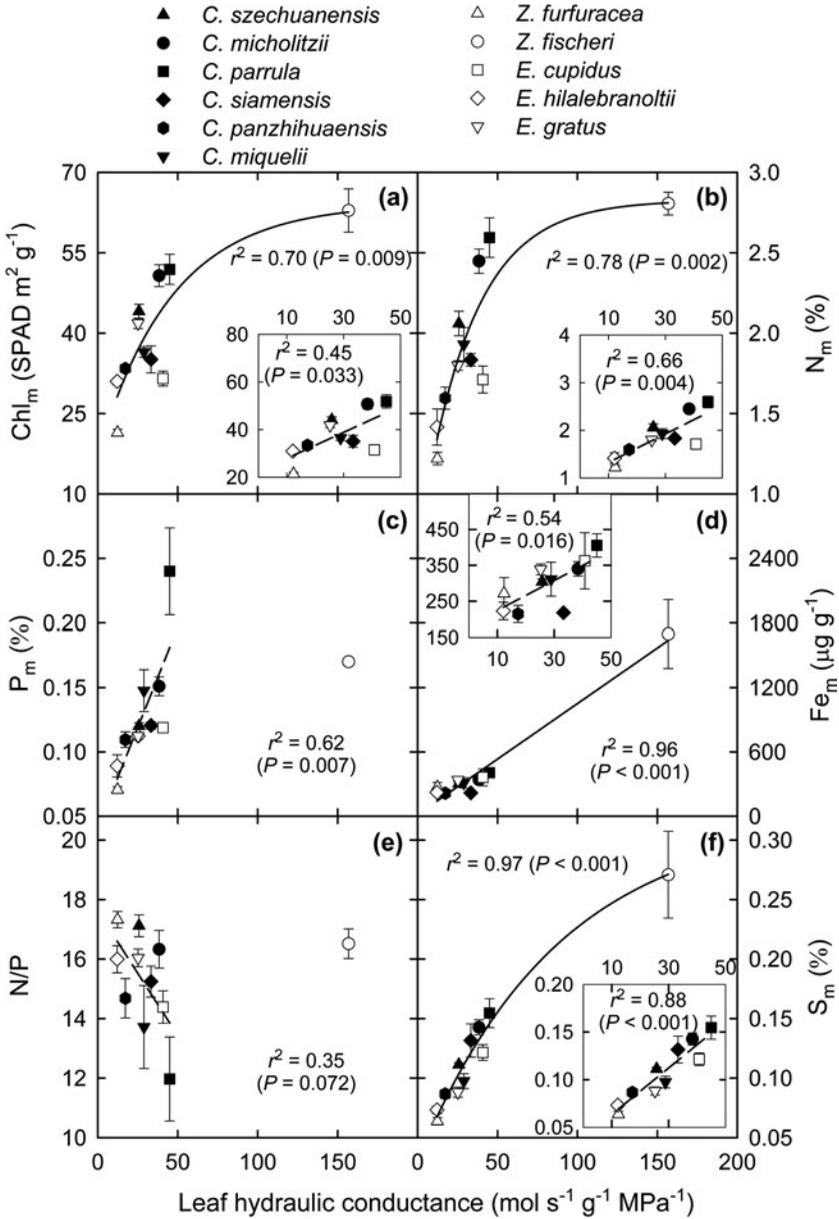


Figure 14-2. The relationships between mass based maximum leaf hydraulic conductance (K_{leaf-m}) and chlorophyll concentration per mass (Chl_m), and between K_{leaf-m} and leaf nutrient concentrations (N_m , Fe_m , P_m , S_m). Solid lines are exponential (a, b, f) or linear (d) equations fitted to the relationships, and broken lines (a-f) are linear regressions with *Zamia fischeri* not included.

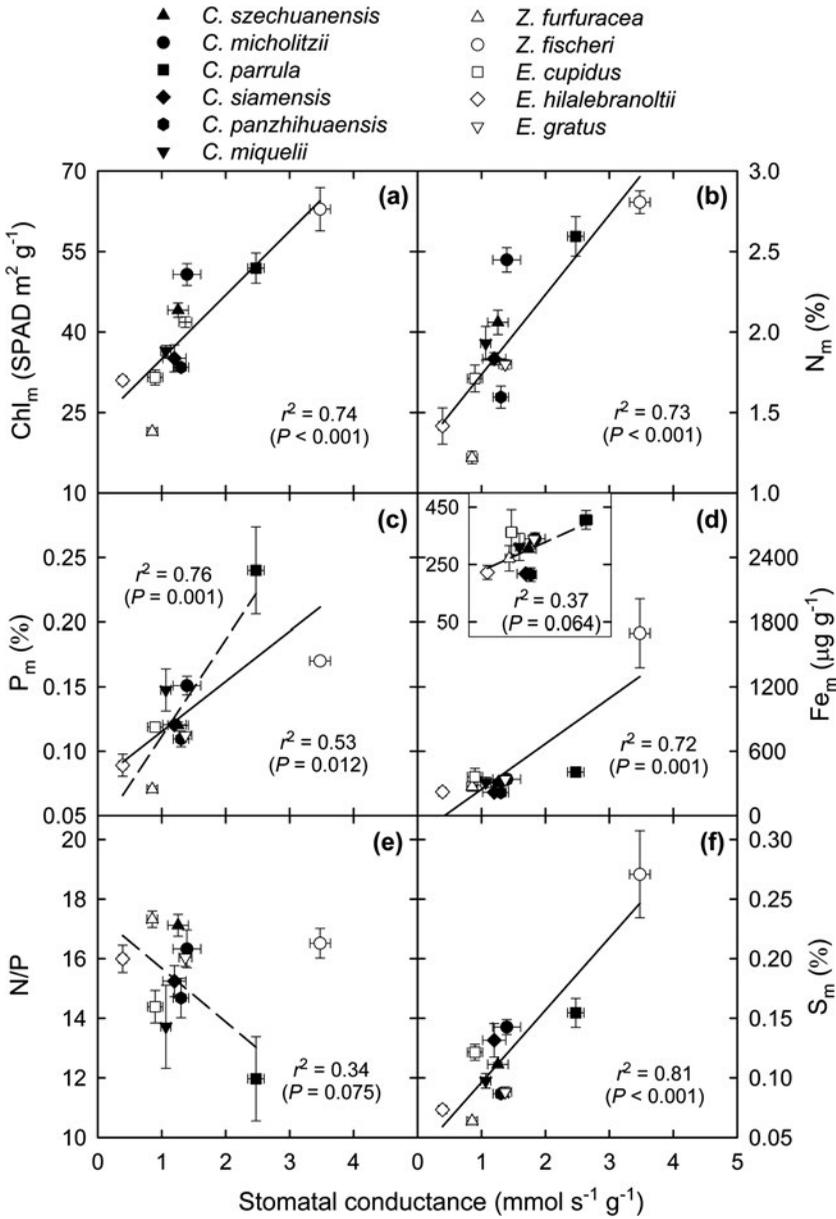


Figure 14-3. The relationships between mass based stomatal conductance (g_{s-m}) and chlorophyll concentration per mass (Chl_m), and between $K_{\text{leaf-m}}$ and leaf nutrient concentrations (N_m , Fe_m , P_m , S_m). Solid lines (a–d, f) are linear equations fitted to the relationships, and broken lines (c, d, e) are linear regressions with *Zamia fischeri* not included.

2006), which would potentially promote nutrient uptake, transport, and distribution. Since nutrient absorption can be promoted by transpiration-driven water/nutrient mass flow through the rhizosphere and from roots to leaves (Barber, 1962; Epstein, 1972; Wright et al., 2003; Cramer et al., 2009), high water uptake and transport efficiencies may facilitate nutrient absorption, translocation, and assimilation in the leaf (Cramer et al., 2008; Cramer et al., 2009). This hypothesis has been frequently proposed for angiosperms, and supported circumstantially (e.g., when xylem sap flow rate is associated with the nutrient concentrations of the xylem sap) in a tropical tree species (Barker & Becker, 1995).

However, an absence of relationship between leaf transpiration rate and nutrient concentrations was reported for maize, sunflower, and pepper (Tanner & Beevers, 1990, 2001; Bower, 2005). In maize, nontranspiration dependent water flow (e.g., guttation generated by root pressure, Münch's phloem counterflow) was suggested to be sufficient for optimal nutrient transport and accumulation in plant tissues (Tanner & Beevers, 2001). Indeed, the facilitation of nutrient accumulation via high water transport may not have as strong an adaptive value for plants with high nutrient use efficiency and low nutrient demands, as for plants with high nutrient demands and low photosynthetic nutrient use efficiency such as the cycads (Zhang et al., 2015). This possibility could be further tested with other plant groups of a wide range of nutrient use efficiencies. Another possibility is that just as the K_{leaf} values of cycads were distinctly lower than angiosperms (Table 14-2), water transport could be more limiting of nutrient transport and assimilation than for fast-growing plants.

In conclusion, we found high variation in leaf hydraulic conductance and leaf nutrient concentrations in the cycad species studied, indicating potential adaptation to habitats with distinct environmental conditions. The positive relationship between leaf nutrient concentrations and leaf hydraulic conductance and stomatal conductance on a leaf mass basis in these cycad species is a first line of evidence indicating that the facilitation of leaf water transport in nutrient uptake and distribution may be important for plant groups of low leaf water transport efficiency and/or low nutrient use efficiency. Future work manipulating leaf water transport and nutrient uptake (e.g., using a range of vapor pressure deficits and/or temperatures for cycads grown in a range of nutrient concentrations) could be used to evaluate this hypothesis mechanistically.

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