

Stoichiometry of ferns in Hawaii: implications for nutrient cycling

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Abstract We asked if element concentrations in ferns differ systematically from those in woody dicots in ways that could influence ecosystem properties and processes. Phylogenetically, ferns are deeply separated from angiosperms; for our analyses we additionally separated leptosporangiate ferns into polypod ferns, a monophyletic clade of ferns which radiated after the rise of angiosperms, and all other leptosporangiate (non-polypod) ferns. We sampled both non-polypod and polypod ferns on a natural fertility gradient and within fertilized and unfertilized plots in Hawaii, and compared our data with shrub and tree samples collected previously in the same plots. Non-polypod ferns in particular had low Ca concentrations under all conditions and less plasticity in their N and P stoichiometry than did polypod ferns or dicots. Polypod ferns were particularly rich in N and P, with low N:P ratios, and their stoichiometry varied substantially in response to differences in nutrient availability. Distinguishing between these two groups has the potential to be useful both in and out of Hawaii, as they have distinct properties which can affect ecosystem function. These differences could contribute to the widespread abundance of polypod ferns in an angiosperm-dominated world, and to patterns of nutrient cycling and limitation in sites where ferns are abundant.

Keywords Pteridophyte · Angiosperm · Plasticity · Phylogeny · Nitrogen-to-phosphorus ratio

Introduction

Structures and metabolic pathways common to all plants provide some underlying consistency to the concentrations and relative abundances—the stoichiometry—of the elements they contain. However, terrestrial plants differ more substantially in stoichiometry within and among species and higher phylogenetic groupings than do other groups of organisms (Sterner and Elser 2002). Causes of this variation include differences in: structure, storage, and defense (Aerts and Chapin 1999; Reiners 1986); growth form and growth rate (Meerts 1997; Shaver and Chapin 1980; Van Arendonk and Poorter 1994); and element availability in the environment (Bowman et al. 2003; Vitousek et al. 1995b). This variation has often been correlated with phylogeny (Broadley et al. 2004, 2001; Kerkhoff et al. 2006; Thompson et al. 1997). Plants differ not only in their element contents under defined conditions, but also in the plasticity of their stoichiometry in differing environments (Knecht and Goransson 2004).

Element stoichiometry influences element cycling, trophic interactions, and decomposition; as such it has long been of interest to agronomists and ecologists (Garten 1976; Waksman and Tenney 1928). The elemental composition of herbivores and decomposers differs substantially from those of the plants they consume and the litter they decompose (Sterner and Elser 2002; Vitousek 2003). These differences affect the biological availability of essential plant nutrients and ultimately the success of plants that differ in element requirements.

Research in phytoplankton has demonstrated that element concentration and ratios are conserved in some phylogenetic groups—mirroring the conditions under which those groups evolved (Quigg et al. 2003). Many ratios that are conserved within phylogenetic phytoplankton groups

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are also conserved among vascular plants. Among angiosperms, concentrations and ratios of some abundant elements (base cations and Si) vary among orders but are relatively consistent within them (Broadley et al. 2004, 2001; Chenery and Sporne 1976; Hodson et al. 2005); similar phylogenetic signals have been observed for N and P in seed plants (Kerkhoff et al. 2006). This conservation of element profiles within plant groups could allow us to predict nutrient-mediated impacts of particular species on nutrient cycling and other ecosystem processes. For example, high concentrations of Si in commelinoid monocots and high N in legumes have significant, but opposite, effects on palatability to herbivores and decomposers (Cornelissen and Thompson 1997; Ma and Yamaji 2006; Ritchie et al. 1998).

Ferns represent a particularly interesting group in which to evaluate both phylogenetic correlates with stoichiometry and the consequences of this variation for ecological processes. Ferns differ substantially from angiosperms in structure and biochemistry, and their ecological uniqueness and importance is well recognized (Page 2002; Rathinasabapathi 2006; Rothwell 1996). Fern phylogenies distinguish a monophyletic clade called “polypod” ferns, consisting of the order Polypodiales, that radiated while angiosperms were becoming dominant (Pryer et al. 2004; Schneider et al. 2004; Smith et al. 2006). All other leptosporangiate ferns can be grouped into “non-polypod” ferns (including tree ferns, filmy ferns, water ferns, gleichenioid, osmundaceous, and schizeoid ferns); the major radiation in non-polypod orders pre-dated the diversification of angiosperms by tens of millions of years. The nutrient physiology of ferns has received relatively little attention (although see Hohne and Richter 1981; Richardson et al. 2005; Wegner et al. 2003), and only As accumulation has been addressed from a phylogenetic perspective (Meharg 2002). Both non-polypod and polypod ferns are widespread and abundant, and even co-dominant with angiosperms in some ecosystems—particularly forested ecosystems where woody dicots comprise much of the overstory. To the extent that polypod and non-polypod ferns differ in stoichiometry and plasticity from each other and from angiosperm forest dominants, this widespread co-occurrence offers the opportunity to determine how phylogenetic group differences in element contents can influence ecosystems. In this paper, we ask if ferns generally, and polypod ferns in particular, differ from other plant groups in element concentrations and/or ratios in ways that could influence the functioning of ecosystems in which ferns are abundant.

We address two main questions in this study

1. Are there differences between ferns and woody dicots, and between fern groups, with regards to stoichiometry when all are grown on the same substrate?
2. Are there differences in the plasticity of element concentrations and ratios among plant groups, when compared across sites that differ substantially in nutrient availability or in response to soil fertilization?

Materials and methods

We carried out this research in the Hawaiian Islands for several reasons. First, ferns are abundant in many ecosystems across the archipelago, with a variety of species dominating the subcanopy or ground layer of forests. The non-polypod ferns *Cibotium* spp. and *Dicranopteris linearis* in particular can contribute substantially (35–75%) to stand-level productivity and nutrient cycling (Raich et al. 1997; Vitousek et al. 1995a), and several polypod genera including *Elaphoglossum*, *Diplazium*, and *Dryopteris* dominate the understory of other stands. Second, the availability of nutrients varies substantially, but in well-understood and well-characterized ways, across the forests of the Hawaiian archipelago (Crews et al. 1995; Vitousek 2004) allowing us to address plasticity of foliar nutrient content. Finally, we have maintained long-term soil fertilization experiments in three sites for at least 13 years, further expanding the range of soil nutrient availability accessible to this analysis.

Fern species were sampled from four sites arrayed along a soil development sequence in which all sites are near 1,200 m elevation and 2,500 mm annual rainfall, but on substrates that range in age from 300 years to 4.1 million years (0.3–4,100 kyears) (Crews et al. 1995). Soils and dominant trees of the 0.3 kyear site are rich in cations and Si, low in available N and P (0.98 kg m⁻² N, 0.036 kg m⁻² organic P in 50-cm soil profiles, Crews et al. 1995), and production there is limited by N (Vitousek et al. 1993). Both the 20- and 150 kyear sites are low in cations and Si and rich in N and P (1.56 kg m⁻² N, 0.155 kg m⁻² organic P, 1.46 kg m⁻² N, 0.202 kg m⁻² organic P, respectively). The 4,100 kyear site is moderately rich in N and P (1.13 kg m⁻² N, 0.112 kg m⁻² organic P), low in cations and Si, and P supply limits plant production (Herbert and Fownes 1995).

The 0.3-, 20-, and 4,100 kyear sites contain replicated factorial fertilization experiments that have been ongoing since 1985, 1993, and 1991, respectively (Vitousek 2004). In addition to unfertilized plots, we sampled ferns from plots fertilized with N (100 kg ha⁻¹ year⁻¹), P (100 kg ha⁻¹ year⁻¹) and a treatment consisting of all other essential plant nutrients (T) that included K (100 kg ha⁻¹ year⁻¹), Ca (100 kg ha⁻¹ year⁻¹), Mg (58 kg ha⁻¹ year⁻¹), S (40 kg ha⁻¹ year⁻¹), Fe (8 kg ha⁻¹ year⁻¹), Mo (0.01 kg ha⁻¹ year⁻¹), and a mixed micronutrient supplement containing Mn, Mo, Zn, Cu, and B.

We collected young, fully developed leaves from the terrestrial ferns that were abundant within each site (Table 1);

these were clipped, dried at 60°C and ground to 40 mesh in a Wiley mill. C and N were analyzed on a Carlo Erba NA 1500 elemental analyzer. Cation digests (P, Ca, K, Mg, Al) were performed by wet ashing of samples in HNO₃ and H₂O₂; extracts were filtered and analyzed on a Thermo Scientific inductively coupled plasma spectrometer (ICP). A subset of samples was sent to the Marine Science Department analytical lab at University of Hawaii Hilo for Si analysis. Samples were dry ashed in a muffle furnace, followed by HF digestion and ICP analysis

Element data for macronutrients (N, P, Ca, K, Mg) for 20 native terrestrial fern species (five non-polypod, fifteen polypod) were compared with data from six common, dominant woody dicot species collected at the four chronosequence sites and from the native dominant tree *Metrosideros polymorpha* collected from all the fertilizer plots. *M. polymorpha* foliage was collected and analyzed at

Table 1 Species included in analyses; dicot species from Vitousek et al. 1995a, b. Voucher specimens are not available

Non-polypod ferns	
<i>Cibotium glaucum</i> (Sm.) Hook. & Arn.	(A, B, C, D) ^a
<i>Cibotium menziesii</i> Hook.	(A, B, C, D)
<i>Dicranopteris linearis</i> Und.	(A, C, D)
<i>Diplazium pinnatum</i> (Kunze) Nakai	(B, D)
<i>Sticherus owyhensis</i> (Hook.) Ching	(D)
Dicots	
<i>Metrosideros polymorpha</i> Gaudich.	(A, B, C, D)
<i>Cheirodendron trigynum</i> A.Heller	(A, B, C, D)
<i>Ilex anomala</i> Hook. & Arn.	(A, B, C, D)
<i>Coprosma</i> spp.	(A, B, C, D)
<i>Vaccinium calycinum</i> Sm.	(A, B, C, D)
<i>Myrsine lessertiana</i> A.DC.	(A, B, C)
Polypod ferns	
<i>Asplenium normale</i> D.Don	(B, D)
<i>Asplenium polyodon</i> G.Forst.	(B, C, D)
<i>Athyrium microphyllum</i> (Sm.) Alston	(B, C, D)
<i>Diplazium sandwichianum</i> (Pr.) Diels	(B, C, D)
<i>Dryopteris fusco-atra</i> Robinson	(C)
<i>Dryopteris glabra</i> (Brack.) Kuntze	(B, C, D)
<i>Dryopteris wallichiana</i> (Spreng.) Hyl.	(B)
<i>Elaphoglossum crassaule</i> Copel.	(D)
<i>Elaphoglossum paleaceum</i> (Hook. & Grev.) Sledge	(A, C, D)
<i>Elaphoglossum parvisquamum</i> Skottsb.	(A)
<i>Nephrolepis cordifolia</i> (L.) C.Presl	(C, D)
<i>Pteridium aquilinum</i> (L.) Kuhn	(D)
<i>Sadleria cyatheoides</i> Kaulf.	(D)
<i>Sadleria pallida</i> Hook. & Arn.	(A)
<i>Sadleria souleyetiana</i> (Gaud.) T.Moore	(A, D)

^a Sites at which species were collected are indicated by letters in parentheses [0.3 kyears (A), 20 kyears (B), 150 kyears (C), 4,100 kyears (D)]

the same time as these fern collections, and found not to differ significantly from earlier publications (Vitousek 1998; Vitousek et al. 1995b). The sampled dicot species include both forest shrubs and trees, and comprise much of the overstory biomass in Hawaiian forests.

To evaluate changes of nutrient content, we calculated species phenotypic plasticity indices (PPI) (Valladares et al. 2000). PPI ranges from 0 to 1 and is calculated as the difference between species maximum and minimum mean values, divided by the maximum. Environmental PPI (PPI_e) values were calculated based on between-site differences in element concentrations for each species that occurred at multiple sites in control (unfertilized) plots. In addition, fertilization PPIs (PPI_f) were calculated for species that occurred in both control and fertilization plots at the same site—where species occurred in control and fertilization plots at multiple sites, the largest PPI_f for those species was used.

Differences among plant groups in nutrient concentrations and PPI within sites were evaluated with *t*-tests adjusted for multiple comparisons. As not all species were found at all sites, significant influences of site nutrient availability on plant concentration of an element or ratio were evaluated with a mixed-model analysis of covariance (ANCOVA) on each plant group separately, with species as a random effect and nutrient availability of that element as a fixed effect. To detect separation of groups based on nutrient concentrations, we submitted mean nutrient concentration data (N, P, Ca, K) for species in control plots at the four sites to a principal component analysis. All statistics were performed on log-transformed data. All statistical analyses were performed in JMP 5.1.2 (SAS Institute, Cary, N.C.).

Results

Differences among groups within sites

At each of the four chronosequence sites, concentrations of some of the macronutrients differed among dicots, non-polypod ferns, and polypod ferns (Table 2). At the relatively N- and P-rich 20- and 150 kyear sites, polypod ferns had significantly more foliar N and P than dicots and non-polypod ferns, which did not differ significantly from each other. Differences among plant groups for those elements were smaller at the low N (0.3 kyears) site, where non-polypod ferns had the highest N, and at the low P (4,100 kyears) site, where non-polypod ferns had the highest P. Across all four sites, polypod ferns had the highest K content, and at all four sites non-polypod ferns had extremely low Ca and Mg content (less than 0.1% foliar Ca at the two oldest sites). Total macronutrient cation (Ca + K + Mg) content was significantly lower in non-polypod ferns than in polypod ferns or dicots at all sites. Dicots,

Table 2 Foliar nutrient concentrations (% dry mass) of non-polypod ferns, polypod ferns, and dicots across a gradient of soil development. Different letters indicate significant differences among groups within a site, calculated by *t*-tests adjusted for multiple comparisons

Element	Group	Site			
		0.3 kyears ^a	20 kyears ^b	150 kyears ^b	4,100 kyears ^c
N	Dicots*	1.09 ± 0.08 a	1.51 ± 0.19 a	1.33 ± 0.12 a	1.24 ± 0.16 a
	Polypod*	1.08 ± 0.02 a	2.96 ± 0.35 b	2.12 ± 0.37 b	1.80 ± 0.20 a
	Non-polypod	1.52 ± 0.23 a	1.54 ± 0.17 a	1.46 ± 0.15 a	1.35 ± 0.13 a
P	Dicots*	0.08 ± 0.01 a	0.10 ± 0.01 a	0.11 ± 0.01 a	0.08 ± 0.01 a
	Polypod*	0.20 ± 0.12 a	0.17 ± 0.02 b	0.35 ± 0.04 b	0.13 ± 0.01 a
	Non-polypod*	0.13 ± 0.01 a	0.09 ± 0.01 a	0.14 ± 0.01 a	0.15 ± 0.04 a
Ca	Dicots*	1.65 ± 0.30 a	1.13 ± 0.24 a	1.09 ± 0.20 a	1.05 ± 0.18 a
	Polypod	0.61 ± 0.18 a	0.33 ± 0.07 b	0.43 ± 0.12 b	0.57 ± 0.09 a
	Non-polypod	0.11 ± 0.02 b	0.10 ± 0.03 c	0.10 ± 0.01 c	0.09 ± 0.01 b
K	Dicots*	1.21 ± 0.34 a	0.76 ± 0.18 a	0.49 ± 0.08 a	1.02 ± 0.27 a
	Polypod	2.00 ± 0.29 a	1.75 ± 0.33 b	1.99 ± 0.21 b	2.33 ± 0.25 b
	Non-polypod*	0.99 ± 0.08 a	0.68 ± 0.11 a	0.65 ± 0.05 b	0.99 ± 0.10 a
Mg	Dicots	0.28 ± 0.02 a	0.31 ± 0.04 a	0.36 ± 0.06 a	0.24 ± 0.05 ab
	Polypod	0.30 ± 0.09 ab	0.30 ± 0.04 a	0.40 ± 0.08 a	0.33 ± 0.04 a
	Non-polypod	0.13 ± 0.02 b	0.13 ± 0.01 a	0.13 ± 0.01 b	0.12 ± 0.01 b
Macro-nutrient cations ^d	Dicots*	3.14 ± 0.52 a	2.27 ± 0.39 a	1.95 ± 0.22 a	2.32 ± 0.42 a
	Polypod*	2.91 ± 0.02 a	2.40 ± 0.32 a	3.08 ± 0.26 b	3.23 ± 0.30 a
	Non-polypod*	1.22 ± 0.05 b	0.92 ± 0.14 b	0.75 ± 0.26 c	1.21 ± 0.10 b
N:P	Dicots*	14.1 ± 1.70 a	15.46 ± 0.58 a	11.89 ± 0.8 a	16.18 ± 1.06 a
	Polypod*	10.8 ± 2.50 a	14.2 ± 2.81 a	6.95 ± 1.27 a	15.10 ± 1.72 a
	Non-polypod	12.4 ± 2.50 a	16.46 ± 1.96 a	11.53 ± 1.76 a	11.1 ± 2.38 a

* $P < 0.05$ (significant effect of site nutrient availability on group nutrient concentration or ratio from a mixed-effects analysis of covariance)

^a Soil is rich in cations and relatively low in N and P

^b Soil is high in N and P and lower in cations

^c Soil is low in P and cations

^d Macronutrient cations include Ca, Mg, and K

polypod ferns, and non-polypod ferns separated with a principal components analyses using N, P, Ca, and K (Fig. 1) at all four sites, although at the youngest site polypod ferns were relatively similar to dicots. Principal components axis 1 (PCA1) explained 46% of the variation, and was

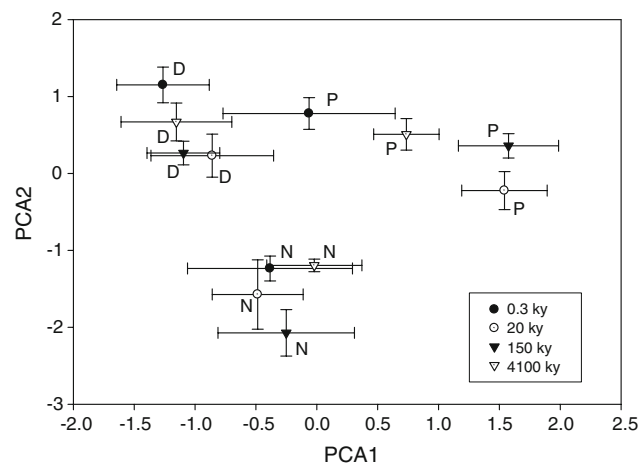


Fig. 1 Principal component analysis of log element concentrations in dicots (*D*), non-polypod ferns (*N*), and polypod ferns (*P*) at four sites, projected onto a plane defined by the first two principal components. The first principal components axis (*PCA1*) and second principal components axis (*PCA2*) explain 46.8 and 26.4% of the variation, respectively: $PCA1 = 0.57N + 0.62P - 0.15Ca + 0.52K$, $PCA2 = -0.18N + 0.05P + 0.90Ca + 0.39K$

dominated by N and P; principal components axis 2 (*PCA2*) explained an additional 26% and was dominated by Ca and to a lesser extent K.

Differences within groups among sites on a natural fertility gradient

Foliar nutrient concentrations varied significantly ($P < 0.05$) with changing nutrient availability along the age gradient across the Hawaiian Islands (Table 2). P and total macronutrient cation concentrations differed significantly in all groups. Individual cations were variably affected, with Mg content not differing significantly in any group, variation in Ca significant only in dicots, and variation in K significant only in non-polypod ferns and dicots. In contrast to polypod ferns and dicots, N in non-polypod ferns did not vary significantly across the sites. The plasticity of element concentrations within species across sites (as measured by PPI) differed among the three groups (Table 3). Non-polypod ferns varied least in N and P content. Dicots had a particularly high PPI with regards to K, as did polypod ferns with regards to P. P concentration more than doubled in polypod ferns in the richest site relative to the poorest, whereas concentrations in non-polypod ferns and dicots increased around 50%. However, principal components analysis (above) demonstrated that groups had a larger and more consistent effect on macronutrient concentrations than

Table 3 Phenotypic plasticity indices for species across sites that differ in soil fertility (PPI_e), or with fertilization (PPI_f). See text for calculation. Different letters indicate significant differences ($P < 0.05$) in PPI among groups

Group	Effect	N	P	Ca	Mg	K
Non-polypod	PPI_e	0.18 ± 0.02 a	0.33 ± 0.08 a	0.59 ± 0.09 a	0.25 ± 0.07 a	0.47 ± 0.10 a
Polypod	PPI_e	0.26 ± 0.09 a	0.57 ± 0.06 b	0.44 ± 0.05 a	0.29 ± 0.07 a	0.15 ± 0.03 b
Dicots	PPI_e	0.28 ± 0.05 a	0.35 ± 0.05 a	0.59 ± 0.05 a	0.40 ± 0.07 a	0.60 ± 0.07 a
Non-polypod	PPI_f	0.13 ± 0.05 a	0.47 ± 0.07 a	0.39 ± 0.12 a	0.20 ± 0.08 a	0.22 ± 0.08 a
Polypod	PPI_f	0.27 ± 0.04 a	0.67 ± 0.02 b	0.31 ± 0.05 a	0.24 ± 0.04 a	0.19 ± 0.04 a

did plasticity in response to variation in nutrient availability (sites), and the three groups separated out significantly at all four sites (Fig. 1).

Element ratios

N:P ratios were variable and did not differ significantly among groups at any site, although there were differences among sites in dicots and polypod ferns (Table 2). The N:P range for polypod ferns across sites was greater (7.5) than that of dicots and non-polypod ferns (both 4.5). We also investigated ratios between macronutrient cations that may substitute for Ca in plant structures. K:Ca and Mg:Ca ratios in ferns were significantly wider than ratios in dicots at all sites. Polypod and non-polypod fern K:Ca ratios averaged 7.2 and 14.4 times wider than dicot ratios, respectively, and Mg:Ca ratios averaged 2.4 and 5.8 times wider.

Responses to fertilization

Fertilization affected foliar nutrient concentrations significantly in both groups of ferns for all five macronutrients ($P < 0.05$). Plasticity in response to fertilization (PPI_f) was similar to plasticity in response to differences in nutrient availability among sites (PPI_e), although fertilization had larger effects on P content, and generally smaller effects on macronutrient cations (Table 3). For seven species that grew at the 150 kyear fertile site (the site naturally richest in P), the 4,100 kyear site (the poorest in P), and in P-fertilized plots in the 4,100 kyear site, P content increased significantly between sites and with fertilization in all five polypod species, and increased only in one of the two non-polypod ferns (and then only with fertilization) (Fig. 2). Polypod fern P concentrations increased from 85 to 250%, whereas non-polypod fern P increased from 2 to 55%.

Other elements

Silica concentrations varied substantially among fern species, but the differences did not segregate between non-polypod and polypod ferns. Three polypod species (*Elaphoglossum paleaceum*, *Dryopteris glabra*, and *Asplenium polyodon*) had

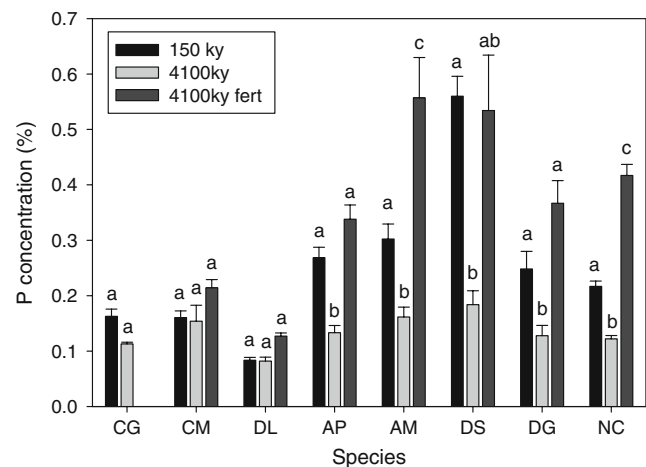


Fig. 2 Responses of P concentrations of eight fern species to differences between sites (150 and 4,100 kyears) that differ substantially in nutrient availability, and with P fertilization at the 4,100-kyear, P-limited site. Different letters indicate significant differences within species ($P < 0.05$). CG *Cibotium glaucum*; CM *Cibotium menziesii*; DL *Dicranopteris linearis*; AP *Asplenium polyodon*; AM *Athyrium microphyllum*; DS *Diplazium sandwichianum*; DG *Dryopteris glabra*; NC *Nephrolepis cordifolia*; CG, CM, DL non-polypod fern species

very low Si content while the other species contained above 0.3% Si. Si concentrations decreased with increasing site age in all eight species investigated (Table 4), consistent with declining Si in soils across this gradient (Vitousek 2004). Al content was very low in all species except *Dicranopteris linearis*; as a result, Al:Si ratios were >1 in that species and far lower in the others. Two other species in the Gleicheniaceae (*Sticherus owyhensis* and *Diplopterygium pinnatum*) also accumulated high levels of Al (unpublished data), but the non-polypod *Cibotium* species did not. The Si:Ca ratio, an indication of potential nutrient substitution (and relative Si accumulation) varied widely between species and across groups, from 1.2 to 10.2 in non-polypod ferns and from 0.03 to 3.1 in polypod ferns.

Discussion

Woody dicots, polypod ferns, and non-polypod ferns have substantially different profiles of element concentrations for

Table 4 Si (% dry mass), Al (p.p.m.), Al:Si, and Ca:Si of several widely distributed ferns at three sites. n.d. Non-detectable

Site	<i>Cibotium glaucum</i> ^a	<i>Cibotium menziesii</i> ^a	<i>Dicranopteris linearis</i> ^a	<i>Athyrium microphyllum</i> ^b	<i>Asplenium polyodon</i> ^b	<i>Dryopteris glabra</i> ^b	<i>Elaphoglossum paleaceum</i> ^b	<i>Sadleria souleyetiana</i> ^b
0.3 kyears	Si 0.443 ± 0.14	0.324 ± 0.023	0.373 ± 0.036	–	–	–	0.064 ± 0.007	1.889 ± 0.377
150 kyears	Si 0.368 ± 0.053	0.252 ± 0.018	0.244 ± 0.032	0.535 ± 0.096	0.034 ± 0.005	0.029 ± 0.002	0.043 ± 0.006	–
4,100 kyears	Si 0.255 ± 0.002	0.079 ± 0.011	0.224 ± 0.065	0.243 ± 0.051	0.027 ± 0.004	0.036 ± 0.011	0.034 ± 0.004	0.218 ± 0.069
0.3 kyears	Al 118.2 ± 14.8	37 ± 10.2	3795 ± 217	–	–	–	3.0 ± 1.5	1.4 ± 1.4
150 kyears	Al 102.6 ± 15.4	42.8 ± 4.6	4653 ± 630	73.9 ± 14.2	14.8 ± 6.6	108.2 ± 24.1	20.6 ± 3.3	–
4,100 kyears	Al 119.3 ± 25.3	32 ± 12.4	2593 ± 500	79.5 ± 29.3	n.d.	17 ± 4.9	18.2 ± 7.6	89.7 ± 39.1
0.3 kyears	Al:Si 0.137 ± 0.113	0.014 ± 0.007	1.029 ± 0.04	–	–	–	0.005 ± 0.002	<0.001
150 kyears	Al:Si 0.028 ± 0.003	0.017 ± 0.001	1.991 ± 0.294	0.016 ± 0.005	0.035 ± 0.015	0.386 ± 0.083	0.048 ± 0.004	–
4,100 kyears	Al:Si 0.047 ± 0.01	0.038 ± 0.011	1.299 ± 0.234	0.032 ± 0.011	n.d.	0.068 ± 0.03	0.051 ± 0.021	0.074 ± 0.038
0.3 kyears	Si:Ca 4.148 ± 1.086	1.219 ± 0.302	6.134 ± 0.660	–	–	–	0.211 ± 0.025	3.141 ± 0.548
150 kyears	Si:Ca 9.179 ± 2.301	10.24 ± 1.411	3.525 ± 1.060	1.16 ± 0.25	0.050 ± 0.018	0.121 ± 0.023	0.155 ± 0.047	–
4,100 kyears	Si:Ca 2.512 ± 0.126	1.455 ± 0.506	2.555 ± 0.316	0.65 ± 0.10	0.028 ± 0.006	0.094 ± 0.027	0.050 ± 0.006	0.489 ± 0.138

^a Non-polypod ferns^b Polypod ferns

elements that contribute substantially to ecosystem structure and functioning. While non-polypod fern N and P concentrations were comparable to those of dicots, as has been demonstrated previously (Richardson et al. 2005; Vitousek et al. 1995b), we observed that polypod ferns often have significantly higher foliar N and P concentrations than other plant groups tested from the same site. Herbaceous angiosperms tend to have higher N and P concentrations than woody angiosperms (Kerkhoff et al. 2006); polypod ferns may function more like herbaceous angiosperms than do tree ferns and other non-polypod ferns. Both non-polypod and polypod ferns resorb similar proportions of N and P as do angiosperms (Allison and Vitousek 2004), and polypod fern litter is often high in N and P content at these sites (unpublished data). Rates of both production and litter decomposition are demonstrably nutrient limited in the infertile 0.3- and 4,100 kyear sites (Harrington et al. 2001; Hobbie and Vitousek 2000), and rapid decomposition of polypod ferns with nutrient-rich litter could increase local nutrient availability.

In contrast to N and P, all measured ferns had low Ca concentrations, remarkably so in the non-polypod ferns. Even the relatively Ca-rich polypod ferns had N:Ca ratios averaging 7 across all sites, higher than angiosperms and even higher than the ratio of those elements in grasses measured at a nutrient-rich site (Neff et al. 2006). Ca concentrations in non-polypod ferns in two sites were below 0.1%, comparable to Ca concentrations in the most nutrient-poor monocots (Broadley et al. 2003; McLaughlin and Wimmer 1999; White and Broadley 2003). Low concentrations of divalent cations in non-polypod ferns are not limited to Hawaii: tree ferns and gleichenioid ferns elsewhere also exhibit low divalent cations but are relatively rich in K compared to co-occurring dicots (Enright and Ogden 1987; Tanner 1977; Vitousek 2003). The availability of Ca can affect the multiple ecosystem functions, including limitations on decomposition rate and interactions with invertebrates (Hobbie et al. 2006; McLaughlin and Wimmer 1999; Silver and Miya 2001). Ca-poor non-polypod ferns often decompose slowly in Hawaii and at other sites, in ways not well explained by traditional predictors of decomposition rate (Allison and Vitousek 2004; Scowcroft 1997; Wardle et al. 2002). Low Ca concentrations in non-polypod fern foliage could slow decomposition and delay the release of nutrients from litter.

Ca concentrations were once thought to be passively controlled by transpiration rates (Bangerth 1979), and indeed they may be when the concentration of Ca in the xylem is high (White and Broadley 2003). However, active exclusion of Ca from the xylem, and so decoupling from transpiration rate, appears to be more important in regulating Ca concentrations (White and Broadley 2003). Ca concentrations as low as those we observe in non-polypod

ferns imply substantial physiological differences from dicots. A major pool of Ca in plants is in cell walls, where Ca crosslinks and stabilizes pectins. Some plants may form covalent crosslinks with phenolic acids in their cell walls, reducing reliance on Ca stabilization (Fry 1986). Extremely low Ca in monocots may also reflect the substitution of Si for Ca in cell walls (Broadley et al. 2003; Cornelissen and Thompson 1997). A survey of Si:Ca in plants demonstrated that many ferns have high ratios, potentially indicative of a monocot-like substitution, and Si:Ca generally decreased between early vascular plants and polypod ferns (Ma and Takahashi 2002). In particular, species in the non-polypod fern group and the group of polypod ferns designated “Athyriales” until recently (Smith et al. 2006) were considered Si accumulators (Si:Ca > 1). We observed relatively high Si and Si:Ca in both of those groups, in contrast to the other species investigated (Table 3). In addition to Si, other cations may substitute for Ca in some processes, and K:Ca and Mg:Ca are both significantly wider in non-polypod ferns than polypod ferns, which in turn have wider ratios than angiosperms (McLaughlin and Wimmer 1999).

Although non-polypod ferns may use a unique combination of cations for essential cell functions, their total cation content is consistently lower than that of polypod ferns and dicots, even when environmental availability is high. This observation raises the possibility that the environments in which ancient ferns and their relatives evolved and diversified were low in available cations, necessitating the evolution of structures requiring few cations—or alternatively, that ways of using Ca in structure had not yet evolved. Conversely, ancient ecosystems may have had high concentrations of toxic multivalent cations. Non-polypod ferns may have retained some ancestral characteristics, as they often persist in environments edaphically inhospitable to other plants due to either extremely low nutrient concentration or high concentrations of toxic elements (Galtier and Phillips 1996; Page 2002). Low relative growth rates are common in plants from nutrient-poor soils, and they often correlate with low element concentrations (Meerts 1997). Ferns are relatively slow growing (Page 2002; Walker and Aplet 1994)—low growth rates, or active selection at the roots, could reduce toxicity due to elements such as iron (Fe) and Al (Snowden and Wheeler 1993). Additionally, Si in plants provides protection against high concentrations of toxic metals (Foy et al. 1978), and high-Al plants generally also have high Si levels (Hodson and Evans 1995). Al accumulation is considered a primitive trait in the angiosperms (Chenery and Sporne 1976), and in fact, we only observed Al accumulation in the Gleicheniales in the non-polypod fern grade. Local Si and macronutrient cation concentrations may affect the success of non-polypod ferns across the range of soil properties in Hawaii; tree ferns generally become decreasingly important with substrate age,

although gleichenioid ferns are common in patches, particularly on slopes, at all but the nutrient-rich 20-kyear-old site.

Polypod ferns are rich—and relatively plastic—in N and P compared to non-polypod ferns, consistent with their diversification after the rise of angiosperms. Angiosperm dominance likely provided a more complex, nutrient-rich, and competitive environment (Schneider et al. 2004), and polypod ferns evolved in direct competition with fast-growing angiosperms in the densely shaded understory of forests (Feild et al. 2003; Feild et al. 2004). As a result, polypod ferns may have evolved increased resource capture, in addition to the evolution of a novel photoreceptor necessary for life in shade (Kawai et al. 2003). Non-polypod ferns, in contrast, exhibit lower levels of nutrient plasticity consistent with evolution in a harsh, nutrient-poor environment (Neff et al. 2006). In our sites, polypod ferns are nearly absent at the N- and P-poor youngest site, but increase in importance in older sites. In low-nutrient, particularly low-P understories, disturbances may release pulses of nutrients, which smaller, more quickly growing polypod ferns can acquire. Additionally, the ability to store luxury nutrients when they become available, and quickly return them to the roots through rapid decomposition rates, could contribute to the transition in understory dominance from non-polypod to polypod ferns along the soil chronosequence. Edaphic gradients influence fern diversity and composition in tropical forests outside of Hawaii as well (Tuomisto and Poulsen 1996; Tuomisto et al. 2002).

Leptosporangiate ferns in Hawaii can be separated into at least two sub-groups, distinct from each other and co-occurring dicots, based on macronutrient concentration and phenotypic plasticity. The relatively small number of non-polypod ferns in Hawaii prevents certainty that among-group differences occur in other systems; however, available data from the literature demonstrate macronutrient cation trends are consistent where non-polypod ferns dominate (Enright and Ogden 1987; Tanner 1977). Contrasts in element concentrations between ferns and angiosperms, and between non-polypod and polypod ferns, have the potential to affect ecosystem-level nutrient cycling. Plant groups can replace each other as understory dominants in space (along environmental gradients) and time (in succession and ecosystem development) in Hawaii and many other tropical and subtropical environments. Moreover, in many areas the fern-dominated understories of Hawaiian forests are being replaced by angiosperm invaders—high-nutrient (N, P, and cations), fast-growing, easily decomposable plants that are adapted to human-altered environments (Allison and Vitousek 2004; Thompson et al. 1997). These angiosperm invasions into non-polypod fern-dominated systems could alter the cycling of Ca and other cations substantially. Distinguishing among plant groups has proved useful in

ecosystem-scale research when groups are correctly designated in regards to the question being asked (Petchey and Gaston 2006; Violle et al. 2007). Identifying generalizable characteristics of groups such as non-polypod and polypod ferns can be useful for predicting how communities and ecosystems change in response to human-induced alterations.

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