Phylogenetic relatedness within Neotropical fern communities increases with soil fertility

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ABSTRACT
Aim To examine the relative importance of gradients in soil fertility and rainfall for the phylogenetic structure of Neotropical forest fern communities, and to quantify how much the results are affected by phylogenetic resolution.

Location Tropical lowland forests in Brazil (central Amazonia) and Panama (the Panama canal watershed).

Methods We inventoried local fern communities at a total of 87 sites to model how their species richness and phylogenetic relatedness varied along gradients in soil fertility and rainfall. We produced a time-calibrated species-level molecular phylogeny of ferns, and quantified the phylogenetic relatedness of species within local communities using Faith’s phylogenetic diversity (PD) and mean phylogenetic diversity (MPD). Calculations were compared for phylogenies resolved to the species and genus levels.

Results (1) We found significant and consistent effects of soil nutrient status on local phylogenetic community structure in both regions. In contrast, phylogenetic structure showed only a weak or no relationship with rainfall. (2) In both regions, MPD declined with increasing soil fertility, which means that fern communities on poor soils consisted, on average, of less closely related lineages than fern communities on rich soils. (3) Different fern genera were over-represented in different sections of the soil nutrient gradient. (4) Qualitatively similar results were obtained whether phylogenies were resolved to the species or the genus level.

Main conclusions Our results highlight the importance of edaphic variation in structuring plant communities over evolutionary time-scales. Within the tropical forests studied, the effects of soil variation on local phylogenetic community structure seem to outweigh those of climate. Several fern genera show strong edaphic niche conservatism to either poor or rich soils, whereas many other genera have radiated to span a rather broad edaphic range. PD-based measures are so dominated by deep relationships that genus-level phylogenies are sufficient to investigate these patterns in tropical fern communities.

Keywords Community phylogenetics, edaphic variation, niche conservatism, phylogenetic overdispersion, phylogenetic underdispersion, precipitation, Pteridophyta.
communities in tropical forests. Adaptive radiation along eco-
logical gradients other than climate is also likely to have con-
tributed to the diversification of rain forest floras (e.g. Fine et al.,
2005, 2013; Tuomisto, 2006). There is, for example, ample evi-
dence for edaphic determinism of community composition (e.g.
Tuomisto & Poulsen, 1996; Tuomisto et al., 2003a,b; Phillips et al.,
2003; ter Steege et al., 2006; Jones et al., 2013), but the degree of conservatism in the edaphic niches of tropical plants
remains under-explored (but see Schreeg et al., 2010; Fine &
Kembel, 2011).

It has become increasingly common to investigate the phylogenetic relatedness of co-occurring species in order to
understand the links between evolution, historical biogeogra-
phy, and ecological processes in shaping local community struc-
ture (e.g. Webb et al., 2002; Emerson & Gillespie, 2008; Vamosi
et al., 2009). Phylogenetic clustering is observed when species in
a community are more closely related to each other than
expected by chance. This has generally been interpreted as evi-
dence of niche conservatism: close relatives are constrained to
occur in similar environments (Webb et al., 2002). Phylogenetic
overdispersion is observed when species in a local community
are less closely related to each other than expected by chance.
This has been suggested to reflect competitive interactions
between close relatives (Webb et al., 2002). Alternative explana-
tions for phylogenetic overdispersion include the idea that older
environments contain proportionately more representatives of
old lineages than do more recent environments (Kooyman et al.,
2011). In general, stable environmental conditions may promote
regional species accumulation through a reduced probability of
extinction, enabling the persistence of old lineages, and perhaps
also by promoting speciation. However, speciation may also be
augmented by environmental variability (Doebeli &
Dieckmann, 2003).

Relatively few studies have examined trends in the phylogenetic structure of tropical plant communities along
environmental gradients other than climate. These have mostly
focused on tropical trees or palms at relatively broad spatial
scales and across coarse habitat categories (Fine & Kembel, 2011;
Eiserhardt et al., 2012; Umaña et al., 2012). Studies including
quantitative soil data have mostly been restricted to local spatial
scales (e.g. Schreeg et al., 2010), although Eiserhardt et al. (2012)
used soil sand content as a surrogate for fertility at broader
scales. Most importantly, these studies illustrate that habitat spe-
cialization, in association with habitat evolution over long time
periods, can leave a signal in phylogenetic community structure.
For example, there is some evidence that past geophysical pro-
cesses are reflected in the phylogenetic structure of western
Amazonian tree communities (Fine & Kembel, 2011).

Rain forests with a modern plant family composition were
apparently established in the Neotropics in the late Palaeocene
(Wing et al., 2009) or early Eocene (Wilf et al., 2003). Geological
history and molecular systematic data suggest that in Amazonia
these early forests may have been adapted to poor soils derived
from the Guiana Shield (Kubitzki, 1989; Frasier et al., 2008).
More fertile soils with a higher clay content began to develop in
Amazonia after the uplift of the Andes and consequent fluvial
inputs of nutrient-rich sediments from the mountains into the
lowlands (Kubitzki, 1989). These became widely distributed
after the drainage of the west Amazonian Pebas wetland system
in the Late Miocene (Räsänen et al., 1995). Within Amazonia
poor soils are both older and more widespread than rich soils, so
poor soils might be expected to have also accumulated more
species there. However, a recent study on pteridophytes (ferns
and lycophytes) found the opposite trend (Tuomisto et al.,
2014). In contrast to the Amazon region, Central America com-
prises a mosaic of volcanic and sedimentary soils derived from
geological formations of generally more recent origin, produced
by its gradual evolution from a chain of volcanic islands to a
continuous land mass upon the closure of the isthmus of
Panama c. 3 Ma (Kirby et al., 2008).

Here we use a phylogenetic hypothesis of unusually high
quality to compare the phylogenetic community structure of
ferns across soil and rainfall gradients in two Neotropical forest
regions with different geological histories: central Amazonia and
central Panama. The younger age and more dynamic recent
history of the Isthmus of Panama can be predicted to have
resulted in more phylogenetically clustered community assem-
blages at a regional level than in central Amazonia, where long-
term geological stability is likely to have resulted in a
proportionately greater representation of older lineages, pro-
ducing less clustered communities. We also test if the diversity
patterns observed in Amazonia (more species in the younger
and less widespread substrates) are repeated at a broader scale,
and whether phylogenetic information can shed light on the
factors behind these patterns. Within each region, we also assess
if (and, if so, how) phylogenetic community structure varies
with local environmental conditions. We furthermore compare
the results obtained with phylogenies resolved to the species and
genus levels to assess to what degree phylogenetic inferences
are affected by the accuracy of information on interspecific
relationships.

METHODS

Field sampling and environmental variables

We inventoried understory ferns in lowland tropical forests in
two geographical areas, central Amazonia (the states of
Amazonas and Roraima in Brazil) and Panama (the Panama
Canal watershed; Fig. 1). All 32 Amazonian sites were at less
than 200 m above sea level (a.s.l.) in non-inundated old-growth
forests. Most sites had tall forests on clayey to loamy soils (terra
firme), but two had white sand soils and forests with a reduced
stature and relatively simple canopy structure (campinarana).
The 55 Panamanian sites were predominantly at less than 300 m
a.s.l. (with one site at 530 m and one at 660 m) and the forests
were of variable ages, from secondary to old growth. Each Pan-
amanian site was represented by a sampling unit of 0.1 ha that
consisted of two parallel transects (100 m × 5 m) separated by
about 35 m. Each Amazonian site was originally sampled using
a transect of 500 m × 5 m, but to match the sampling unit size
Figure 1 Locations of 55 fern inventory plots in Panama and 32 in central Amazonia (white triangles). The grey scale represents altitude according to Shuttle Radar Topographic Mission data.

used in Panama only the central 200 m were used in the present analyses.

Understorey ferns were defined as ferns growing either on the ground or as epiphytes or climbers on the lower parts of tree trunks. To be included in the inventory, a fern individual had to have at least one green leaf longer than 10 cm, and this had to be less than 2 m above ground if the individual was epiphytic or climbing. In the data analyses, no separation was made between individuals on different substrates. All individuals were identified to species or given a field name if the species name was not known. To verify the identifications, representative voucher specimens were collected of all entities with a different field name. Duplicates of the Amazonian material are deposited in two herbaria in Brazil (SP, full set; INPA, fertile specimens) and one in Finland (TUR). Duplicates of the Panamanian material are deposited in two herbaria in Panama (PMA, full set; STRI) and one in Finland (TUR). The specimens were first sorted into morphospecies that were thought to correspond to biological species, and then assigned species names with the help of taxonomic literature and by comparisons with existing Neotropical fern material at TUR. Some closely related species were lumped before analysis because they were not consistently distinguished in the field. Unnamed morphospecies were treated as good species if they were found to be both monophyletic and genetically distinct from their nearest relative (details explained below).

Geographical coordinates for each sampling unit were obtained using a hand-held GPS. Estimates of annual mean precipitation for all sites were extracted from the WorldClim database (Hijmans et al., 2005; http://www.worldclim.org/bioclim) at a resolution of 2.5 arcmin using the DIVA-GIS software (Hijmans et al., 2001).

Composite surface soil samples were collected in each sampling unit to determine the concentration of extractable bases in the soil (Ca, Mg, and K). The samples were air-dried and sieved through a 2-mm mesh before analysis. In Amazonia, three soil samples were taken along each transect, one close to each end and one in the middle (see Tuomisto et al., 2003a, for further details on the methodology). The cations were extracted with 1 M ammonium acetate at pH 7 at MTT Agrifood Research (Jokioinen, Finland). In Panama, two soil samples were taken at each site within a square 1-ha plot that contained both fern inventory transects (see Jones et al., 2013, for further details). The cations were extracted with the Mehlich-III method at the soil laboratory of the Smithsonian Tropical Research Institute (STRI, Panama City, Panama). Before numerical analyses, the cation concentrations of samples from the same site were averaged to obtain a single cation value for each sampling unit.

Because the MTT and STRI soil laboratories used different protocols for extracting the cations, 23 of the 110 Panamanian soil samples were used as calibration samples and analysed in both laboratories. These samples were selected to represent the entire range of soil cation concentrations in the Panama data. A linear regression model was parameterized with the calibration samples to predict MTT-equivalent values of log-transformed soil cation concentration on the basis of the STRI-measured values ($R^2 = 0.996$). The regression equation was applied to those soil samples that were only analysed at STRI; for the 23 samples analysed at both laboratories, the measured values from MTT were used.

In most analyses, we used annual rainfall and log-transformed soil cation concentration as continuous variables. However, for those analyses requiring an environmental classification the soil cation concentration gradient was divided into four classes and the precipitation gradient into three classes (Table 2). Precipitation class limits were chosen to coincide with breaks in the distribution of the observed values in the data, whereas the log-transformed soil cation gradient was simply divided evenly into four classes. The poorest-soil class consisted of Amazonian sites only and the richest-soil class of Panamanian sites only, but the two intermediate classes contained sites from both areas. The rainfall classes were geographically less biased, although the wettest class was dominated by Panamanian sites.

**DNA extraction and sequencing**

For each species we extracted DNA from one to three specimens. We amplified and sequenced the plastid atpB, atpA, and rps4 sequences produced from these extractions for other purposes. Laboratory work followed established protocols (Lehtonen, 2011). Altogether, this resulted in 413 atpB, 17 atpA, 5 rps4, and 30 rps4 sequences (GenBank accession numbers KJ628500–KJ628963).

**Phylogenetic analyses**

To produce a phylogeny of as high a quality as possible, we used the large dataset analysed in Lehtonen (2011) and added to it both the new DNA sequences described above and sequences made available in GenBank release 184 (15 June 2011). Details on dataset construction are given in Lehtonen (2011).
We tested the monophyly of our species by performing an equally weighted parsimony analysis based on 500 ‘new technology’ search replications in TNT 1.1 (Goloboff et al., 2008). Whenever a species was resolved as monophyletic, we discarded any duplicate samples and retained only one specimen, except when the same species was present in both Panama and Brazil. In this case, a specimen from each region was retained. If unnamed morphospecies were not diagnosable from their DNA sequences, they were lumped until they formed a genetically distinguishable monophyletic unit. We lacked DNA samples of six observed species. For five of these, we were able to use a sequence from GenBank but one species that occurred in a single transect in central Amazonia had to be excluded from the analyses.

After removing duplicate samples, the final data set consisted of 3137 operational terminal units (OTU), which here will be referred to as ‘species’ for simplicity. These represent about 25% of the global fern flora (Moran, 2008) and 4392 aligned base pairs of molecular data (rbcL 1316 bp, rps4 379 bp, atpB 1188 bp, atpA 1509 bp). The final data set was analysed under the maximum likelihood (ML) criterion using the parallel Pthreads-version of the computer program RAxML 7.3.0 (Stamatakis, 2006; Ott et al., 2007). The search was initiated with 500 rapid bootstrap replications followed by a thorough ML search on the full data matrix (-T 16 -f a -x 12345 -p 12345 -R 500 -m GTRGAMMA). Free model parameters were estimated by RAxML under the GTR + Γ model.

The obtained ML phylogeny was pruned to retain only those species that occurred in our Panamanian and Amazonian field data using the command sampleprune in PHYLOCOM (Webb et al., 2008). Pruning preserved the topological relationships and branch lengths of the original ML tree, but greatly reduced the number of terminal taxa and hence allowed time calibration. The pruned phylogeny was time calibrated with a penalized likelihood method in r8s 1.70 (Sanderson, 2003). The root node age was fixed to 354 Ma following Pryer et al. (2004), and the ages of a further 64 internal nodes were fixed according to their age estimates as given by Schuettpelz & Pryer (2009). The final ultrametric tree was used in all subsequent analyses.

Community structural analyses

The phylogenetic structure of the fern community in each sampling unit was quantified using three different metrics. Species richness is simply a count of the terminal taxa in the final ultrametric tree that were encountered in the sampling unit. Phylogenetic diversity (PD) is the total length of the branches connecting these terminal taxa (Faith, 1992), and mean phylogenetic diversity (MPD) is PD divided by species richness (Webb et al., 2002). Because we used a dated phylogeny, both PD and MPD are in practice measured in units of time (millions of years). In fact, MPD/2 can be interpreted as the average time that has passed since a randomly chosen species pair from the phylogeny diverged from a common ancestor. Each metric was calculated separately for each sampling unit using PHYLOCOM version 4.2 (Webb et al., 2008).

We tested for two kinds of phylogenetic bias in the fern communities. First, we tested whether particular clades were significantly over-represented or under-represented in different parts of the environmental gradients. For each sampling unit we generated a pool of 999 reference communities randomly drawn from the final ultrametric tree, each consisting of the number of species originally observed, using the ‘nodesig’ command in PHYLOCOM (Webb et al., 2008). Then we tested for clade over- or under-representation in sampling units belonging to each of the four soil cation concentration classes and three rainfall classes. Because small clades have a high probability of being absent from random communities, under-representation is in practice only detectable at deeper phylogenetic levels even if it occurs throughout the phylogeny (Parra et al., 2010). For this reason, we focus mostly on over-representation.

The second phylogenetic bias we tested for was whether MPD in the sampling units differed from random expectation within each study region. This was done by randomizing species identities within each of the two regional species lists separately. Local communities were considered significantly clustered if their MPD values were within the lowest 2.5% of the values obtained for randomized communities, and significantly dispersed if the values were within the highest 2.5%.

Linear and second-order polynomial regression analyses were run to test whether species richness, PD and MPD were significantly related to gradients in soil cation concentration or annual rainfall.

All analyses were done using species presence–absence data. The sensitivity of the analyses to phylogenetic resolution was tested by collapsing all intrageneric nodes in the phylogeny and repeating the analyses using genus-level resolution.

RESULTS

The total number of species encountered (gamma richness) was 106 in our Panamanian data and 66 in our central Amazonian data. The average number of genera per sampling unit was seven for Panama and six for central Amazonia; on average, 1.5 species per genus were observed per sampling unit in both regions. The average number of species per sampling unit (alpha richness) was 10.5 for Panama and 8.9 for central Amazonia (Table 1). Consequently, beta richness (i.e. gamma/alpha) was 10.1 for the Panamanian data and 7.4 for the central Amazonian data. The higher total number of species encountered in Panama therefore reflected both a higher species density at the local scale and a higher heterogeneity among the sampling units. The higher heterogeneity, in turn, was consistent with the fact that the number of sampling units was larger in Panama, and that both the rainfall gradient and, especially, the soil cation concentration gradient were longer (Table 1). The geographical extent of sampling, however, was far smaller in Panama than in central Amazonia (Fig. 1).

Analyses of both PD and MPD gave very similar results whether the phylogeny was resolved to the species level or to the genus level (Fig. 2). In the following, we focus on the results for species resolution.
We found that PD was strongly correlated with species richness. Species richness and PD both had a hump-shaped response to soil cation concentration in Panama, and also when data from both regions were combined. In central Amazonia, the trends were not statistically significant (Fig. 3, Table 2). MPD decreased with increasing soil cation concentration in both Panama and central Amazonia, as well as for the combined data. Soil and rainfall gradients were not correlated with each other (correlation coefficient $-0.12$ in Panama and $-0.06$ in central Amazonia), hence their effects were modelled separately. All diversity metrics were better explained (higher $R^2$ value) by the regression model with soil cation concentration than by the one with annual rainfall (Table 2). In Panama, PD and MPD increased with annual rainfall, and when both areas were considered together species richness and PD also increased with rainfall. No significant trends with rainfall were observed within central Amazonia.

Two central Amazonian sampling units had phylogenetically clustered fern communities, and these were the ones with the highest soil cation concentrations (Fig. 3). Similarly, phylogenetically clustered communities in Panama were mostly

### Table 1 General description of study sites.

<table>
<thead>
<tr>
<th></th>
<th>Panama</th>
<th>Amazonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of 0.1-ha plots</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Total species richness</td>
<td>106</td>
<td>66</td>
</tr>
<tr>
<td>Species richness per plot</td>
<td>$11 \pm 5$ (3–22)</td>
<td>$9 \pm 4$ (2–18)</td>
</tr>
<tr>
<td>Genus richness per plot</td>
<td>$7 \pm 3$ (2–15)</td>
<td>$6 \pm 2$ (2–12)</td>
</tr>
<tr>
<td>Soil cation concentration (cmol(+) kg$^{-1}$)</td>
<td>$23.03 \pm 18.50$ (0.55–65.62)</td>
<td>$0.30 \pm 0.43$ (0.07–2.58)</td>
</tr>
<tr>
<td>Annual precipitation (mm)</td>
<td>$2582 \pm 403$ (1866–3421)</td>
<td>$2388 \pm 261$ (1827–2974)</td>
</tr>
</tbody>
</table>

Soil cation concentrations for Panama have been adjusted to ensure comparability with values for central Amazonia (see Field sampling and environmental variables for details). Means ± SD are given, with the total range in parentheses.
Table 2  Explanatory power (adjusted $R^2$) of log-transformed soil cation concentration and total annual rainfall in linear and second-order polynomial regression models with fern species richness, phylogenetic diversity (PD) or mean phylogenetic diversity (MPD) as the dependent variable.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Region</th>
<th>Log(soil cations) linear</th>
<th>Log(soil cations) quadratic</th>
<th>Annual rainfall linear</th>
<th>Annual rainfall quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richness</td>
<td>Amazonia</td>
<td>0.07/0.03</td>
<td>0.03/0.04</td>
<td>-0.03</td>
<td>-0.01/-0.02</td>
</tr>
<tr>
<td>Richness</td>
<td>Panama</td>
<td>0.02/0.04</td>
<td>0.30***</td>
<td>0.07*/0.16**</td>
<td>0.07/0.15</td>
</tr>
<tr>
<td>Richness</td>
<td>All</td>
<td>0.01</td>
<td>0.16***/0.20***</td>
<td>0.07***/0.13**</td>
<td>0.06/0.12</td>
</tr>
<tr>
<td>PD</td>
<td>Amazonia</td>
<td>-0.01</td>
<td>0.03/0.05</td>
<td>-0.03</td>
<td>-0.04</td>
</tr>
<tr>
<td>PD</td>
<td>Panama</td>
<td>0.11**</td>
<td>0.35***/0.36***</td>
<td>0.16*/0.14**</td>
<td>0.14/0.12</td>
</tr>
<tr>
<td>PD</td>
<td>All</td>
<td>0.00</td>
<td>0.20**</td>
<td>0.09***/0.07**</td>
<td>0.08/0.06</td>
</tr>
<tr>
<td>MPD</td>
<td>Amazonia</td>
<td>0.29***/0.28**</td>
<td>0.30**</td>
<td>-0.03</td>
<td>-0.04</td>
</tr>
<tr>
<td>MPD</td>
<td>Panama</td>
<td>0.35****/0.36***</td>
<td>0.38/0.39</td>
<td>0.06*/0.05</td>
<td>0.07/0.05</td>
</tr>
<tr>
<td>MPD</td>
<td>All</td>
<td>0.42****/0.43***</td>
<td>0.47***/0.48**</td>
<td>-0.01</td>
<td>0.00</td>
</tr>
</tbody>
</table>

When two values are shown, the first value refers to analyses at species-level resolution and the second value to analyses at genus-level resolution. Regressions were run both for the two geographical regions separately and for all data combined. Statistical significance: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

Table 3  Breakdown of the 87 sample plots by geographical region, soil cation concentration and annual rainfall, with the average values of fern species richness, phylogenetic diversity (PD) and mean phylogenetic diversity (MPD) shown for the plots belonging to each category.

<table>
<thead>
<tr>
<th>Region</th>
<th>Plots</th>
<th>Species</th>
<th>PD</th>
<th>MPD</th>
<th>MPDrnd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Amazonia</td>
<td>32</td>
<td>8.9</td>
<td>1281</td>
<td>380</td>
<td>384</td>
</tr>
<tr>
<td>Panama</td>
<td>55</td>
<td>10.5</td>
<td>1313</td>
<td>311**</td>
<td>335</td>
</tr>
<tr>
<td>Soil cation concen-</td>
<td>0.07–0.28 cmol(+) kg$^{-1}$</td>
<td>27</td>
<td>8.6</td>
<td>1281</td>
<td>389</td>
</tr>
<tr>
<td>tration</td>
<td>0.42–1.87 cmol(+) kg$^{-1}$</td>
<td>9</td>
<td>8.9</td>
<td>1281</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td>2.58–11.47 cmol(+) kg$^{-1}$</td>
<td>15</td>
<td>13.5</td>
<td>1686</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>11.97–65.62 cmol(+) kg$^{-1}$</td>
<td>36</td>
<td>9.7</td>
<td>1160</td>
<td>284***</td>
</tr>
<tr>
<td>Annual rainfall</td>
<td>0.07–0.28 cmol(+) kg$^{-1}$</td>
<td>27</td>
<td>8.6</td>
<td>1281</td>
<td>389</td>
</tr>
<tr>
<td></td>
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<td>1160</td>
<td>284***</td>
</tr>
</tbody>
</table>

MPDrnd gives the random expectation for MPD generated by randomly shuffling species labels across the phylogeny 999 times. Statistical significance of departures of MPD from MPDrnd: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

For geographical locations of the plots, see Fig. 1.

on high-cation soils, whereas dispersed communities were on intermediate soils. Patterns in relation to rainfall were less clear, but phylogenetically clustered communities in Panama were absent from the wettest sites and phylogenetically dispersed communities from the driest ones. No phylogenetically dispersed communities were observed in central Amazonia.

The mean MPD of the fern communities in our sampling units averaged 311 Ma in Panama and 380 Ma in central Amazonia (Table 3). The difference (69 Ma) is significantly larger than expected by chance (49 Ma). An even larger difference was observed between the sampling units belonging to the most cation-rich of the four edaphic classes (average MPD 284 Ma) and those belonging to the most cation-poor class (389 Ma). This difference (105 Ma) was more than twice as large as the difference expected by chance (49 Ma). In other words, species in Amazonian communities and on cation-poor soils were found to be more distantly related than species in Panamanian communities and on cation-rich soils.

The nodal significance test revealed that some fern genera were concentrated on specific parts of the soil cation gradient (Fig. 4). Lindsaea, Trichomanes, and especially Triplophyllum were over-represented in communities growing on the cation-poor soils. Adiantum was common in all soil categories but had its highest representation on cation-rich soils, whereas Thelypteris and Tectaria were clearly over-represented in the most cation-rich soil class. Species in the other lineages were more broadly distributed across the soil cation concentration gradient.

The relative species richness of genera specializing on poor soils (Lindsaea, Trichomanes, Triplophyllum) steadily decreased towards cation-rich soils whereas the relative species richness of genera specializing on rich soils (Tectaria, Thelypteris) increased (Fig. 5). The relative species richness of genera specializing on rich soils varied considerably even in the richest soils, whereas the poorest soils were largely dominated by genera specializing on poor soils. Relatively few biases were identified in relation to annual rainfall, with the clearest pattern at the genus level being the over-representation of Thelypteris in the intermediate rainfall class.

**DISCUSSION**

**Impact of soil and rainfall gradients on phylogenetic community structure**

We found significant and consistent effects of soil nutrient status on the phylogenetic community structure of ferns. These results are in striking contrast with the view that soil niches are too labile to leave a detectable phylogenetic signal (Swenson, 2013). Within
both regions, fern communities in richer soil habitats exhibited a higher degree of phylogenetic relatedness than those in poorer soil habitats. In Panama, phylogenetic relatedness tended to decline with increasing rainfall, although the effect was weaker than that of soil cation concentration. Significant phylogenetic turnover with rainfall has also been detected in Panamanian tree communities (Hardy et al., 2012), but trends in the phylogenetic relatedness of trees have not been examined along either rainfall or edaphic gradients. We found no detectable effects of rainfall on the phylogenetic community structure of ferns in central Amazonia. This difference between the two regions is not surprising given that all of the central Amazonian sites were in evergreen forests, whereas some of the Panamanian sites were in semi-deciduous forests. It may also reflect the poorer quality of digital climate data in Amazonia, where meteorological stations are extremely sparse (Hijmans et al., 2005).

Eiserhardt et al. (2012) found that palm communities on extremely nutrient-poor white sand soils in western Amazonia tended to be phylogenetically overdispersed when compared with the regional species pool, whereas communities on richer upland and floodplain soils tended to be clustered. This result parallels the trend we observed in mean MPD, even though the MPD values of our individual poor-soil communities did not deviate from random expectation. Fine & Kembel (2011) found that the choice of which phylogenetic diversity index to use determined whether phylogenetic overdispersion or random structure was found in the tree flora of white sand soils in Peruvian Amazonia. Kluge & Kessler (2011) investigated the phylogenetic structure of tropical fern communities along an elevational gradient in Costa Rica. They did not find any clear pattern, and hypothesized that communities may be structured more with respect to local habitat conditions than the elevational gradient itself. Our results provide support for the importance of local conditions.

**Poor soils are inhabited by more distantly related species than rich soils**

Our results from both regions are consistent with the idea that older lineages tend to be disproportionately represented
in fern communities on poor soils relative to those in richer soils, as has been found for tropical trees in Peruvian Amazonia (Fine & Kembel, 2011). Furthermore, we observed that the lineages making up local communities are older, on average, in central Amazonia than in Panama. Because the poorest soils were found in central Amazonia and the richest soils in Panama, it is difficult to cleanly separate between the effects of soils and geographical location. Nevertheless, the fact that the differences in average lineage relatedness was clearly greater between the poorest and richest soils (105 Ma) than between the two study regions (69 Ma) suggests that soil may be more important than geographic region for the observed pattern.

The differences in MPD are largely driven by the basal phylogenetic position of the genera specializing on poor soil, *Lindsaea* and *Trichomanes*, which were more consistently present in poor-soil sites than was the derived poor-soil specialist genus *Triplophyllum*. All three genera were relatively poorly represented in Panama, as has previously been noted for *Triplophyllum* (Prado & Moran, 2008). Whether this is caused by the lack of suitable soil conditions or other factors such as historical dispersal limitation remains to be clarified. Similarly, genera such as *Tectaria* and *Telopea* that specialize on rich soils were very species poor in our central Amazonian sites, as has been observed in earlier inventories in the region (Tuomisto & Poulsen, 1996; Zuquim et al., 2009). This is in agreement with the generally low cation concentrations of central Amazonian soils. In conclusion, the difference between Panama and central Amazonia in the commonness of these genera seems partly attributable to different soil properties, which in turn are determined by the distinct geological histories of the two regions.

White sands have been suggested to represent the ancestral habitat for Amazonian species (e.g. Kubitzki, 1989; Frasier et al., 2008), with much recent speciation occurring in younger and more fertile soils. However, in our phylogeny there is no evidence that crown-group diversification differs temporally between groups specializing on poor and rich soils. Poor-soil specialists do tend to belong to older lineages, but the main diversification within these lineages appears to be no more ancient than that of the rich-soil specialists. Further studies that specifically address the timing of diversification, and also take into account taxa from other geographical areas, are needed to clarify this point.

Poor soils in both regions had relatively low fern species richness, as has been found in earlier studies (Tuomisto et al., 2014). Overall, the richness–soil fertility relationship was hump-shaped across all sites, and also within Panama. Low species richness in the richest soils in Panama may, however, be due to limited water availability, since these include shallow soils with a low water-holding capacity on limestone, sandstone and other sedimentary rocks. The tree layer on these soils includes a significant proportion of deciduous, drought-adapted species, despite the relatively high annual rainfall at some sites (Bohlman, 2010). It would therefore be interesting to repeat these analyses using actual measurements of plant-available moisture rather than annual rainfall as a surrogate.

**Edaphic niche evolution and conservatism**

Different fern genera were over-represented in different sections of the soil nutrient gradient, in agreement with patterns reported previously (Tuomisto & Poulsen, 1996). Hence, for several genera our results are consistent with phylogenetic conservatism of edaphic niches. However, for at least as many genera the observed pattern was more consistent with niche evolution or adaptive radiation along the soil fertility gradient: a given lineage was represented by different species on different kinds of soils.

Although species turnover also occurs along a rainfall gradient in both regions (Jones et al., 2013; Zuquim et al., 2014), our results in relation to the rainfall gradient were less conclusive. Little evidence was found for either climatic niche conservatism or niche evolution within the rainfall range covered by our sampling. This perhaps reflects the fact that the rainfall gradient was relatively shorter than the soil fertility gradient when compared with the physiological tolerance of fern species.

In this respect it is noteworthy that the proportion of the local flora that belongs to genera specializing on poor soils closely followed soil cation concentration: sites in the most cation-poor quarter of the soil gradient were largely dominated by such species. Similarly, the proportion of species belonging to genera specializing on rich soils tended to increase with increasing soil cation concentration, but in this case the variation was much larger. In addition, genera specializing on rich soils never attained as high a dominance in the poor-soil sites as those specializing on poor soils did at the opposite end of the gradient. In other words, most fern species on very poor soils belong to specialist genera, whereas rich soils support communities with a mixture of rich-soil specialists and generalist genera. This indicates that poor soil environments may be harder for ferns to cope with and adapt to. As a result, there may be a bias in the prevalence of niche shifts within lineages, with more lineages shifting from poor to rich soils than vice versa. This could explain the higher observed species diversity in rich soils despite the older age of poor soils in Amazonia (Tuomisto et al., 2014), and perhaps also the decrease in MPD towards rich soils observed in our data. Since the specialist genera recognized here are pantropical in their distribution, a more rigorous analysis of their niche evolution is needed at the global extent.

**Phylogenetic resolution and community phylogenetic analyses**

It has been suggested that highly resolved and accurate phylogenies are critical in studying the interface between community ecology and evolution (Kress et al., 2009). However, our analyses produced similar results with genus-resolution and full-resolution phylogenies. This is partly explained by the
fact that we observed an average of only 1.5 congenic species per community, and partly by the PD measure being dominated by deeper relationships, such that the short terminal branches make little difference to its value at the community level. Similarly, Fine & Kembel (2011) reported that phylogenies resolved to the family level gave very similar results to genus-level phylogenies in the case of tropical trees. Simulations have also shown that loss of terminal resolution in a phylogeny has little effect on measures of community PD (Swenson, 2009). The implication is that future studies using PD-based measures can be conducted with well-resolved backbone phylogenies even when species-level information is not available.

**CONCLUSION**

Our results suggest that soil nutrient gradients are more important than rainfall gradients in defining the phylogenetic structure of fern communities within Neotropical lowland forests. Several genera were concentrated in specific parts of the soil cation concentration gradient, which is consistent with the idea of phylogenetic niche conservatism. Two of the three genera specializing on poor soils were phylogenetically basal, and communities on poor soils were composed, on average, of more distantly related species than were communities on rich soils. The proportion of species belonging to genera that were soil specialists was higher in communities on poor soils than in those on rich soils. Sites on the poorest quarter of the soil gradient were largely dominated by genera specializing on poor soils, in contrast to the richest quarter where the majority of species belonged to generalist genera.

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**BIOSKETCH**

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