Oecologia
DOI 10.1007/s00442-013-2591-1

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Physiological mechanisms drive differing foliar calcium content in ferns and angiosperms

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Received: 14 June 2012 / Accepted: 4 January 2013

Abstract Recent evidence points to ferns containing significantly lower contents of foliar calcium and other cations than angiosperms. This is especially true of more ancient 'non-polypod' fern lineages, which predate the diversification of angiosperms. Calcium is an important plant nutrient, the lack of which can potentially slow plant growth and litter decomposition, and alter soil invertebrate communities. The physiological mechanisms limiting foliar calcium (Ca) content in ferns are unknown. While there is a lot we do not know about Ca uptake and transport in plants, three physiological processes are likely to be important. We measured transpiration rate, cation exchange capacity, and leaching loss to determine which process most strongly regulates foliar Ca content in a range of fern and co-occurring understory angiosperm species from a montane Hawaiian rainforest. We found higher instantaneous and lifetime (corrected for leaf lifespan) transpiration rates in angiosperms relative to ferns. Ferns preferentially incorporated Ca into leaves relative to strontium, which suggests that root or stem cation exchange capacity differs between ferns and angiosperms, potentially affecting calcium transport in plants. There were no differences in foliar Ca leaching loss between groups. Among the physiological mechanisms measured, foliar Ca was most strongly correlated with leaf-level transpiration rate and leaf lifespan. This suggests that interspecific differences in a leaf's lifetime transpiration may play a significant role in determining plant nutrition.

Keywords Transpiration · Calcium:strontium · Cation exchange capacity · Cation leaching · Decomposition · Leaf lifespan

Communicated by Fernando Valladares.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-013-2591-1) contains supplementary material, which is available to authorized users.

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Introduction

Ferns are an ancient plant lineage, representing nearly all growth forms and filling most ecological niches, including terrestrial trees, aquatic floating plants, and understory epiphytes and herbs. Ferns are found worldwide and can be the dominant plant species in some ecosystems, contributing substantially to standing biomass, annual productivity and nutrient cycling (Enright 1999; Walker 1994; Vitousek et al. 1995a; Raich et al. 1997; Marrs et al. 2000). Although ferns can be important to ecosystem processes, research on this cosmopolitan group has more often focused on understanding the abiotic and biotic factors that regulate their modern distributions and persistence over evolutionary time (e.g., Tuomisto et al. 2002; Kessler et al. 2011; McElwain 2011; Qian et al. 2012), rather than on how the functional traits of ferns feedback to influence ecosystem processes in the habitats in which they occur (but see Dearden and Wardle 2008; Vitousek et al. 2009).

There is mounting evidence of striking differences in element content and biochemistry between ferns and other vascular plant groups, although the physiological and biogeochemical consequences of these differences are not

Published online: 16 February 2013

fully understood. Ferns exhibit low concentrations of cations, particularly calcium (Ca), even in places where they have similar or higher nitrogen (N) and phosphorus (P) content to co-occurring angiosperms (Ma and Takahashi 2002; Richardson et al. 2005; Amatangelo and Vitousek 2008). These differences may have effects on ecosystem processes in habitats where ferns are important. Ferns in general decompose more slowly than would be expected given standard indicators of tissue quality (Perez-Harguindeguy et al. 2000; Allison and Vitousek 2004; Amatangelo and Vitousek 2009). It has been proposed that low Ca content may limit decomposition of fern litter (Amatangelo and Vitousek 2009). Calcium is correlated with root decomposition, soil acidity, and soil invertebrate activities, which ultimately affect nutrient cycling in forest systems (Silver and Miya 2001; Reich et al. 2005).

Calcium is an essential macronutrient for plants; in healthy tissue, it is found at concentrations from 0.1 to 6 % of dry weight (Epstein and Bloom 2005). Calcium plays important roles in intercellular signaling, stomatal regulation, and cell wall stabilization (White and Broadley 2003). Recent work has demonstrated that Ca also reduces cavitation risk in woody vascular plants by strengthening pit membranes, which regulate water flow between adjacent xylem vessels and also prevent the spread of embolism (Herbette and Cochard 2010).

Given its importance at scales ranging from individual plants to whole ecosystems, the processes regulating the uptake and transport of Ca in plants are surprisingly unresolved. Calcium can be incorporated into plants both passively and actively. Many studies have found evidence for passive transport of Ca through the xylem, driven by transpiration (Wiebe et al. 1978; Tibbitts 1979; Barber 1995; White 2001; Marschner 2002; White and Broadley 2003; Epstein and Bloom 2005). However, other studies have found directional movements of Ca that are not linked to transpiration, likely caused by active transport of Ca across membranes (Lee et al. 1983; De Guzman and Dela Fuente 1984; Singh and Jacobson 1979). Whether Ca is taken up and transported via active or passive mechanisms, the amount of Ca in leaves should be a function of Ca uptake from the soil, translocation and loss, where translocation is defined as the long-distance transport of water and solutes within the plant body.

Calcium is an immobile element, meaning that it is not resorbed from a leaf once it has been assimilated (Karley et al. 2000) and, therefore, foliar Ca content should be directly correlated with how much water is taken up over a leaf's lifetime. This appears to have only been examined in transpiring fruit (e.g., Montanaro et al. 2010). Leaf lifetime transpiration is influenced by several factors, including stomatal conductance (the degree that leaf pores are open to the atmosphere to allow gas exchange between the leaf and atmosphere), photosynthetic rate, and the leaf's lifespan. The degree to which the stomata of seedless vascular plants (ferns, lycophytes) respond to environmental cues including drought is currently under debate (Ruszala et al. 2011; McAdam and Brodribb 2012a). Recent studies have suggested that fern stomata respond passively to leaf water potential rather than to abscisic acid (ABA) signaling (McAdam and Brodribb 2012b). If stomata are unresponsive to ABA, the need to accumulate Ca for this purpose may be reduced. Ferns are very diverse in their xylem anatomy and organization (Carlquist and Schneider 2001, Brodersen et al. 2012) with most species displaying both vessels and tracheids with various levels of interconnections. More primitive vessels and tracheids in some fern species may not conduct water as efficiently (Watkins et al. 2010; but see Pittermann et al. 2011), resulting in lower transpiration rates (Woodhouse and Nobel 1982; Brodribb and Holbrook 2004); however, differences in leaf lifetime transpiration between ferns and angiosperms have not been examined.

During the passive transport of water from root to leaf, divalent cations such as Ca, strontium (Sr), and barium (Ba) undergo a series of exchange reactions at negatively charged sites on cell walls, which is referred to as the cation exchange capacity (CEC). Differential uptake of cations during transport influences foliar concentrations of these elements. Studies measuring Ca:Sr ratios of various plant tissues have found that plant species differ in the absorption of Ca and Sr in root and stem xylem cell walls. Overall, Sr is retained by cation exchange sites in stems to a greater extent than Ca, resulting in increased translocation of Ca relative to Sr in the foliage of woody (Poszwa et al. 2000; Dasch et al. 2006) and herbaceous (Smith 1971; Veresoglou et al. 1996) angiosperm species. The degree of discrimination against Sr in foliage varies twofold, implying that root and stem CEC varies among species, ultimately influencing foliage Ca content.

While Ca is not resorbed from leaves, it can be lost through leaching that occurs as Ca is stripped from leaves during rain events. Leaching losses can be substantial: 30 % of North American temperate forests investigated leached over 50 % of net annual Ca uptake (McLaughlin and Wimmer 1999). Calcium leaching occurs due to exchange of cuticle and cell wall calcium and by diffusion of ions from the transpiration stream (Mecklenburg et al. 1966). Differences in leaching loss rates among species therefore depends on both the thickness and morphology of the leaf cuticle that prevents the exchange of material and gases between leaves and the atmosphere (Tukey 1970; Zamierowski 1975) and the structure of cell walls. The few studies that have examined leaf morphology in ferns report both mesomorphic and xeromorphic leaf types which display significant variation in leaf thickness and specific leaf

weight (Kessler et al. 2007; Watkins et al. 2007). Additionally, the structure of plant cell walls has been shown to be distinct between ferns and other plant groups (Popper and Fry 2004); however, little is known about cuticle morphology in ferns. Thus, the role that leaf morphology plays in differential Ca leaching from fern and angiosperm leaves is unclear.

In this study, we measured three processes (transpiration rate, cation exchange capacity, leaching loss) that may regulate foliar Ca content in a range of fern and co-occurring understory angiosperm species. We conducted our experiment in a montane Hawaiian rainforest. Fern species make up a relatively large percentage of Hawaii's plant species (~16 %; Palmer 2003) and contribute substantially to productivity and nutrient cycling in Hawaiian forests (Vitousek et al. 1995a; Raich et al. 1997). Thus, understanding what limits foliar Ca in ferns and how ferns influence nutrient cycling is important to deciphering the ecology of Hawaiian forests. Our objective was to determine the relative importance of these processes in explaining low foliar Ca in ferns relative to co-occurring angiosperm species.

Materials and methods

We conducted this experiment in the Ola'a tract of Hawaii Volcanoes National Park on the Island of Hawaii. Ola'a is situated at 1,176 m with approximately 16 °C MAT and 1,500 mm MAP (Crews et al. 1995). The Thaptic Udivitrand soils at Ola'a were formed from tephra associated with the Kilauea volcano—a thin layer of ash from a 1790 eruption overlays a deeper deposit that is 2,100 years old (Vitousek 2004). Soils at Ola'a are high in available cations and have moderate availability of nitrogen and phosphorus as reflected in the foliage of the dominant tree species, *Metrosideros polymorpha* (Vitousek 2004; Vitousek et al. 1995b). Vegetation is dominated by ohia (*M. polymorpha*), tree ferns (*Cibotium* spp.), and *Cheirodendron trigynum* (Kitayama and Mueller-Dombois 1995).

We selected nine fern and six angiosperm species (Table 1). Fern phylogenies distinguish a monophyletic clade called 'polypod' ferns, consisting of the order Polypodiales, which radiated while angiosperms were becoming dominant (Pryer et al. 2004; Schneider et al. 2004; Smith et al. 2006; Schuettpelz and Pryer 2009). All other leptosporangiate ferns can be grouped into 'non-polypod' ferns (including tree ferns, filmy ferns, water ferns, gleichenioid, osmundaceous, and schizeoid ferns); radiation in non-polypod orders predated the diversification of angiosperms by tens of millions of years. We included both polypod and non-polypod ferns to ensure a wide range of calcium stoichiometry and life forms, since foliar Ca is lower in non-polypod ferns than polypod ferns in Hawaii (Amatangelo and Vitousek 2008). We selected the most common non-epiphytic understory to mid-canopy fern and angiosperm species at the site. Among ferns, two non-polypod ferns and one polypod fern form root-mat 'trunks': tree ferns (*Cibotium menziesii*, *Cibotium glaucum*), and the polypod fern *Sadleria pallida*. Angiosperm species varied phylogenetically and morphologically: monocots *Hedychium gardnerianum* and *Cyperus haspan*, dicot herb *Peperomia macraeana*, and dicot shrubs *Broussaisia arguta*, *Cyrtandra platyphylla*, and *Perrottetia sandwicensis*.

Table 1 List of species used in the study

Angiosperms	Ferns
Broussaisia arguta	*Asplenium polyodon*
Cyperus haspan	*Athyrium microphyllum*
Cyrtandra platyphylla	*Cibotium glaucum*
Hedychium gardnerianum	*Cibotium menziesii*
Peperomia macraeana	*Dicranopteris linearis*
Perrottetia sandwicensis	*Diplazium sandwichianum*
	Nephrolepis multiflora
	Pneumatopteris sandwichensis
	Sadleria pallida

Nomenclature follows Wagner et al. (1999)

In October 2006, we tagged five individuals per species. On each individual, two leaves per plant were tagged and leaf births and death rates were monitored over a 12-month period. Leaf longevity was calculated as in Ackerly (1999). We were unable to obtain leaf longevity data for two species. We lost our leaf tags on the sedge, *C. haspan*, possibly as a result of animal disturbance. Multiple attempts to tag and monitor leaves of *P. sandwicensis* failed because leaves succumbed to pathogen infestation before we could determine natural leaf longevity.

Physiological and chemical analyses were performed on recently mature leaves or subpinnae. Photosynthetic rate, transpiration rate, and chlorophyll fluorescence were measured with a LI-6400 portable gas exchange system (LI-COR, Lincoln, NE, USA). When leaves were too small to fill the chamber, the cuvette leaf area was determined and used to area-correct gas exchange data. Measurements were taken between 0800 and 1130 hours with chamber relative humidity between 60 and 70 % to ensure transpiration rates were not low due to mid-day stomatal closure resulting from high vapor pressure deficit (VPD). Ambient CO_2 concentration and light levels were held constant at 400 μL L^{-1} and 500 μmol photon s^{-1}, respectively. Leaf temperature was allowed to fluctuate and varied between 16 and 21 °C. Measurements were taken after 10 min, by which time photosynthesis and transpiration had achieved steady-state. Leaf lifetime transpiration was approximated

by multiplying leaf-level transpiration rate (mol H_2O m^{-2} s^{-1}) by leaf longevity (days). While this is not an accurate representation (because transpiration rate will vary diurnally and seasonally), this provided us with a relative measure of leaf lifetime transpiration.

After physiology measurements, leaves were harvested, scanned for leaf area, and dried to calculate leaf mass per area (LMA). An additional recently mature leaf or subpinnae was harvested from each individual and analyzed for Ca concentration and Ca:Sr ratio. Leaves were dried and ground to 40 mesh. Calcium and Sr digests were performed by wet ashing of samples in nitric acid and hydrogen peroxide; extracts were filtered and analyzed on a thermo-scientific inductively coupled plasma spectrometer (ICP). Throughout, we use the term 'concentration' to refer to a parameter expressed per unit dry mass and 'content per area' for the area basis. Foliar calcium content was unconventionally expressed per unit area in order to make units comparable to area-based physiological data.

We assessed foliar Ca leaching following the protocol in Lee and Weber (1979) and Chapin and Kedrowski (1983). Fresh–dry weight conversions were made for all species and 0.25 g of leaf dry weight (±25 %) was placed in 120 mL distilled water (pH 4.5) in specimen cups. We used freshly harvested, recently mature leaves or subpinnae. Samples were shaken at 130 rpm for 30 min and 40 mL of leachate was extracted with a syringe and stored at 20 °C prior to analysis. Leaves were dried at 60 °C for 72 h to determine dry weight. Leachate was analyzed for Ca on an atomic absorption spectrometer (Perkin-Elmer 603) and data were expressed as mg g^{-1} or mg m^{-2}.

Differences in gas exchange and foliar traits among fern and angiosperm groups were evaluated with *t* tests. Data that violated the ANOVA assumptions of normality and homogeneity of variance were Box–Cox transformed. Linear regressions were performed to evaluate the relationships among transpiration, foliar Ca content per area and foliar Ca:Sr. We examined correlations between leaching loss and the residuals of foliar Ca content per area and transpiration (instantaneous and lifetime) to see if leaching could explain variation in foliar Ca not accounted for by transpiration. To minimize the effect of outliers, we also analyzed the data using Spearman's rank correlation and used these significance values to evaluate the results. ANOVA, linear regressions, and Spearman's correlations were performed in JMP 8.0 (SAS Institute Inc., Cary, NC, USA).

We used stepwise model selection by Akaike information criterion (AIC) to determine which variables contributed most to variation in foliar Ca content per area. The initial model consisted of transpiration rate (mol H_2O m^{-2} s^{-1}), leaf lifespan (days), leaching loss (mg m^{-2}), and foliar Ca:Sr. Models were evaluated using stepAIC in the MASS package in R (http://www.r-project.org, version 2.13.2).

Results

Foliar Ca content per area, photosynthetic rate and transpiration rate were higher in angiosperms than ferns (Fig. 1a–c; Table 2). Patterns were similar for foliar Ca concentration (data not shown). Leaf lifespan was similar between groups; thus, our index of leaf lifetime transpiration (leaf-level transpiration multiplied by leaf lifespan) followed patterns of instantaneous transpiration with higher rates for angiosperms compared to ferns (Fig. 1e; Table 2). The amount of Ca leached from leaves was similar between groups when expressed on either an area or mass basis (Fig. 1f).

Across all species, foliar Ca content per area was positively correlated with transpiration rate (Fig. 2a), although this relationship was primarily driven by one angiosperm species, *B. arguta*. When the data were analyzed with Spearman's rank correlation, the relationship was not significant. Foliar Ca content was positively correlated to our index of lifetime transpiration (transpiration rate multiplied by leaf lifespan; Fig. 2b) and this result was not affected by outliers. There were no significant correlations between leaching loss and the residuals of foliar Ca (area- and mass-based) and transpiration (instantaneous and lifetime).

Foliar Ca:Sr was significantly different across species, with higher ratios found in ferns compared to angiosperms (Fig. 3a). When foliar Ca:Sr was standardized by our index of leaf lifetime transpiration, there was a larger difference between the two groups, with higher ratios found in ferns compared to angiosperms (Fig. 3b).

Foliar Ca content per area was best predicted by a model with leaf-level transpiration rate and leaf lifespan (highest AIC value, $r^2 = 0.61$, $P = 0.004$). Leaching loss and Ca:Sr were not important explanatory variables for foliar Ca.

Discussion

Transpiration played a significant role in determining foliar Ca across the broad range of fern and angiosperm species we investigated. These results extend those of previous studies (reviewed in White and Broadley 2003) and confirm that transpiration rates likely contribute to the very low foliar Ca observed in Hawaiian fern species. Our finding that ferns had lower transpiration rates than angiosperms concurs with those from Kagawa et al. (2009), who found that tree ferns had low sap flow relative to angiosperm species in similar Hawaiian forests. While

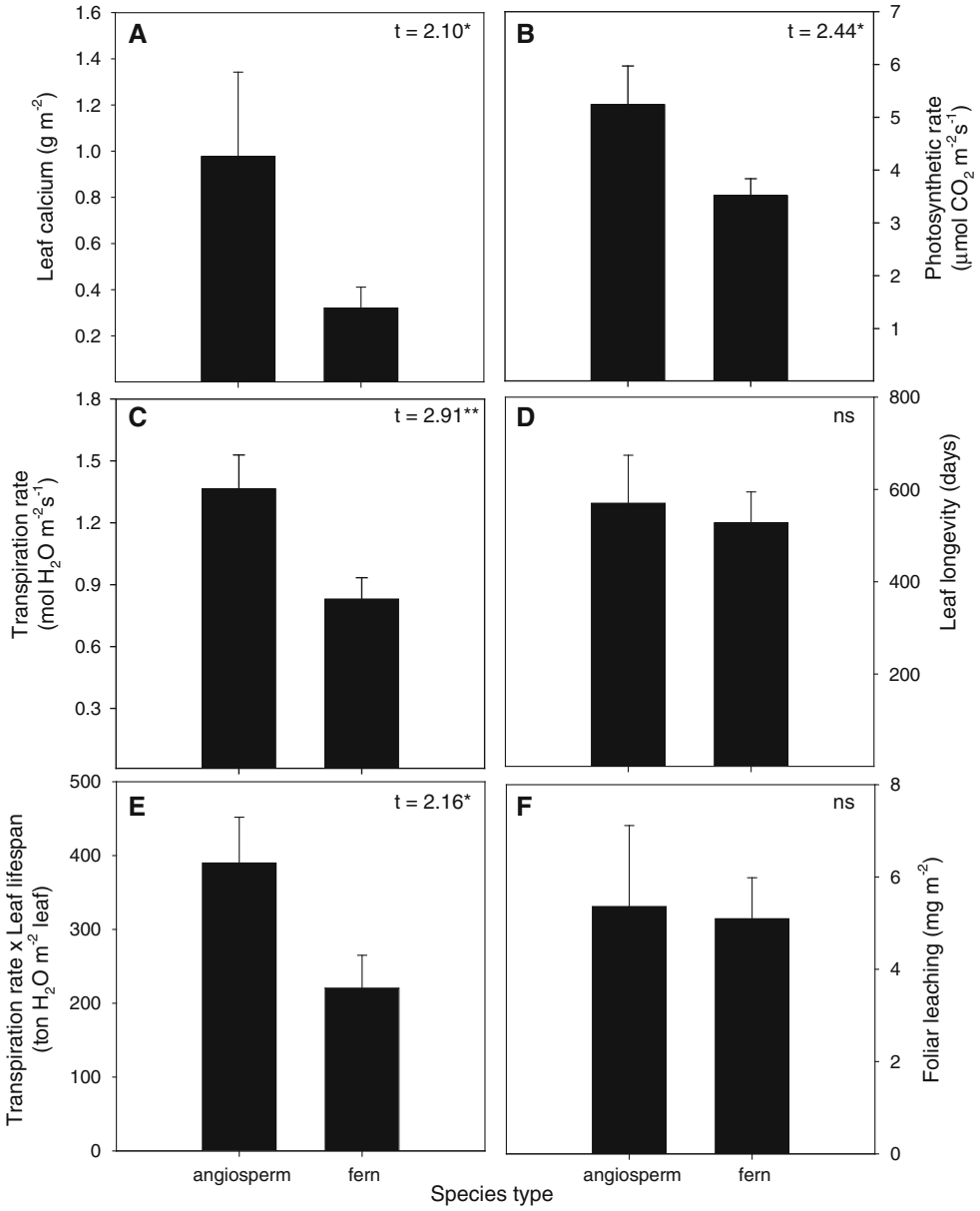

Fig. 1 Foliar Ca content per area (**a**), photosynthetic rate (**b**), transpiration rate (**c**), leaf longevity (**d**), index of leaf lifetime transpiration rate (**e**), and Ca leaching loss (**f**) for angiosperms ($n = 6$ species) and ferns ($n = 9$ species). Data are means and standard error. *T* statistics are reported at $^{*}P < 0.05$ and $^{**}P < 0.01$. *ns* no significant difference between groups

previous work has assumed that the tracheid-based vein network of ferns is inefficient in transporting water to the mesophyll (Woodhouse and Nobel 1982; Brodribb and Holbrook 2004), there is growing evidence that many fern species contain wide and efficient vessels, including species from several of the genera (*Dicranopteris*, *Cibotium*, *Nephrolepis*, *Asplenium*, *Sadleria*) examined in this study (Schneider and Carlquist 1998, 1999a, b; Carlquist and Schneider 2000). However, ferns vary in their xylem organization and connectivity. For example, Brodersen et al. (2012) found that *Pteridium aquilinum*, a drought deciduous pioneer species, had wide and efficient conduits with a large degree of connectivity, while *Woodwardia fimbriata*, a slow-growing evergreen species with long-lived fronds, had small conduits with low connectivity to limit embolism spread. Thus, the degree to which transpiration in ferns is limited by xylem morphology remains to be elucidated.

Furthermore, recent work on fern–water relationships has demonstrated that ferns are very sensitive to changes in water status (Brodribb and Holbrook 2004; Brodribb et al. 2009) and it is possible that low transpiration in ferns could be partially mediated by stomatal responses. Specifically, ferns responded to water stress by closing their stomata at much higher water potentials than angiosperms, which decreases the vulnerability of xylem to tension-induced cavitation in ferns and, consequently, transpiration rate. Recent work suggests that fern stomata respond passively to leaf water potential rather than high levels of ABA (McAdam and Brodribb 2012b). However, precipitation in

Table 2 Leaf-level data for angiosperm and fern species

Species	Species type	Foliar Ca	Foliar Ca:Sr	*A*	*E*	LLS	*E* × LLS
Broussaisia arguta	Angiosperm	3.29 (0.26)^{a}	272 (12)^{c}	8.35 (0.74)^{a}	2.05 (0.19)^{a}	529 (49)$^{b–e}$	561.6 (72.7)^{a}
Cyperus haspan	Angiosperm	0.29 (0.04)^{gh}	337 (15)^{c}	4.64 (0.92)^{bc}	1.32 (0.10)$^{a–d}$	–	–
Cyrtandra platyphylla	Angiosperm	1.49 (0.07)^{cd}	380 (34)^{bc}	4.82 (0.49)^{bc}	1.54 (0.26)^{ab}	334 (48)$^{d–f}$	267.2 (58.7)^{ab}
Hedychium gardnerianum	Angiosperm	0.60 (0.12)$^{e–h}$	529 (137)^{bc}	6.19 (0.078)^{ab}	1.19 (0.21)$^{b–e}$	578 (74)$^{a–e}$	355.5 (75.4)^{ab}
Peperomia macraeana	Angiosperm	1.90 (0.31)^{bc}	415 (36)^{bc}	4.11 (0.70)^{bc}	0.87 (0.07)$^{b–e}$	841 (151)^{a}	377.3 (66.9)^{ab}
Perrottetia sandwicensis	Angiosperm	2.25 (0.14)^{b}	190 (19)^{c}	3.32 (0.064)^{bc}	1.22 (0.12)$^{b–e}$	–	–
Asplenium polyodon	Polypod fern	1.08 (0.15)$^{d–f}$	469 (36)^{bc}	2.41 (0.49)^{c}	0.62 (0.07)$^{c–e}$	702 (30)$^{a–c}$	225.6 (28.8)^{ab}
Athyrium microphyllum	Polypod fern	0.41 (0.04)$^{f–h}$	547 (90)^{bc}	3.05 (0.54)^{c}	1.13 (0.15)$^{b–e}$	149 (12)^{f}	87.1 (13.2)^{b}
Cibotium glaucum	Non-polypod fern	0.20 (0.03)^{h}	1,394 (230)^{a}	4.08 (0.68)^{bc}	0.55 (0.11)^{de}	666 (53)$^{a–c}$	190.2 (39.7)^{ab}
Cibotium menziesii	Non-polypod fern	0.13 (0.02)^{h}	1,227 (138)^{a}	5.08 (0.51)^{bc}	0.66 (0.03)$^{c–e}$	586 (29)$^{a–d}$	200.1 (13.5)^{ab}
Dicranopteris linearis	Non-polypod fern	0.10 (0.02)^{h}	498 (46)^{bc}	4.22 (0.53)^{bc}	0.84 (0.08)$^{b–e}$	429 (42)$^{c–f}$	186.7 (25.2)^{ab}
Diplazium sandwichianum	Polypod fern	0.55 (0.07)$^{f–h}$	637 (104)^{b}	2.66 (0.29)^{c}	0.89 (0.05)$^{b–e}$	279 (22)^{ef}	128.3 (12.1)^{b}
Nephrolepis multiflora	Polypod fern	1.21 (0.17)^{de}	314 (13)^{c}	2.45 (0.30)^{c}	0.40 (0.07)^{e}	607 (33)$^{a–d}$	126.7 (23.5)^{b}
Pneumatopteris sandwichensis	Polypod fern	0.98 (0.10)$^{d–g}$	323 (16)^{c}	3.36 (0.30)^{bc}	0.99 (0.09)$^{b–e}$	592 (29)$^{a–d}$	305.2 (31.3)^{ab}
Sadleria pallida	Polypod fern	0.88 (0.07)$^{d–g}$	359 (31)^{c}	4.35 (0.91)^{bc}	1.39 (0.42)$^{a–c}$	739 (112)^{ab}	533.3 (180.9)^{a}

Data are means with standard error in parentheses ($n = 5$ or 6 individuals per species). Species means with the same lowercase letter are not significantly different from each other at $P < 0.05$. Trait abbreviations: Foliar Ca (%), *A* photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), *E* transpiration rate (mol H_2O m^{-2} s^{-1}), *LLS* leaf lifespan (days), *E* × *LLS* index of leaf lifetime transpiration (t H_2O m^{-2})

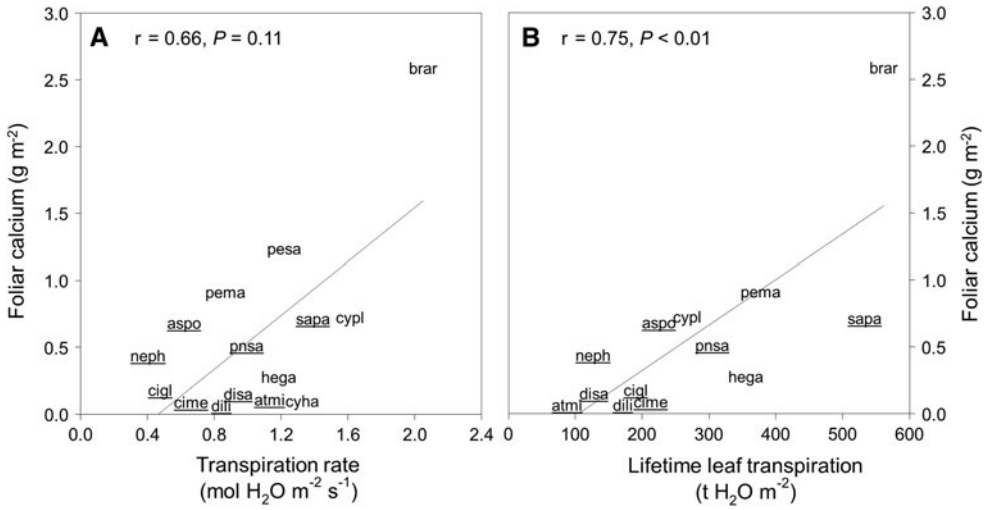

Fig. 2 The relationships between foliar Ca content per area and transpiration rate (**a**) and index of leaf lifetime transpiration rate (**b**). Data are species means ($n = 5$–6 plants per species). Four-letter species codes are the first two letters of the genus and species name. Ferns are *underlined* and angiosperms are normal text

our Hawaiian forest system is high (1,500 mm $year^{-1}$) and it is likely that factors other than stomatal regulation, such as xylem morphology, xylem organization, or leaf vein architecture, contribute the most to low transpiration in these fern species. For example, ferns have low vein density relative to angiosperms and this has been shown to contribute to low photosynthetic rate, stomatal conductance, and leaf hydraulic conductance (Brodribb et al. 2007; Boyce et al. 2009). Future work should examine anatomical indices of transpiration capacity, such as vein and stomatal density, and xylem morphology and connectivity, across fern and angiosperm species.

Our study was the first to examine differences in Ca translocation among ferns and angiosperms. Ca:Sr ratios in the soil at this site average 185 (range 137–294 depending on soil depth; Wiegand et al. 2005). The foliar values observed in this study (270–1,429) are primarily above this range suggesting that increased translocation of Ca relative to Sr occurred in all species. Our results show that ferns preferentially incorporate Ca into leaves relative to Sr to a

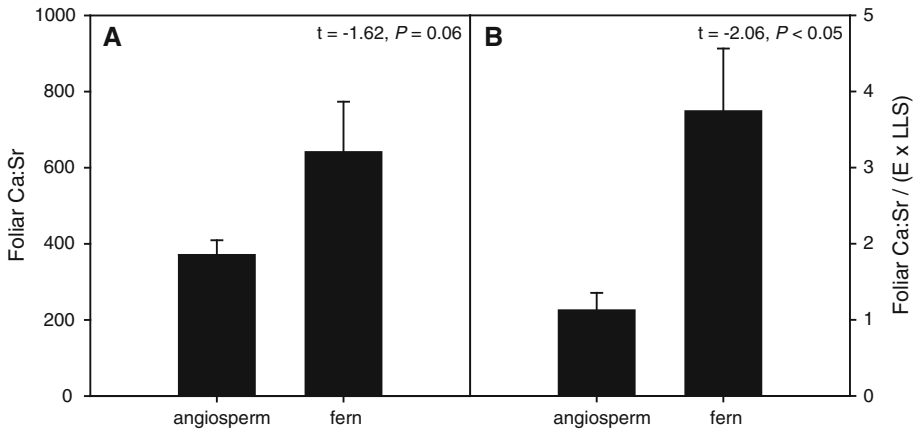

Fig. 3 Foliar Ca:Sr (**a**) and foliar Ca:Sr standardized for leaf lifetime transpiration (**b**) for angiosperms ($n = 6$ species) and ferns ($n = 9$ species). Our index of leaf lifetime transpiration is leaf-level transpiration rate (E) multiplied by leaf lifespan (LLS). Data are means and standard error. T statistics and P values are reported

greater degree than angiosperms, suggesting that CEC differs among ferns and angiosperms. Plant height is a potential explanatory factor for foliar differences in Ca:Sr as longer xylem pathways amplify differential translocation of Ca and Sr. While we did not measure height for our species, we obtained average height values for each species in the literature (Valier 1995; Wagner et al. 1999; Palmer 2003) and found an insignificant positive correlation between height and foliar Ca:Sr and no correlation between height and foliar Ca (area- or mass-based; see Online Resource). The correlation between height and foliar Ca:Sr was driven by the two tallest species in our dataset (*Cibotium* tree ferns) which had very high Ca:Sr ratios. Despite these potentially interesting differences between xylem length and function in ferns and angiosperms, the results from our multiple regression indicated that transpiration rate and leaf lifespan were more important than differences in Ca translocation in predicting foliar Ca.

While stand-level Ca leaching losses may be sizeable in this system, we found no differences in leaf-level leaching loss among fern and angiosperm species in this study. Absolute leaching losses of the species were similar, but this represented variable losses (0.2–5 %) of total foliar Ca, with ferns losing a significantly larger percentage of foliar Ca. The amount of total foliar Ca lost through leaching is comparable to losses reported elsewhere ($\sim$1 %; McLaughlin and Wimmer 1999). Although we found no significant differences between species in the laboratory, species differences in plant height and canopy position could affect leaching loss. Leaves higher in the canopy receive more direct contact with precipitation, which then flows as stem flow to the soil, bypassing other individuals (Xiao et al. 2000). Shorter individuals receive lower amounts of direct precipitation and higher amounts of throughfall, which may have higher solute content. Lower amounts of direct precipitation and a larger fraction of high-solute throughfall could reduce leaching losses in shorter-statured vegetation, although we could not find any study that has examined this. Our leaching experiment was performed with distilled water and does not reflect any differences in precipitation due to canopy position.

We focused primarily on passive mechanisms contributing to foliar Ca differences, but an active factor that may also play a role is the accumulation of Ca oxalate crystals. Calcium oxalate accumulation is hypothesized to play a role in Ca storage, the maintenance of ionic equilibrium, and defense against herbivores (Franceschi and Horner 1980). The amount of Ca oxalate in leaves can be high: one species within Cactaceae was 85 % Ca oxalate by dry weight (Franceschi and Horner 1980). McNair (1932) reported that 215 higher plant families contain species with Ca oxalate crystals, including four of the six angiosperm families sampled here. The two monocot families from which we included species, Zingiberaceae (*H. gardnerianum*) and Cyperaceae (*C. haspan*), were not found to accumulate crystals. Those species coincidentally had the lowest foliar Ca content of all angiosperm species we studied—monocots typically have low cation content (Broadley et al. 2003). Ca oxalate crystals have been identified in two fern families: Cyatheaceae and Marattiaceae (Prychid et al. 2003, Baran and Rolleri 2010). Phytoliths (mineral structures of plant origin) have been found in other fern groups, including three genera examined here (*Athyrium*, *Dicranopteris*, and *Diplazium*), but it is unclear if these minerals are Ca oxalate crystals, starch grains, tannins; or silica bodies (Mazumdar 2011). Quantifying Ca oxalate crystals was beyond the scope of our study, but may contribute to higher Ca content in some angiosperms relative to ferns. If so, this weakens support for a strong role of transpiration in leaf Ca content.

It is possible that other ecological factors affecting Ca availability and loss may contribute to species differences in foliar Ca. In addition to the physiological aspects we measured, Ca uptake is also a function of Ca availability in the soil in both ferns and angiosperms (Amatangelo and Vitousek 2008). Calcium could be patchily distributed on

the forest floor, perhaps due to calcium 'pumping' from deeper soil into foliage and then deposition in surface layers through leaching or decomposition (Tukey 1970; McLaughlin and Wimmer 1999; Dijkstra 2003). However, our site has very high Ca availability due to its young soil age, so these effects are likely to be minor. In addition to heterogeneous horizontal distribution, variable vertical distribution of soil Ca availability could affect plant access to this nutrient. At our site, soil Ca concentrations are 9.2 mg g^{-1} on the surface (0–5 cm) and then increase linearly from 49.8 mg g^{-1} at 5–12 cm to 64.8 mg g^{-1} at 62–85 cm (B.A. Wiegand, personal communication). We did not measure the rooting depths of our species and it is possible that variation in foliar Ca among species reflects differences in soil Ca availability due to rooting depth. Third, the presence of both soil and leaf fungi (mycorrhizae and endophytes, respectively) can influence the uptake and loss of nutrients. Ferns have known mycorrhizal and endophytic associates (Berch and Kendrick 1982; Arnold 2008), but differences in fungal infection rates were not examined here. Lastly, it is possible that ferns excrete unnecessary Ca in the spores, which are incidentally at the xylem terminus.

Our study focused on a subset of species found at a nutrient-rich volcanically derived site. Further research should be performed in other systems to evaluate whether our results are generalizable across climates and substrates. Additionally, we included both polypod and non-polypod ferns in this study, but there is a wide range in the relative importance of these two fern groups both across Hawaiian forests and more generally across systems worldwide. Since differences in stoichiometry within ferns may be linked to their evolutionary history (Amatangelo and Vitousek 2008), it is possible that some of the physiological mechanisms we investigated here could also vary between these two plant groups. Future research should include broader representation of the non-polypod ferns and directly compare them with polypod ferns.

In conclusion, we found that inter-specific differences in foliar Ca were most closely linked to a leaf's lifetime transpiration, as represented by leaf-level transpiration rate and leaf lifespan in our study. This result has important implications for plant nutrition and biogeochemical cycling in a changing world. Increases in atmospheric CO_2 concentration and drought associated with climate change will likely lower stomatal conductance and, consequently, transpiration rates (e.g., Cramer et al. 2001). Lower transpiration results in lower Ca content of plant tissues and this will have implications for nutritional quality of crops as well as rates of biochemical cycling (Loladze 2002). Given that low Ca content may limit the rate of fern litter decomposition (Amatangelo and Vitousek 2009), further reductions in Ca content could further reduce rates of biogeochemical cycling. Thus, inter-specific differences in transpiration may play a significant role in how climate change influences decomposition rates and this merits further study.

Acknowledgments We thank Radika Bhaskar, Laura Schreeg, Erika Sudderth, Lawren Sack's laboratory group, Fernando Valladares, and two anonymous reviewers for comments on the article and Peter Vitousek, Heraldo Farrington, Linda Pratt, and Doug Turner for logistical, field and laboratory support. We greatly appreciate the support of the staff and volunteers at Resources Management Division, Hawaii Volcanoes National Park.

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