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RESEARCH ARTICLE

Prospecting the presence of aluminum-accumulating species inhabiting temperate rainforests from southern Chile

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Abstract
Background and aims Aluminum (Al) is toxic to most agricultural plants in acidic soils. However, many native species growing on acidic soils are Al tolerant. Indeed, Al-accumulating species maintain high Al concentrations in their leaves without harm. We surveyed native plants across different temperate rainforests of Chile to determine whether Al accumulator species occur in these regions and assessed how soil chemistry correlates with their ability to accumulate Al.
Methods Mature leaves or fronds were collected from 107 native trees, shrubs, climbers, herbs, mosses, epiphytes and ferns growing in four sites of south-central Chile. Chemical analyses were performed on these organs and adjacent soils and were compared among the different sites.
Results Soils from the four collection sites ranged from slightly acidic (pH 6.2, Al saturation of 1.3%) to very acidic (pH 4.7, Al saturation of 70%). Only 7% of the plants sampled were designated as Al-accumulators, including species from the Proteaceae, Dryopteridaceae, Lycopodiaceae, Gleicheniaceae and Polytrichaceae families. Most Al-accumulators were found in the soil with the lowest pH and highest soil Al saturation. However, some of the Al-accumulating plants accumulated similar concentrations of Al in their leaves despite large differences in pH and soil Al saturation.
Conclusions Al-accumulating species were found in the temperate forests of southern South America and soil chemistry can only partly explain the differences in Al accumulation in different sites. These new Al-accumulator species can be investigated further to reveal the mechanisms and genes controlling this trait.

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Keywords Acidic soils · Native species · Proteaceae · South America

Introduction

Aluminum (Al) is the most abundant metal in the Earth's crust and present in the organs of most plants and animals, including humans (Rengel 2004). The vast majority of Al in the soil is incorporated in minerals or chelated by compounds where it poses little harm to plants. However, as soil pH declines the minerals begin to dissolve and release Al ions into the soil solution including $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and Al^{3+}. The relative proportion of these ions varies as pH declines but in highly acidic soils ($pH < 4.8$) the toxic Al^{3+} cations begin to dominate (Hajiboland et al. 2023; Von Uexküll and Mutert 1995). Many crop species are sensitive to Al^{3+} because it disrupts a range of cellular processes including root-cell division and elongation. Therefore, a primary symptom of Al^{3+} toxicity in plants is the inhibition of root growth (Horst et al. 2010; Silva et al. 2019) which adversely affects nutrient acquisition and increases the susceptibility of plants to drought.

Plants that grow naturally on acidic soils have evolved mechanisms to resist and tolerate the harmful effects of H^+ and Al^{3+}, and some even benefit from the presence of Al in the soil or nutrient solution (Bojórquez-Quintal et al. 2017; Bressan et al. 2021; Muhammad et al. 2018; Silva et al. 2023; Sun et al. 2020). A small proportion of Al-tolerant plants accumulate Al in their organs as an internal detoxification strategy (Haridasan 1982; Kochian et al. 2015; Timpone and Habermann 2022; Zaia et al. 2022) and plants with leaf Al concentration above 1000 mg Al kg^{-1} dry mass (DM) are classified as 'Al-accumulators' (Chenery 1948; Jansen et al. 2002). Examples of common commercial plants that accumulate these high concentrations of Al include tea (*Camellia sinensis*), buckwheat (*Fagopyrum esculentum*) and *Hydrangea macrophylla*. A different mechanism of Al resistance that has been evidenced in a wide range of plants, including many crop species, involves the exudation of organic anions like malate and citrate from roots. These organic anions are thought to protect the growing roots by binding with the Al^{3+} ions to form non-toxic complexes in the apoplast or to help sequester them internally (Ryan et al. 2011; Xia et al. 2010).

A few studies have surveyed natural ecosystems for Al accumulators and those that are available have focused on the shrubs and trees from the acidic soils of tropical areas (Jansen et al. 2002; Haridasan (2008) such as the Cerrado in South America (de Oliveira Carvalho Bittencourt et al. 2020; Silva et al. 2023; Souza et al. 2015; Timpone and Habermann 2022). Temperate forests, by contrast, have received even less attention (Ginocchio and Baker 2004) despite the rich diversity of species growing on the acidic soils in those regions. For example, only a single study has investigated the temperate forests of southern South America despite many of them having acidic soils (pH 4.4—6.1) with high Al saturation (up to 74%) (Delgado et al. 2018). That study identified three members of the Proteaceae as Al-accumulators (Delgado et al. 2019).

Other Proteaceae species accumulate high concentrations of Al in their leaves as can members of the Cunoniaceae, Monimiaceae and Lauraceae families and some non-flowering species (Jansen et al. 2002). So far Al accumulators have not been recorded among the gymnosperms (Chenery 1949; Metali et al. 2012; Webb 1954). While the forests of southern South America include many species from these families (Donoso 2006) it is not known whether any of them accumulate Al. However, since this trait is more abundant in some phylogenetic groups than others (Jansen et al. 2002; Metali et al. 2012), it is possible that the South American forests do contain species that show high Al concentration.

The present study surveyed the common plant species in four different temperate rainforests across Chile. Aboveground organs were analysed for Al and other mineral nutrients, and these were compared with the soil chemistry at each location. We described the presence of Al-accumulator species in the temperate forests of South America. In addition, we tested whether (1) soil chemistry is the major factor driving Al accumulation above 1000 mg kg^{-1}DW, and (2) the accumulation of high concentrations of Al in leaves and fronds alters the nutritional status of Al-accumulators compared with non-accumulators.

Material and methods

Study sites

The study was conducted in four contrasting temperate rainforests in south-central Chile (Fig. 1). These include: (1) Rucamanque Ecological and Cultural Park (38°39′34"S—72°36′20"W) with an average 376 m above sea level (m.a.s.l.), (2) Conguillio National Park (38°40′00″S—71°39′00″W) which is 700 to 3,125 m.a.s.l. (3) Oncol Park (39°41′59"S—73°19′34"W) with an average 550 m.a.s.l, and (4) Vicente Perez Rosales National Park (41°10′20″S—72°26′56″W) with an altitude ranging from 137 to 3,470 m.a.s.l. The samples at Conguillo were collected at 1,137 m.a.s.l., and the samples at V.P. Rosales was collected at 137 m.a.s.l. Soils in these regions typically have low pH, low effective cation exchange capacity (ECEC), elevated Al saturation (m%) and limited macro- and micronutrient availabilities. The soils from Rucamanque, Conguillio and V. P. Rosales all developed from volcanic ashes, but among these, the Rucamanque soil was most developed (Almonacid-Muñoz et al. 2022). Soils from Conguillio and V. P. Rosales are classified as young soils with volcanic scoria in some areas (Lillo et al. 2011; Martínez 1985). Finally, soil from the Oncol site is derived from metamorphic materials upon which volcanic ash has been deposited or mixed (Hernández et al. 2012).

Collection of leaves and soil samples

Leaves, thalli or fronds of flowering and non-flowering plants from a range of Phyla and growth habits (herbs, epiphytes, trees, shrubs, climbers, ferns,

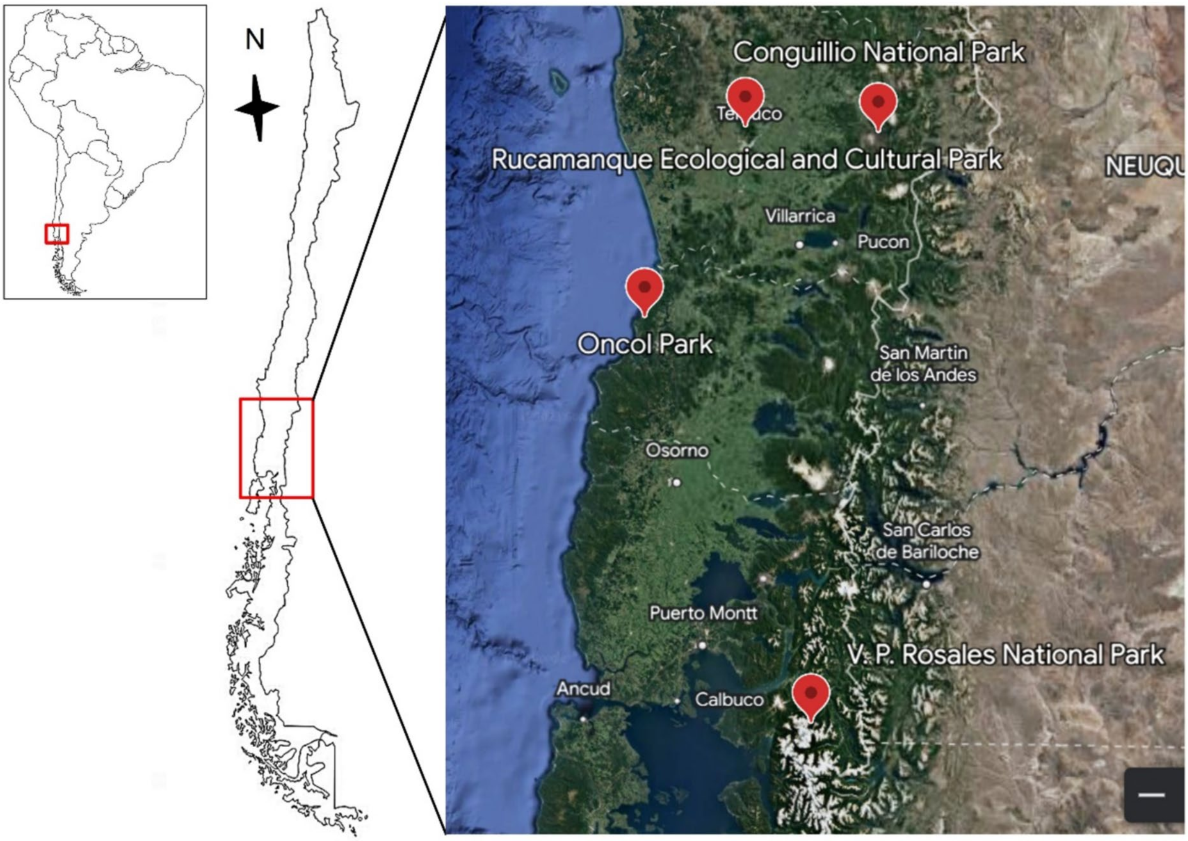

Fig. 1 Location of sites where leaves and soil samples were collected in this study: Rucamanque Ecological and Cultural Park (La Araucanía Region), Conguillio National Park (La Araucanía Region), Oncol Park (Los Rios Region) and Vicente Perez Rosales National Park (Los Lagos Region)

mosses and one hemiparasitic) were sampled from the four sites during Spring 2022 and 2023. Aboveground plant samples and soil samples were randomly collected along a ~ 2000 m transect within the sites. Three to six individual plants were sampled for each species, ensuring a minimum separation of 5 m between them.

Leaves from tall trees were collected at a maximum of 2 m above ground. Mature, healthy, fully expanded leaves with petioles were collected from the four quadrants of each individual (N, S, E, and W) in order to represent the whole plant canopy. Samples from the four quadrants were also collected from the mosses. All samples were stored, labeled in paper bags and taken to the laboratory for further chemical analyses.

A minimum of three soil samples at 20 cm depth were collected at each of the four experimental areas. The soil samples were put in plastic bags, labeled, and stored for subsequent chemical analysis.

Chemical analyses of leaves and soils

Leaf, frond and thallus samples were washed with deionized water and then dried in an oven at 60 °C for 72 h. Aluminum, potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), Copper (Cu), Iron (Fe) and Zinc (Zn) were quantified using an atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., SavantAA, Sigma, Dandenong, Victoria, Australia). For this, the dried samples were ground and subsamples of approximately 0.5 g were ashed at 500 °C for 8 h and subsequently digested using hydrochloric acid (2 M). The same extract was used to measure P, which was quantified colorimetrically using the vanado-phosphomolybdate method and read at an absorbance of 466 nm in a UV–visible spectrophotometer (Epoch 2 Microplate Spectrophotometer, BioTek, USA). To measure N concentration, 0.2 g of dried sample was digested with concentrated sulfuric acid (96%), steam distilled using the Kjeldahl system (Behrotests K40, Behr S4, Behrlabor-technik, Germany) and finally titrated with sulfuric acid (0.025 mol L^{-1}) and pH indicator (bromocresol green + methyl red). All the methods used to quantify plant elements are well described by Sadzawka et al. (2004).

For soil chemical analyses, the soil was air dried, sieved (2 mm) and chemically analyzed as follows. Soil P (Olsen) was measured by extracting soil with $NaHCO_3$ (0.5 M, pH 8.5) (Olsen and Sommers 1982) and determined colorimetrically applying the phospho-antimonyl-molybdenum blue complex method (Drummond and Maher 1995). Soil pH was determined in a 1:2.5 soil to distilled water ratio. The mixture of soil and water was stirred vigorously for 5 min at 500 rpm, then allowed to stand for at least 2 h, and finally the pH was measured using a pH meter (Orion model 410 A, USA). Exchangeable cations (Ca^+, K^+, Na^+, Mg^+, Al^+) were measured by atomic absorption spectrophotometer (GBC Scientific Equipment Pty Ltd., SavantAA, Sigma, Dandenong, Victoria, Australia) and the effective cation exchange capacity (ECEC) was calculated by the sum of the cations (SC), and soil Al saturation (m%) was calculated as $m\% = (100 \times Al^{3+})/(SC + Al^{3+})$. Mineral N in the soil was determined after distillation in the presence of Devarda alloy and MgO of the extracts prepared with 5 g of soils and KCl (2 M). Organic matter was quantified using the sodium dichromate oxidation method in the presence of H_2SO_4. All the methods to measure soil parameters are described by Sadzawka et al. (2006).

Data analysis

Soil fertility parameter values were not compared between experimental areas. The leaf Al concentration of 1000 mg Al kg^{-1} DM was used to separate between Al-accumulating and non-accumulating species (Chenery 1948; Jansen et al. 2002). A t-Welch test with *p* values adjusted with Bonferroni (to avoid Type I errors) was used to compare mean Al and nutrient concentration between Al-accumulating and non-accumulating species. This test is suitable to analyze unequal sample sizes, as in the present study, without variance homogeneity (Ruxton 2006). Welch t-test comparisons were performed using the R software platform (version R-4.4.2, (R Core Team 2024)).

Because the Al concentration in leaves of some species could vary according to the percentage of Al saturation in the soil, we standardized the leaf Al concentration by site using the formula *Leaf Al (as average proportion from site) = log (Individual leaf [Al]/log (Site average leaf [Al])* as proposed by Lambers et al. (2021). This approach allowed for more accurate comparisons of Al concentrations across different growth forms and taxonomic groups. To make these comparisons, we performed Welch´s *F*-tests analysis using the software SPSS 22.0 (IBM Corp. Chicago, IL, USA).

Results

Soil fertility

Soils collected from the four forest sites varied in pH and chemistry. Soil pH (H_2O) ranged from 6.2 at Conguillio to 4.7 at Oncol, while Al saturation varied from 0.5% at Rucamanque to 70.3% at Oncol (Table 1). The ECEC was greatest at Rucamanque (16 ±3.6 cmolc kg^{-1}) and lowest at V. P. Rosales (2.8 ±0.8 cmolc kg^{-1}). Organic matter was highest at Oncol (19.3 ±2.7%), followed by Rucamanque (11.8 ±1.8%), Conguillio (8.9 ±4.1%), and V. P. Rosales (2.8 ±0.6%) (Table 1). Despite having the highest organic matter content, the Oncol soil was most acidic and had the greatest Al saturation, classifying it as a dystrophic soil.

Leaf Al and mineral analyses

Of the 104 species sampled across all four sites, only seven species (7%) had Al concentrations in their leaves or fronds exceeding 1000 mg Al kg^{-1} DW and, therefore, could be designated Al-accumulators (Table 2). These species included trees from the Proteaceae family (*Gevuina avellana* and *Lomatia dentata*), ferns from the Dryopteridaceae (*Megalastrum spectabile*), Gleicheniaceae (*Gleichenia quadripartite, Gleichenia criptocarpa*) and *Lycopodiaceae* (*Lycopodium paniculatum*), and a moss from Polytrichaceae (*Dendrologotrichum dendroides*) (Fig. 2). The highest concentrations of Al were measured in the Proteaceae and Gleicheniaceae families (Fig. S1) and the average Al concentration in the Al-accumulators was 23-fold greater than the non-accumulating species. None of the epiphytes, hemiparasites, shrubs, herbs or climber plants that were sampled showed high Al concentration, and neither did any of the five gymnosperms tested. When these data were standardized in an attempt to account for the different soil

Table 1 Chemical analyses of soil collected at Rucamanque, Conguillio, Oncol, and Vicente Perez Rosales, natural habitats of Southern South American species, Chile

Soil Properties	Location (Latitude)			
	Site 1	Site 2	Site 3	Site 4
	Rucamanque38°39′34"S −72°36′20"W n =6	Conguillio 38°40′00″S −71°39′00″W n = 3	Oncol 39°41′59"S −73°19′34"W n = 6	V. P. Rosales 41°10′20″S 72°26′56″W n =3
Mineral N (mg kg^{-1})	13.4 (2.0)	9.7 (2.4)	6.7 (0.9)	28.7 (6.1)
P-Olsen (mg kg^{-1})	3.6 (1.3)	8.3 (2.7)	4.4 (0.5)	12.3 (1.2)
K (mg kg^{-1})	n.d	97.8 (39.4)	139.5 (5.21)	46.9 (11.7)
Organic matter (%)	11.8 (1.8)	8.9 (4.1)	19.3 (2.7)	2.8 (0.6)
pH (H_2O)	5.9 (0.2)	6.27 (0.22)	4.7 (0.03)	5.6 (0.4)
Ca ($cmol_c$ kg^{-1})	12.4 (3.0)	10.3 (6.0)	0.48 (0.1)	0.5 (0.0)
Mg ($cmol_c$ kg^{-1})	2.6 (0.5)	1.6 (0.9)	0.5 (0.1)	2.0 (0.9)
Na ($cmol_c$ kg^{-1})	0.1 (0.0)	0.1 (0.02)	0.13 (0.01)	0.1 (0.0)
K ($cmol_c$ kg^{-1})	0.8 (0.3)	0.25 (0.1)	0.33 (0.03)	0.1 (0.0)
Al ($cmol_c$ kg^{-1})	0.1 (0.0)	0.03 (0.01)	4.35 (0.4)	0.2 (0.1)
Sum of cations ($cmol_c$ kg^{-1})	15.9 (3.6)	12.3 (6.9)	1.5 (0.2)	2.6 (0.9)
ECEC (cmolc kg^{-1})	16.0 (3.6)	12.4 (6.9)	5.8 (0.5)	2.8 (0.8)
Al saturation (m%; %)	0.5 (0.3)	1.39 (1.1)	75.1 (2.8)	7.0 (5.6)

ECEC effective cation exchange capacity

Each value corresponds to a mean of samples (± standard error)

Table 2 Range and average of aluminum (Al) concentration of Al-accumulating species growing in the temperate forests of southern South America, in Chile

Species	Family	Range [Al] (mg kg^{-1} DM)	Average [Al] (mg kg^{-1} DM)	*n*	Reference
Gevuina avellana	Protecaeae	3062—7047	5688	8	This study
		1738—12732	5216	32	Delgado et al. 2019
Lomatia dentata	Protecaeae	1008—1128/954—3656	1080/1804	3/8	This study Delgado et al. 2019
Orites myrtoidea	Protecaeae	805—1545	1081	3	Delgado et al. 2019
Megalastrum spectabile	Dryopteridaceae	345—2031	1313	8	This study
Gleichenia quadripartite	Gleicheniaceae	4956—6767	5758	3	This study
Gleichenia criptocarpa	Gleicheniaceae	6113—8429	7088	4	This study
Dendrologotrichum dendroides	Polytrichaceae	454—4222	1765	7	This study
Lycopodium paniculatum	*Lycopodiaceae*	3502—6296	4569	6	This study

n = number of individuals sampled

characteristics at each site (see Lambers et al. 2021), the moss (Bryophyta) showed the highest concentrations of Al (Fig. 3a, b).

All except one of the seven Al-accumulators identified here were sampled from the Oncol site which had the lowest soil pH and greatest Al saturation (Table 1). No Al accumulators were found in the Conguillo site where soil pH was highest, and Al saturation was low. Four of the Al accumulators from Oncol also grew at another location. Three of these showed similar high Al concentrations at both sites despite large differences in soil chemistry. For example, *Gevuina avellana* (Proteaceae) and *M. spectabile* (Dryopteridaceae) grew at Oncol and Rucamanque and accumulated similar concentration values of Al at these sites despite large differences in soil pH and a 140-fold difference in soil Al saturation. Similarly, *L. paniculatum* showed the same concentrations of Al at Oncol and V. P. Rosales (~ 4,500 mg Al kg^{-1} DM), again, despite Oncol being more acidic and with a greater soil Al saturation. The fourth Al accumulator growing at more than one site was the moss *D. dendroides* which was sampled at Oncol and V. P. Rosales. While this species accumulated significantly more Al at Oncol than V. P. Rosales (2,789 and 996 mg Al kg^{-1} DM, respectively) the concentration at V.P. Rosales was still very high and reached the ~1000 mg Al kg^{-1} DM threshold for Al accumulators.

We also compared some of the non-accumulator species growing at different sites to assess how soil characteristics affected them. Three non-accumulator species were common to Oncol and V.P. Rosales (*Blechnum chilense* and *Hymenophyllum tortuosum*, *Weinmannia trichosperma*). The ferns *B. chilense* and *H. tortuosum* accumulated significantly more Al at the more acidic Oncol site (128 ±8 mg kg^{-1} and 623 ±90 mg kg^{-1} DM, respectively) compared to plants growing in V.P. Rosales (29 ±5 mg kg^{-1} DM and 98 ±13 mg kg^{-1} DM, respectively). However, the differences for *W. trichosperma* were relatively small and less than 30%. One non-accumulating species collected at the Conguillo site also grew at Oncol (*Berberis darwinii*), presenting less Al accumulation at the Conguillo (132 ±5 mg kg^{-1} DM) compared to Oncol site (308 ±69 mg kg^{-1} DM) (Fig. 2).

Elemental analyses of the plant aboveground organs showed that, on average, the Al-accumulating species had more than two-fold greater Fe concentrations than the non-accumulators, but approximately half the concentrations of Mn, P, and Ca and smaller decreases in K, Cu and N (Table 3). The only exception was in the Al-accumulating tree species, *Lomatia dentata,* where the Mn concentration (2,352 mg Mn kg^{-1} DM) was eight-fold greater than the average of non-accumulators. A heatmap comparing the normalized concentrations of major mineral nutrients for each species at each site is shown in Fig. 4.

Discussion

Al-accumulating species inhabit the temperate rainforest from southern South America

We surveyed the temperate rainforests from southern South America to identify native species that

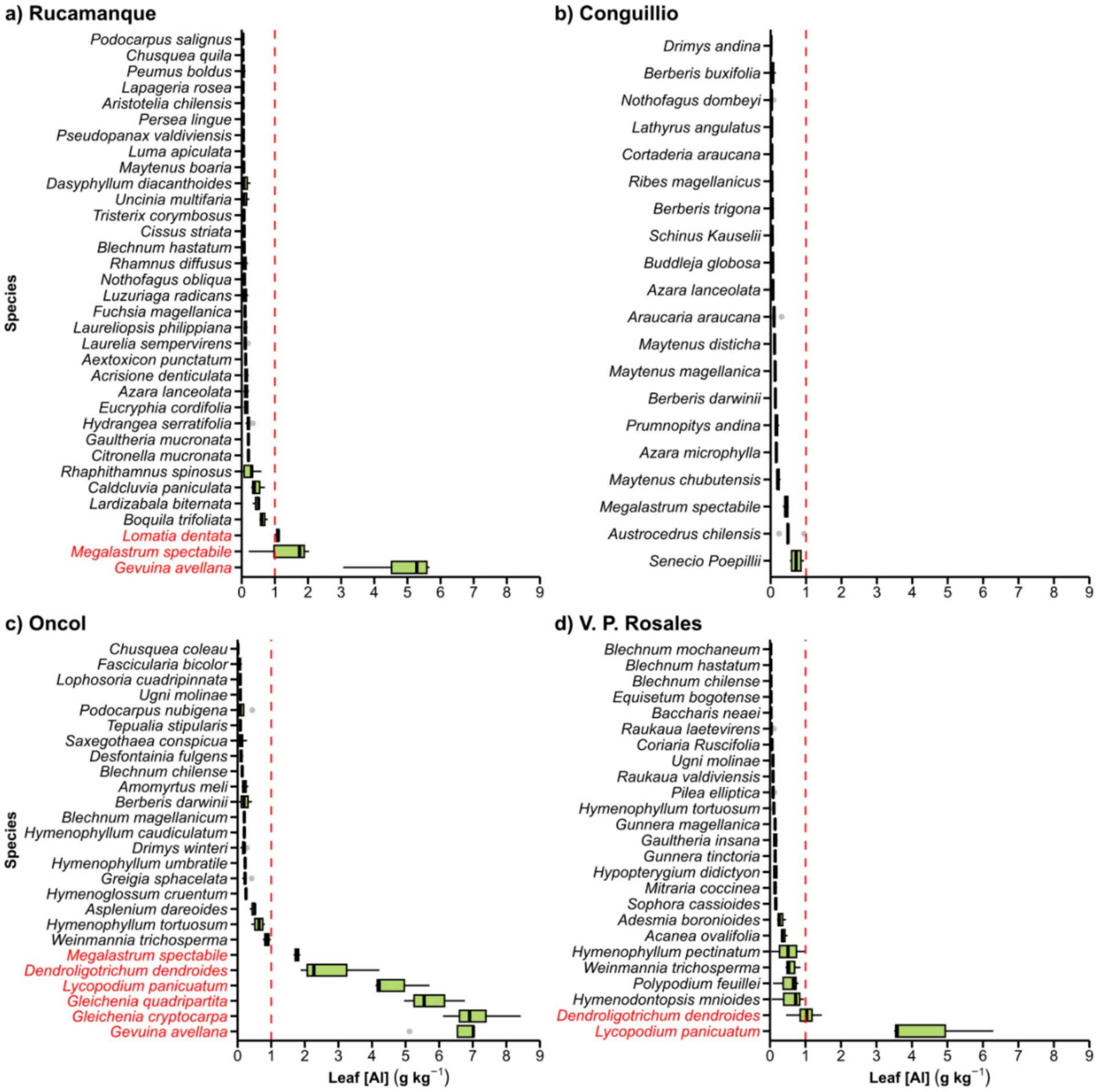

Fig. 2 Boxplots of leaf aluminum (Al) concentrations in mature leaves of plant species sampled in their natural habitats of southern South American: **a** Rucamanque Ecological and Cultural Park, **b** Conguillio National Park, **c** Oncol Park, and **d** Vicente Perez Rosales National Park. Vertical dashed red line represents the limit of 1000 mg Al kg^{-1} DM at which a species is considered an Al-accumulator

accumulate high concentrations of Al in their aboveground organs. Two of the 62 angiosperms sampled (3.2%), four of the 12 pteridophytes and one of the three mosses sampled were Al-accumulators (Figs. 2 and 3). These findings are consistent with previous reports whereby only a small percentage of angiosperms, including ~5% of all eudicots and less than 1% of monocots, are Al accumulators (Chenery 1949; Jansen et al. 2002), while many more ferns (~ 43%) show this trait (Chenery 1949; Schmitt et al. 2017). Therefore, the present study generally corroborates previous estimates for the proportion of each taxonomic group showing this trait.

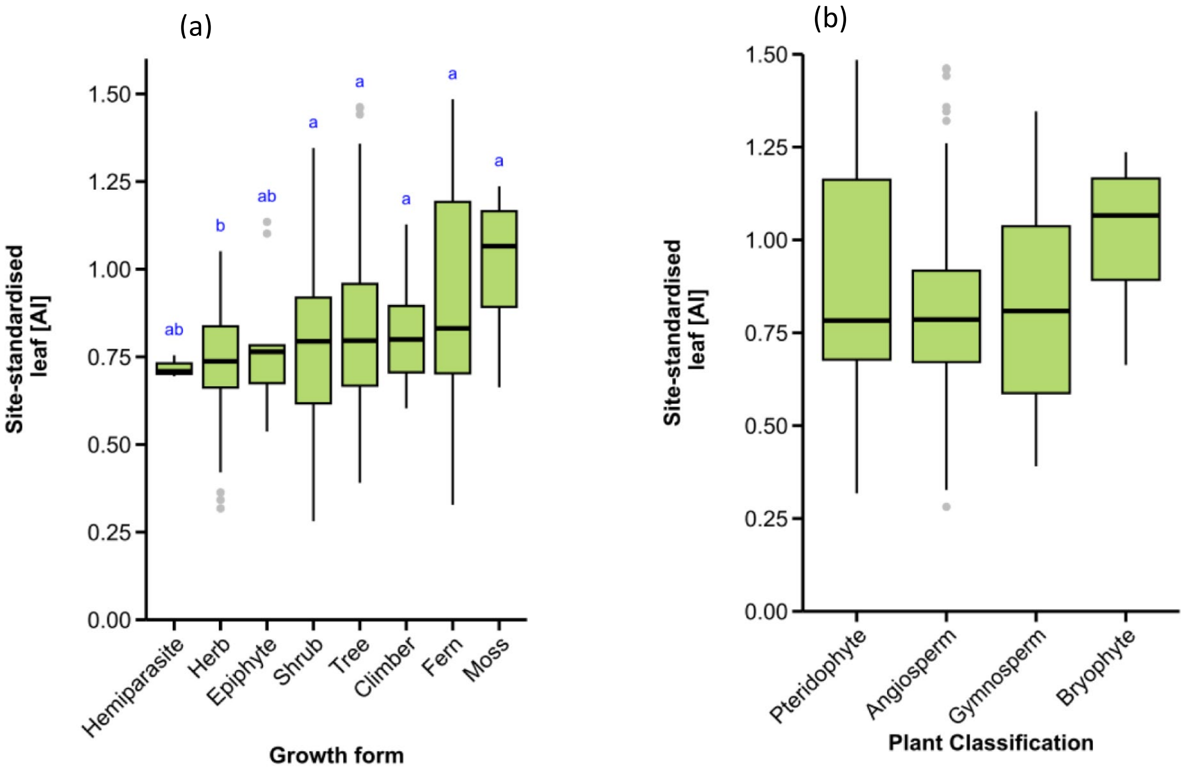

Fig. 3 Box plots of leaf aluminum (Al) concentrations in mature leaves of plant species according growth form (**a**) and plant classification (**b**). These data were standardized according to Lambers et al. (2021) which attempts to account for the different soil characteristics between sites so that leaf Al concentrations can be compared among the different growth habits and taxonomic groupings

Table 3 Average aluminum (Al) and nutrient concentration [Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn)] of Al-accumulating and non-accumulating species

Element concentration	Al-accumulator	Al non-accumulator	p-value	t-Welch test significance
Al (mg kg^{-1})	4411.5 ± 346.6	187.9 ± 9.3	9.1×10^{-19}	***
Mn (mg kg^{-1})	156.4 ± 12.8	315.6 ± 20.5	1.4×10^{-10}	***
Cu (mg kg^{-1})	4.6 ± 0.5	5.6 ± 0.2	3.3×10^{-02}	*
Zn (mg kg^{-1})	12.0 ± 1.9	19.3 ± 1.1	9.1×10^{-04}	***
Fe (mg kg^{-1})	214.8 ± 79.0	98.6 ± 6.9	1.5×10^{-01}	ns
P (mg g^{-1})	0.7 ± 0.1	1.2 ± 0.0	7.0×10^{-08}	***
K (mg g^{-1})	6.5 ± 1.2	9.6 ± 0.4	1.8×10^{-02}	*
Ca (mg g^{-1})	4.4 ± 0.9	9.4 ± 0.4	7.8×10^{-06}	***
Mg (mg g^{-1})	2.1 ± 0.3	2.6 ± 0.2	2.4×10^{-01}	ns
Na (mg g^{-1})	1.0 ± 0.2	0.6 ± 0.1	4.2×10^{-02}	*
N (mg g^{-1})	11.4 ± 0.5	14.1 ± 0.4	5.2×10^{-05}	***

t-Welch test significance: ns = not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. For the comparison of leaf Mn concentration between Al-accumulating and non-accumulating species, we excluded *Lomatia dentata*, as it is considered an outlier species

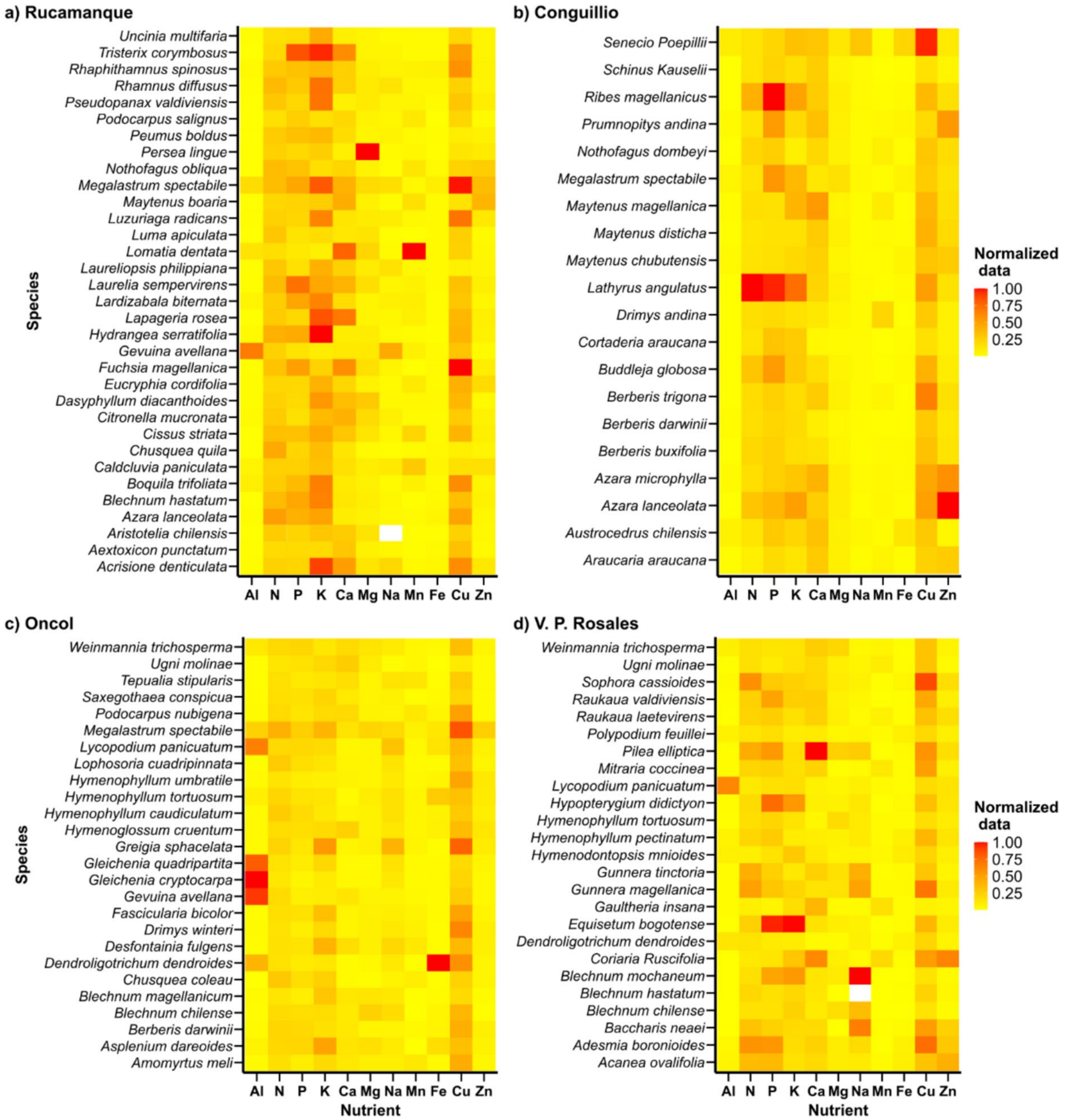

Fig. 4 Heatmap representing the normalized leaf concentrations of aluminum (Al) and nutrient concentration [Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn)] of Southern South American plant species growing in their natural habitats in Chile: **a**) Rucamanque Ecological and Cultural Park, **b**) Conguillio National Park, **c**) Oncol Park, and **d**) Vicente Perez Rosales National Park. Leaf Al and nutrient concentrations were normalized according to the average of each element per species divided by the highest average of each element considering all sites

We confirmed earlier work by Delgado et al. (2019) by showing that *Gevuina avellana* and *L. dentata*, common Proteaceae species in the temperate rainforests of Chile, are Al-accumulators. Other studies have measured high Al concentrations in the leaves of Proteaceae species (Chenery 1948; Jansen et al. 2002; Kukachka and Miller 1980) but they all used simple colorimetric assays which are qualitative and less precise at measuring the Al concentrations in tissue. To the best of our knowledge, the present study and that by Delgado et al. (2019) are the only ones to quantitatively measure leaf Al concentrations in Al-accumulators plants within this family (Table 2). However, not all members of the Proteaceae are Al accumulators because *Roupala montana* is not an Al-accumulator despite growing in the highly acidic soils of the Cerrado region of Brazil where many woody species from the Melastomataceae, Rubiaceae, and Vochysiaceae show very high concentrations of Al (Haridasan 1982). Although it is becoming clear that Al accumulation is a common feature among the Proteaceae, it is not a universal trait in the family.

Ferns belonging to families Gleicheniaceae (*Gleichenia quadripartite, G. cryptocarpa*) and *Lycopodiaceae* (*Lycopodium paniculatum)* were identified as Al-accumulating species (Table 2). Other species of Gleicheniaceae (*Sticherus nudus* (Moritz ex.Reichardt) Nakai) and Lycopodiaceae (*Lycopodium clavatum* L.) show this trait including some growing on acidic soils in the "cloud forests" of Venezuela (Olivares et al. 2009). Indeed, high Al concentrations have been described in numerous fern taxa including Marattiales, Gleicheniales, Cyatheales (Schmitt et al. 2017) and Lycopodinae (Chenery 1949). However, this is the first report demonstrating that *Megalastrum spectabile* (Dryopteridaceae) is an Al accumulator (Table 2).

The present study also found that the moss *Dendroligotrichum dendroides* (Polytrichaceae) is an Al-accumulator. This confirms a much earlier report by Chenery (1949) who measured Al concentration in different specimens from the Kew Herbarium in England and found that *D. dendroides* accumulated 1,320 mg Al kg^{-1} DM in its leaves, which is similar to our measurements of samples from Oncol (2,789 and 996 mg Al kg^{-1} DM) and V.P. Rosales (996 mg Al kg^{-1} DM).

Six of the seven Al-accumulating species identified were collected at the Oncol site which was the most extreme of all the sites due to its lowest pH and greatest soil Al saturation. To assess how soil chemistry might influence the accumulation of Al it was useful to compare how the same species performed at different sites. Four of the Al-accumulating species found at the highly acidic Oncol site also grew at other locations. Interestingly, three of them accumulated very similar amounts of Al despite very large differences in soil pH and Al saturation. The only exception was *D. dendroides* that accumulated 2.8-fold greater Al at Oncol than V.P. Rosales. *Lycopidium paniculatum* showed similar Al concentrations at Oncol and V. P. Rosales, while *G. avellana* and *M. spectabile* similar Al concentrations at Oncol and Rucamanque. The fern *M. spectabile* also grew at Conguillo and, although the Al concentrations in the fronds were ~30% those measured in Oncol, this difference was relatively small compared with the 50-fold difference in Al saturation of the two soils. Similar findings were reported for two woody plants from Brazil, *Qualea grandiflora* and *Q. parviflora* (Vochysiaceae) (Haridasan 1982; Silva et al. 2023). These species show similar concentrations of Al in their leaves whether they were grown on a calcareous soil with an Al saturation of 3.6% or on an acidic latosol with an Al saturation of 63% (Nogueira et al. 2019).

Several of the non-accumulating species sampled also grew at different locations. The evergreen shrub *Berberis darwinii* grew at Oncol and Conguillo and accumulated significantly more Al in their leaves at the more severe Oncol site than at Conguillo (Fig. 2). Similarly, three non-accumulating species common to Oncol and V.P. Rosales (*B. chilense*, *H. tortuosum* and *W. trichosperma*) all accumulated more Al at the Oncol site. The ferns *B. chilense* and *H. tortuosum* accumulated four- and six-fold more Al at the more acidic Oncol site. However, the differences for *W. trichosperma* were relatively small and less than 30% (Fig. 2). Therefore, no consistent relationship was observed between soil chemistry (pH and Al saturation) and Al accumulation in the leaves and fronds. The same conclusion was made previously by others after studying Al-accumulators from the Vochysiaceae (de Andrade et al. 2011; Nogueira et al. 2019), Proteaceae (Delgado et al. 2019) and Melastomataceae (Timpone et al. 2025) families. However, results from the present study enable us to refine this observation further because we observed different responses in the Al-accumulating and non-accumulating species. Specifically, the non-accumulating species did accumulate much more Al when soil Al saturation was greater and therefore did show a positive relationship between soil Al saturation and Al in the leaves. By contrast, most Al-accumulating species did not show relationship because they could maintain high concentrations of Al in their leaves or fronds despite very large differences in available Al in the soil. A recent phylogenetic study focusing on Al accumulation in *Miconia* species (Melastomataceae) found that, even within a group characterized by high Al accumulation, certain clades displayed significantly lower leaf Al concentrations, and this variation did not appear to be associated with soil Al saturation (Timpone et al. 2025). This variation in Al accumulation suggests that other factors beyond soil Al saturation, including a complex interplay of environmental, genetic, and ecological factors, may drive differences in plants exhibiting Al accumulation. Further research on both Chilean and global floras is essential to better understand Al accumulation in plants, its distribution patterns, calcifuge behavior, and the relationships between soil chemistry and phylogeny.

The term “facultative (hyper) accumulator”, refers to species that accumulate metals depending on their availability in the soil (Van der Ent et al. 2013). By this definition, *D. dendroides* could all be designated as facultative Al accumulator because although it accumulated more than 1000 mg Al kg^{-1} DM at the Oncol and V.P. Rosales sites it accumulated almost three-fold more Al at the Oncol site. By contrast, *L. paniculatum* and *G. avellana* are not facultative accumulators since they showed similar concentrations of Al despite large differences in soil chemistry.

Leaf nutrient status in Al-Accumulating plants

Another result from this study is the lower concentrations of mineral nutrients in the leaves and fronds of Al-accumulating species compared with non-accumulating species, including N, P, K, Ca, Mn, and Cu (Table 3). Similar results were found in plants from the Cerrado vegetation in Brazil, where leaves of non-accumulating plants showed higher macronutrient (N, P, K, Ca, Mg) concentrations compared with Al-accumulating plants (Souza et al. 2015). These findings suggest that the accumulation of Al to high concentrations come with trade-offs in nutrient acquisition, possibly due to physiological or metabolic constraints associated with managing the toxic Al ions. It is possible that non-accumulating plants could be using some physiological strategies to exclude Al and avoid its uptake, such as the exudation of carboxylates by their roots, which play a crucial role in solubilizing P and detoxifying Al (Chen and Liao 2016). In this context, several authors have observed a positive relationship between foliar Mn concentration and the release of carboxylates by the roots (Lambers et al. 2021; Pang et al. 2018; Yan et al. 2025). Our results demonstrated that non-accumulating plants exhibited two-fold higher foliar Mn concentration in comparison with Al-accumulating ones. Perhaps some of the non-accumulating plants (Al excluders) could be releasing carboxylates from their roots at a higher rate than the Al-accumulating plants as a strategy to exclude Al. Although it is important to mention that Al-accumulating and non-accumulating species exude carboxylates (de Oliveira Carvalho Bittencourt et al. 2020; Delgado et al. 2021) so other regulators are likely involved.

The results reveal distinct patterns of nutrient accumulation among the species (Fig. 4, Suplemmentary tables). Interestingly, we found that some Al-accumulating plants also show high concentrations of other metals. *Lomatia dentata* exhibits high concentrations of Mn (~ 3,452 mg kg^{-1} DM), while *D. dendroides* shows high concentrations of Fe (~ 3,452 mg kg^{-1} DM at the Oncol site) in their leaves (Fig. 4, Suplemmentary tables). Several authors have reported that Al can alleviate the toxicity of metals such as Mn and Fe (Bojórquez-Quintal et al. 2017; Hajiboland et al. 2013; Muhammad et al. 2016, 2018; Watanabe et al. 2006). These authors concluded that Al alleviates the toxicity of Mn or Fe because Al exerts an antagonistic effect on the uptake of these metals (and vice-versa). We speculate that the high Al accumulation in *L. dentata* and *D. dendroides* may be influenced by the presence of Mn and Fe potentially through mechanisms that mitigate metal toxicity. This could occur via competition for uptake pathways or alteration of root exudates affecting metal solubility. Further investigation into the physiological and molecular interactions between Al, Mn, and Fe uptake and accumulation in these species could provide deeper insights into their nutrient management.

Conclusions

This study demonstrated that Al-accumulating plants do occur in the temperate rainforests from southern South America. We confirmed that two Proteaceae species (*G. avellana* and *L. dentata*) and a moss

(*D. dendroides*) are Al accumulators and identified four new Al-accumulating ferns belonging to families Dryopteridaceae (*Megalastrum spectabile*), Gleicheniaceae (*Gleichenia quadripartite* and *G. criptocarpa*) *and Lycopodiaceae* (*Lycopodium paniculatum*). Our findings indicate that soil pH and Al availability can influence the accumulation of Al, but the relationship is more significant among non-accumulators than among Al-accumulators. Finally, we found that soil chemistry and the accumulation of high concentrations of Al significantly affected the uptake and accumulation of other essential mineral nutrients in leaves.

Our findings highlight the value of exploring plant diversity in natural environments in order to identify species with unique adaptations to challenging conditions. The discovery of Al-accumulating species in the temperate rainforests of southern South America opens new avenues for research into plant resistance mechanisms. A greater understanding of the mechanisms and genes involved could help protect native forest systems and subside biotechnological strategies aimed at improving the acidic-soil tolerance of crops.

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Declarations

Competing interests Gustavo Habermann is Section Editor for Plant and Soil and the peer-review process for this article was independently handled by another member of the journal editorial board.The authors declare that they have no conflict of interest.

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