Variation of Foliar Calcium and Magnesium in Six Fern Species at Different Elevations

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Abstract. Several efforts have been made to understand nutrient ecology worldwide. However, Ca and Mg have received less attention, despite their function in important biological processes of plants, such as growth and photosynthesis. Few studies focus on fern nutrient ecology of foliar Ca and Mg. Moreover, none has investigated the variation of these elements along elevational gradients. Herein, we analysed if there were differences of the foliar Ca and Mg contents at each elevation. We found significant differences at the interspecific and intraspecific level variation of foliar Ca and Mg contents at each elevation. In terms of the relationship between elevation and nutrient content, we found that two species showed contrasting trends of foliar Ca with elevation. Content of Ca decreased with elevation in Adiantum humile, while it increased in Maxonia apiifolia. Regarding Mg, it decreased with elevation in Adiantum humile, Thelypteris biformata and Maxonia apiifolia. However, these results were not statistically supported. An exhaustive sampling effort will provide insight on variation of foliar Ca and Mg along tropical elevation gradients and the factors influencing patterns of variation.

1. Introduction
Ferns are widely distributed globally, but their diversity peak is found in humid tropical regions [1]. Most studies on ferns in elevational gradients are focused on studying the distribution of species, diversity patterns and the causes of these [2-4]. In contrast, the nutrient ecology of ferns along elevational gradients has received little attention. There are two studies, but they are concentrated in foliar contents of carbon (C), nitrogen (N), and phosphorus (P) [5,6]. Wegner et al. [5] found that foliar C:N of fern communities decreased with elevation. However, at the specific level, when two species of the genus Elaphoglossum were analyzed, they did not show any pattern with elevation [5]. This lack of pattern in foliar C:N with elevation was also found in epiphytic ferns [6]. Regarding P, Cardelús and

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Mack [6] found the lack of pattern with elevation, but tree ferns displayed highest values than epiphyte ferns.

Patterns of variation in concentration of Ca and Mg have received less attention and no studies on these elements were found in elevational gradients. Ca is known to be involved in plant growth and developmental processes [7]. While Mg plays an important role in the photosynthesis process since it regulates the activity of key photosynthetic enzymes [8]. Amatangelo and Vitousek [9] compared the difference of foliar Ca and Mg concentrations between ferns and woody dicots in several soil types and found that ferns have lower concentration of Ca, but Mg concentrations are similar. Funk and colleagues [10] studied the role of Ca in transpiration rate, cation exchange capacity, and leaching loss in co-occurring ferns and angiosperm. They found no difference in transpiration rates and leaching loss between the two group of plants, but cation exchange capacity is not relative between ferns and angiosperms.

Due to the lack of information on the foliar Ca and Mg contents in ferns along elevational gradients, herein we evaluated six species of ferns to determine if there are differences of the foliar Ca and Mg contents between species and different elevations.

2. Material and Methods

2.1. Study Area

We sampled 8 sites located every 500 m along a tropical elevation gradient in Ecuador, from lowland forests at 400 m to forest patches close to the timberline at 4000 m asl. (Figure 1). Temperature regularly declines with elevation; the lapse rate was found to be 0.56°C per 100m elevational distance [11]. Humidity Index has a unimodal relation with elevation and the highest values of precipitation are found at 2000 m [11].

At each elevation, we sampled 3 plots of 400 m², with a total of 24 plots. We collected species separately for terrestrial and epiphytic ferns as described in Salazar et al. (2015) [11]. All species were deposited at the herbarium of the Pontificia Universidad Católica del Ecuador (QCA).

2.2. Selection of Species and Leaf Collections

We found a total of 91 species of terrestrial ferns. However, for this work, we chose the species present in at least three elevations. The six species with these characteristics are shown in the Table 1. For the chemical analyzes of the foliar Ca and Mg of each species, we collected at least one leaf per species at every elevation. The chemical analyses were performed in the laboratory at the University of Göttingen.

Figure 1. Map of the study area. Positions of all sites along the elevational gradient are indicated with green stars.
Table 1. Species of terrestrial ferns recorded in at least three elevations, detailed in the second column.

| Species                        | Elevation (m asl) |
|--------------------------------|------------------|
| Adiantum humile                | 1500, 1000, 500  |
| Cyathea tortuosa               | 2500, 2000, 1000, 500 |
| Cyclodium meniscioides         | 1500, 1000, 500  |
| Danaea moritziana              | 2500, 2000, 1500 |
| Maxonia apiifolia              | 2000, 1500, 1000 |
| Thelypteris bifformata         | 2000, 1500, 1000 |

2.3. Statistical Analyses
The relationships between foliar Ca and Mg contents and elevation were analyzed using ANOVA and linear regressions. All statistical analyses and graphics were carried out with the software environment R (version 4.0.2, R Core Team, 2020).

3. Results and Discussions

3.1. Variation of Foliar Ca and Mg
We found interspecific and intraspecific variation of foliar Ca and Mg contents at each elevation (ANOVA for foliar Ca content, F = 2.728, p = 0.0484, for foliar Mg content, F= 5.16, p= 0.00276). This variation has also been reported in other studies [9,10]. However, it is necessary an exhaustive sampling to reach stronger conclusions. The values of foliar Ca and Mg are detailed in the Table 2.

Table 2. Values of foliar Ca and Mg of the six species at each elevation.

| Elevation (m a.s.l.) | Species                        | Foliar Ca mg/g | Foliar Mg mg/g |
|----------------------|--------------------------------|----------------|----------------|
| 1500                 | Adiantum humile                | 1.05891862     | 1.74786507     |
| 1500                 | Adiantum humile                | 2.39702106     | 2.01104746     |
| 1500                 | Adiantum humile                | 0.80795839     | 1.07138998     |
| 1000                 | Adiantum humile                | 0.73366302     | 0.88927031     |
| 500                  | Adiantum humile                | 6.07394006     | 3.26477101     |
| 2500                 | Cyathea tortuosa               | 2.46572699     | 2.02948454     |
| 2500                 | Cyathea tortuosa               | 1.40729444     | 1.46154618     |
| 2000                 | Cyathea tortuosa               | 4.38467148     | 4.02908137     |
| 2000                 | Cyathea tortuosa               | 6.32438879     | 4.60959073     |
| 2000                 | Cyathea tortuosa               | 2.95832211     | 3.18503064     |
| 2000                 | Cyathea tortuosa               | 3.48140585     | 3.09545778     |
| 1000                 | Cyathea tortuosa               | 3.32524119     | 2.23372312     |
| 1000                 | Cyathea tortuosa               | 2.33007346     | 2.36771954     |
| 1000                 | Cyathea tortuosa               | 2.2254804      | 1.67648292     |
| 1000                 | Cyathea tortuosa               | 2.42166055     | 1.54681039     |
| 1000                 | Cyathea tortuosa               | 1.84872609     | 1.57580737     |
| 500                  | Cyathea tortuosa               | 1.94389674     | 2.15456601     |
| 500                  | Cyclodium meniscioides         | 2.13794253     |               |
| 1000                 | Cyclodium meniscioides         | 0.0070038      | 1.85268411     |
| 1500                 | Cyclodium meniscioides         | 6.17547017     | 2.33268806     |
### 3.2. Variation of Foliar Ca and Mg at the Different Elevations

Our linear regressions analyses revealed that no species present a significant relationship between elevation and foliar Ca and Mg content. This might be due to small sample sizes, and/or that the species are not widely distributed along the elevation gradient. The latter would show that although Ca and Mg are important elements for growth [7,8], it does not influence the distribution of these species. Therefore, other factors such as temperature or humidity might be more prominent in determining the distribution of the species in the altitudinal gradient [11].

Nonetheless, we found contrasting trends in different species regarding the foliar contents of Ca and Mg along elevation. Foliar Ca contents showed a positive trend with elevation in only two species, although with opposite direction: *Adiantum humile* and *Maxonia apiifolia* (Figure 2a, 2d). In *Adiantum humile*, concentration of foliar Ca decreased with elevation ($p = 0.12$, adjusted $R^2 = 0.59$), whereas in *Maxonia apiifolia*, it increased with elevation ($p = 0.36$, adjusted $R^2 = 0.27$). We did not observe any trends in *Cyathea tortuosa*, *Danaea moritziana* and *Thelypteris biformata* (Figure 2b, 2c, 2d). Regarding foliar Mg contents, we observed a negative trend with elevation in two species: *Adiantum humile* and *Thelypteris biformata* ($p = 0.28$, adjusted $R^2 = 0.36$ for *A. humile*; $p = 0.4$, adjusted $R^2 = 0.6$ for *T. biformata*; Figure 2a). We found a positive relation with elevation in *Maxonia apiifolia* ($p = 0.27$, adjusted $R^2 = 0.36$; Figure 3e, 3f). No trends were observed in *Cyathea tortuosa*, *Cyclodium meniscioides* or *Danaea moritziana* (Figures 3b, 3c, 3d).

| Elevation | Species               | Ca Concentration | Mg Concentration |
|-----------|-----------------------|------------------|------------------|
| 1500      | *Cyclodium meniscioides* | 0.66693191       | 3.50155633       |
| 2500      | *Danaea moritziana*    | 3.3337622        | 2.4362301        |
| 2000      | *Danaea moritziana*    | 1.9498227        | 1.97793159       |
| 2000      | *Danaea moritziana*    | 7.59616001       | 5.21692282       |
| 1500      | *Danaea moritziana*    | 3.3337622        | 2.4362301        |
| 1500      | *Danaea moritziana*    | 1.9498227        | 1.97793159       |
| 1500      | *Danaea moritziana*    | 1.9498227        | 1.97793159       |
| 2000      | *Danaea moritziana*    | 1.9498227        | 1.97793159       |
| 2000      | *Maxonia apiifolia*    | 5.05312263       | 5.91451378       |
| 2000      | *Maxonia apiifolia*    | 0.25588898       | 2.4731991        |
| 1500      | *Maxonia apiifolia*    | 1.84117246       | 4.14308842       |
| 1500      | *Maxonia apiifolia*    | 1.18746498       | 3.90335824       |
| 1000      | *Maxonia apiifolia*    | 0.06801711       | 1.26950231       |
| 2000      | *Thelypteris biformata*| 4.14738146       | 1.82839331       |
| 1500      | *Thelypteris biformata*| 2.96566111       | 1.71265563       |
| 1000      | *Thelypteris biformata*| 4.58240968       | 2.60212816       |
Figure 2. Variation of foliar Calcium of 5 species of ferns between different elevational levels.

Figure 3. Variation of foliar Magnesium of 6 species of ferns between different elevational levels.
4. Conclusions
To our knowledge, this is the first study to evaluate foliar Ca and Mg of tropical fern species along an elevational gradient. We found differences in the foliar content of this elements within and between species at different elevations. Although, we found interesting trends of variation along elevation in the studied species, these were not significant. We consider that a greater sampling effort, especially of the species that showed an apparent trend with elevation will provide more robust insights on how it varies across elevations. Furthermore, it would be also interesting to study the relation between growth of several species of ferns with the content of foliar Ca and Mg, and the availability of Ca and Mg in the soil.

Acknowledgments
We thank D. Torres, L. Cotugno, R. Güdel, E. Gortaire, W. Santillán, W. Pérez and people from the local communities for their valuable help, support, and enthusiasm during the fieldtrips. We thank institutional support of Ministerio del Ambiente y Agua of Ecuador (MAAE).

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