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Soil nutrient-related trait variation in *Leiothrix* (Eriocaulaceae) species from Brazilian rock outcrops

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Abstract

Campos rupestres are shaped by nutrient limitation and strong heterogeneity, traditionally interpreted within a phosphorus-centered framework. We evaluated how soil nutrient variation relates to plant morphology and biomass allocation in three endemic *Leiothrix* (Eriocaulaceae) species inhabiting *campos rupestres* of the Serra do Cipó, Brazil. Using linear mixed-effects models, we investigated relationships between vegetative traits, ramet biomass partitioning, and soil nutrient concentrations. Soil nutrient effects were species- and trait-specific. In *L. crassifolia*, Fe showed predominantly negative associations with plant and leaf dimensions. Cu, B, and K were positively associated with vegetative growth and biomass traits. Zn negatively affected root biomass and belowground allocation. *Leiothrix spiralis* exhibited intermediate responsiveness, with Fe positively associated with leaf traits and Zn and Mn showing negative relationships. *Leiothrix mucronata* displayed limited responsiveness, with nutrient effects restricted mainly to belowground traits. *Leiothrix crassifolia* showed high responsiveness, whereas *L. mucronata* exhibited the weakest responses. These contrasting patterns reveal substantial interspecific variation in soil nutrient responsiveness within a single genus and suggest that specific soil nutrients are associated with functional differentiation among closely related species. Our findings highlight the importance of considering multiple soil nutrients to better understand plant–soil interactions and trait variation in oligotrophic rock outcrop ecosystems.

Keywords: biomass partitioning; clonal plants; ecological strategies; edaphic heterogeneity; functional traits; oligotrophic systems; rupestrian grasslands.

Introduction

Rock outcrop ecosystems are among the most environmentally restrictive terrestrial habitats, characterized by shallow and discontinuous soils, low water retention, and intense thermal and radiation stress, which together impose severe edaphic constraints on plant growth (Nascimento *et al.*, 2021). In many quartzitic outcrops, soil nutrient availability is extremely low, especially phosphorus (P), reinforcing strong nutritional limitations and favoring the evolution of conservative

ecological strategies (Oliveira *et al.*, 2015). In addition, these systems may exhibit significant concentrations of potentially toxic elements such as nickel, chromium, cobalt, and zinc, creating chemically stressful environments that select for tolerance and exclusion mechanisms in plants (Wójcik *et al.*, 2017). These combined abiotic filters favor distinctive morphological and allocation traits, including compact rosette growth forms, specialized rooting systems, and conservative biomass allocation patterns associated with persistence under extreme environmental conditions (Teodoro *et al.*, 2019). Consequently, rock outcrop ecosystems harbor highly specialized floras with high levels of endemism and represent important natural laboratories for understanding plant adaptation and diversification in nutrient-poor landscapes (Hopper, 2009).

In southeastern Brazil, *campos rupestres* constitute one of the most emblematic rock outcrop ecosystems, forming a mosaic of herbaceous and shrubby vegetation associated with shallow soils and rocky substrates at high elevations (Scarano, 2007). Distributed mainly along the Espinhaço Mountain Range, these ecosystems combine severe oligotrophy with pronounced environmental heterogeneity at fine spatial scales (Silveira *et al.*, 2016; Zappi *et al.*, 2017). Despite occupying less than 1% of the Brazilian territory, *campos rupestres* harbor nearly 15% of the country's plant diversity and exceptionally high levels of endemism (Zappi *et al.*, 2017; Colli-Silva *et al.*, 2019). Within this ecosystem, the Serra do Cipó region is recognized for its remarkable species richness and functional diversity, strongly structured by variation in soil and microclimatic conditions (Fernandes *et al.*, 2020; Miola *et al.*, 2021). Across *campos rupestres*, edaphic properties are considered major factors shaping plant community structure, ecological strategies, and evolutionary diversification (Silveira *et al.*, 2016; Zappi *et al.*, 2017; Demetrio & Coelho, 2018; Miola *et al.*, 2020; Demetrio *et al.*, 2026).

Species of the genus *Leiothrix* (Eriocaulaceae), one of the characteristic herbaceous groups of *campos rupestres*, provide an important model for understanding plant responses to environmental heterogeneity in rock outcrop systems. Previous studies demonstrated that life-history attributes, growth dynamics, reproductive strategies, and clonal architecture in *Leiothrix* are strongly associated with soil conditions and microenvironmental variation (Coelho *et al.*, 2006; Coelho *et al.*, 2008a;b; Coelho *et al.*, 2014). Such soil factors also influence species distribution and abundance within the genus, suggesting that fine-scale edaphic heterogeneity contributes to ecological differentiation among closely related taxa (Demetrio & Coelho,

2018). In addition, variation in substrate structure, vegetation cover, and soil conditions has been associated with differences in reproductive modes and persistence strategies among sympatric species (Coelho *et al.*, 2008b). Together, these findings support the view that soil conditions act as major ecological filters shaping plant morphology, biomass allocation, and species coexistence in *campos rupestres*.

Despite the recognized importance of soil conditions in rock outcrop ecosystems, most previous studies have focused on broad edaphic gradients such as substrate type, moisture availability, or general fertility rather than disentangling the effects of specific soil chemical components (Negreiros *et al.*, 2014; Coelho *et al.*, 2006; Coelho *et al.*, 2008a;b; Coelho *et al.*, 2014; Demetrio & Coelho, 2018). Additionally, in *campos rupestres* and other old, climatically buffered, infertile landscapes (OCBILs), research on nutrient limitation has traditionally emphasized the importance of P because of its extremely low availability and central role in constraining plant productivity and physiological performance (Lambers *et al.*, 2010; Silveira *et al.*, 2016; Lambers, 2022). In *Leiothrix*, recent evidence demonstrated that soil phosphorus availability influences biomass allocation patterns and trade-offs among vegetative organs, particularly affecting belowground investment and storage structures (Demetrio *et al.*, 2026). However, the strong emphasis on phosphorus has also limited broader investigations into how other soil nutrients contribute to plant trait variation and ecological differentiation in nutrient-poor rock outcrop environments.

In addition to phosphorus, other soil nutrients may exert important influences on plant functional strategies in *campos rupestres*. Macronutrients such as potassium (K), calcium (Ca), and magnesium (Mg) are directly associated with osmotic regulation, membrane stability, photosynthetic processes, and tissue development (Hawkesford *et al.*, 2012), whereas micronutrients including iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and boron (B) are essential for enzymatic activity, respiration, redox regulation, and cell wall formation (Hänsch & Mendel, 2009; Broadley *et al.*, 2012). In oligotrophic and metal-rich substrates, the availability of these nutrients may vary substantially across short spatial scales, and their effects on plants can range from limitation to toxicity depending on local concentrations and species-specific tolerances (Kazakou *et al.*, 2008; Broadley *et al.*, 2012). Nevertheless, these nutrients are rarely incorporated explicitly into ecological analyses of plant functional strategies in rock outcrop ecosystems, where nutrient effects are more commonly treated as integrated

measures of fertility or environmental harshness (Hopper, 2009; Lambers *et al.*, 2010; Silveira *et al.*, 2016).

Plant responses to nutrient availability are also expected to vary among species according to differences in ecological strategies related to resource acquisition, stress tolerance, and persistence (Grime, 2001; Reich, 2014). Trait-based approaches integrating morphological variation and biomass allocation patterns provide a useful framework for investigating such strategies because they allow the identification of coordinated responses to environmental constraints through differences in plant form and resource investment (Westoby *et al.*, 2002; Violle *et al.*, 2007). In ecosystems characterized by strong environmental filtering and fine-scale edaphic heterogeneity, such as campos rupestres, species-specific nutrient responses may therefore contribute to ecological differentiation and coexistence among closely related taxa (Hopper, 2009; Silveira *et al.*, 2016).

In this study, we investigated how variation in soil nutrients influences biometric traits and biomass allocation in three endemic species of the genus *Leiothrix*, characteristic elements of *campos rupestres* vegetation. Focusing on populations occurring in quartzitic outcrops of Serra do Cipó, we evaluated the effects of soil micronutrients (Fe, Zn, Mn, Cu, and B) and macronutrients (K, Ca, and Mg) on vegetative dimensions and above- and belowground biomass components. We hypothesized that soil nutrients show significant associations with plant morphology and allocation patterns in rock outcrop environments and that species differ in their responsiveness to nutrient availability, potentially indicating contrasting ecological strategies associated with resource acquisition and conservation.

Materials and Methods

Study system

We conducted this study in Brazilian *campos rupestres* (rupestrian grasslands), montane grassland ecosystems that cover approximately 83,000 km^2, corresponding to about 0.83% of Brazil's total surface area (Fernandes *et al.*, 2018). Rather than constituting a single vegetation type, *campos rupestres* form a complex mosaic of plant communities (Morellato & Silveira, 2018), whose floristic composition and structure we know to be closely associated with substrate origin and chemical composition (Zappi *et al.*, 2017).

These ecosystems occur predominantly on ancient, highly weathered substrates and have been classified as Old, Climatically Buffered, Infertile Landscapes (OCBILs; Hopper, 2009; Silveira *et al.*, 2016), a designation that reflects both their extreme nutrient poverty and their exceptionally high levels of plant species richness and endemism.

There is a pronounced morphological convergence among plant assemblages in *campos rupestres*, with many species exhibiting rosette-forming growth habits or tightly overlapping leaves. These traits are commonly associated with stress tolerance in shallow, nutrient-poor soils. Eriocaulaceae stands out as one of the most common and ecologically important groups in *campos rupestres* (Giulietti & Hensold, 1990). Within this family, the genus *Leiothrix* comprises 39 described species (Giulietti, 2023), and the Espinhaço Mountain Range represents its main center of diversity (Giulietti & Hensold, 1990). Because of their abundance, endemism, and ecological differentiation, we consider *Leiothrix* species to be suitable models for investigating the ecology of herbaceous plants in OCBIL systems. Individuals of *Leiothrix* typically grow as basal rosettes from which floral scapes bearing capitate inflorescences emerge.

The species included in this study differ markedly in their reproductive strategies. We classify *Leiothrix mucronata* (Bong.) Ruhland and *L. crassifolia* (Bong.) Ruhland as a rhizomatous, seed-producing (RS) species, whereas *L. spiralis* (Bong.) Ruhland exhibits a pseudoviviparous rooted (PR) reproductive mode (Coelho *et al.*, 2007; Coelho *et al.*, 2008b). In RS species, sexual reproduction via seed production represents the primary pathway for offspring establishment (Coelho *et al.*, 2008b). In contrast, PR species reproduce asexually through the formation of plantlets on their inflorescences; these propagules establish upon contact with the soil by producing new roots and may either remain temporarily connected to the parental rosette via the floral scape or become independent (Coelho *et al.*, 2006). We note that all three species are also capable of vegetative propagation through rhizome sprouting (Coelho *et al.*, 2007).

Floral scape length also varies among species. Floral scapes of *L. crassifolia* and *L. mucronata* are generally shorter than those of *L. spiralis* (Coelho *et al.*, 2007). Toward the end of the reproductive season, scapes often curve, bringing the flower heads into contact with the soil surface, which results in seed deposition near the parental rosettes (Coelho *et al.*, 2008a). Although rosettes of *L. spiralis* are morphologically similar to those of *L. crassifolia*, establishment via rhizomes sprouting in *L. spiralis* is rarely observed (Coelho *et al.*, 2007). In this species, pseudoviviparous propagules form late in

the reproductive cycle, after flower heads have fully matured and reached the soil surface (Coelho *et al.*, 2007).

All studied species are represented by reference material deposited in the Universidade Federal de Minas Gerais Herbarium (BHCB), under the registration numbers 50367 (*L. crassifolia*), 80762 (*L. mucronata)*, and 166413 (*L. spiralis*).

Terminology

Clonal plants may produce offspring through both sexual and asexual pathways. We refer to individuals originating from seeds as genets, whereas we define ramets as vegetatively produced units that are physiologically independent of the parental individual (Harper, 1977). In *Leiothrix* species, we define a ramet as a single rosette together with its associated root system and rhizome. Accordingly, all measurements of plant size and dry mass in this study refer to individual ramets rather than to genets.

Operationally, we considered a ramet to be a rosette attached to an unbranched rhizome segment, not connected to any other rosette, and possessing its own root system. This definition allowed us to maintain consistency in trait measurements and to minimize ambiguity regarding individual boundaries in clonal plants.

Data sampling

We sampled three *Leiothrix* species using square plots of 100 m^2, each subdivided into 100 quadrats of 1 m^2. We selected this quadrat size because it is commonly employed in ecological studies of *Leiothrix*, given the small stature of individuals and their limited dispersal capacity (Coelho *et al.*, 2006; Coelho *et al.*, 2008a; Coelho *et al.*, 2014). In each monospecific stand, we positioned a sampling grid to encompass a continuous patch dominated by the target species. Within each grid, we randomly selected six 1 m^2 quadrats for sampling.

Within each selected quadrat, we randomly harvested ten ramets and collected a composite soil sample. For each species (*L. spiralis*, *L. crassifolia*, and *L. mucronata*), we sampled three independent areas, resulting in a total of 18 soil samples and 180 ramets per species. This sampling design allowed us to characterize local soil conditions while minimizing potential effects of dispersal limitation and patchy ramet distribution. At the time of collection, all sampled ramets were non-flowering and originated from clearly separated plant clumps, with sufficient spacing to ensure that they belonged to different

rhizome systems. Moreover, we confirmed that all sampled individuals were produced via vegetative rhizome sprouting, as described in the species accounts.

All field sampling and collection of biological material were conducted under authorization from the Brazilian Institute for Biodiversity Conservation (ICMBio), issued through the SisBio system (permit no. 38040), in accordance with national legal and ethical regulations for research involving biological resources and sampling within protected areas. In addition, access to Brazilian native genetic heritage for scientific research purposes was duly registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), in compliance with Brazilian legislation (Law no. 13,123/2015 and its regulations), under registration number AEF8111, issued by the Conselho de Gestão do Patrimônio Genético (CGen). We provide a map with geographic coordinates of all sampling sites in Figure 1.

Plant trait measurements

To ensure complete collection of belowground structures, we carefully excavated ramets using a gardening shovel inserted at least 10 cm from the rosette and to the maximum attainable depth. The sandy texture of the soils and the loose anchorage of *Leiothrix* plants facilitated excavation of the entire root and rhizome systems. Based on previous studies and our own field observations, we note that *Leiothrix* species possess shallow root systems, typically confined to the upper 10 cm of the soil profile (Coelho *et al.*, 2007; Coelho *et al.*, 2008a), which allowed us to reliably collect all belowground organs.

After excavation, we placed ramets in paper bags and transported them to the laboratory at the Universidade Federal de Lavras. We gently washed plants to remove adhering soil particles. After cleaning, we conducted all morphological measurements in the laboratory. For each ramet, we measured the rosette diameter (cm) using a digital caliper. To obtain representative estimates of leaf morphology while accounting for within-ramet variability, we randomly selected four fully developed leaves per ramet and measured their length and maximum width (cm) using a caliper. Similarly, to characterize root morphology, we randomly selected four root segments per ramet and measured their length. For each ramet, we calculated mean values for leaf length, leaf width, and root length and used these mean values in subsequent analyses.

Following morphological measurements, we separated each ramet into leaves, roots, and rhizomes. We oven-dried all plant components at 60 °C for 72 h, or until constant mass, and weighed them using an analytical balance with a precision of 0.0001 g. We calculated biomass allocation (g) as the proportion of total ramet dry mass allocated to each organ by dividing the dry mass of leaves, roots, or rhizomes by the total dry mass of the ramet.

Soil sampling and analyses

For each selected 1 m^2 quadrat, we collected soil samples from the 0–15 cm layer, corresponding to the superficial soil horizon commonly used in ecological studies evaluating soil fertility and nutrient availability (Robertson *et al.*, 1999; Poorter *et al.*, 2012). Soil sampling was performed using a cylindrical hand soil auger measuring 15 cm in height and 2.5 cm in diameter, corresponding to approximately 73.6 cm^3 of soil per subsample. Within each quadrat, five subsamples were collected: one from the center and one from each of the four corners. These subsamples were pooled to produce a single composite soil sample representative of each quadrat, totaling approximately 368.2 cm^3 of soil per quadrat and yielding at least 100 g of soil for chemical analyses.

In the laboratory, roots and coarse organic fragments were removed by sieving the samples through a 2 mm mesh. Soil samples were then manually homogenized and oven-dried at 60 °C until constant mass before being stored in sealed plastic bags for subsequent analyses. All soil analyses were conducted at the Soil Fertility Laboratory of the Universidade Federal de Lavras following standardized protocols for tropical soils.

Soil concentrations of zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), magnesium (Mg), calcium (Ca), potassium (K), and were determined using the Mehlich-1 extraction method, which is widely applied to acidic tropical soils with low cation-exchange capacity. Boron (B) concentrations were determined by hot-water extraction. All analyses followed the standardized procedures described by Teixeira *et al.* (2017).

Data analysis

All analyses were conducted separately for each *Leiothrix* species to account for species-specific responses to soil nutrient variation. Initially, we considered the effects of eight soil nutrients, including the micronutrients Fe, Zn, Mn, Cu, and B, and the macronutrients K, Ca, and Mg, on plant biometric traits and biomass allocation

variables. Prior to model fitting, correlations among all soil nutrients were evaluated to assess potential collinearity and guide variable selection. Pearson correlation coefficients were calculated using the cor function from the *Hmisc* package (Harrell, 2024), and statistical significance was assessed using the cor.mtest function. Calcium and Mg showed relatively strong correlations with some micronutrients, particularly Zn and Mn (Ca–Zn: $r = 0.79$, $p < 0.001$; Ca–Mn: $r = 0.67$, $p = 0.03$; Mg–Zn: $r = 0.63$, $p = 0.04$; Mg–Mn: $r = 0.52$, $p = 0.15$), indicating substantial covariance among these variables. In addition, Mg showed very limited variation across sampling units, providing little explanatory information and generating instability during preliminary model fitting. Therefore, Ca and Mg were excluded from subsequent analyses to reduce redundancy among predictors and avoid overparameterization. Phosphorus was also evaluated during exploratory analyses but showed weak and non-significant correlations with all analyzed nutrients (Table S1). The complete correlation matrix and correlogram are provided in the supplementary material (Table S1; Fig. S1). As no retained pairwise correlation exceeded commonly accepted thresholds for problematic collinearity ($|r| > 0.7$), final models therefore included Fe, Zn, Mn, Cu, B, and K as predictors.

The effects of these soil nutrients on plant biometric traits and biomass allocation variables were evaluated using linear mixed-effects models (LMMs) implemented in the glmmTMB framework (Brooks *et al.*, 2017). The response variables included plant diameter, leaf length, leaf width, root length, leaf dry mass, root dry mass, rhizome dry mass, the root:shoot ratio, and the belowground:aboveground dry mass ratio.

Prior to modeling, response variables were visually inspected using histograms and Q–Q plots to assess distributional assumptions. When necessary, response variables were log- or square-root transformed to improve normality and homoscedasticity. Exploratory analyses indicated that all response variables were continuous and did not exhibit strong zero inflation. Accordingly, all models were fitted using a Gaussian error distribution with an identity link function (family = gaussian(link = "identity")). Additionally, soil nutrient concentrations were standardized (mean = 0, SD = 1) before analysis to facilitate model convergence and comparison of effect sizes among predictors.

For each response variable, we first fitted a null model including only random intercepts (~ 1 + (1 | Area/Quadrat)), representing variation attributable to the

hierarchical sampling design. We then fitted a full model including all selected soil nutrients as fixed effects (~ Zn + Fe + Mn + Cu + B + K + (1 | Area/Quadrat)). Random effects were specified as quadrats nested within sampling areas, matching the structure of the data.

Model comparison between null and full models was conducted using likelihood ratio tests based on changes in deviance, as implemented via the anova function in *glmmTMB*, and supported by comparisons of Akaike's Information Criterion (AIC). When the full model did not provide a significantly better fit than the null model ($p > 0.05$), the response variable was considered not to be influenced by soil nutrient variation. For response variables in which the full model was supported, reduced models were subsequently fitted by retaining only predictors with significant effects ($p < 0.05$). These reduced models were used to obtain final parameter estimates, standard errors, z values, and associated p values. When model reduction did not improve model fit, results from the full model were retained.

All analyses were performed in the R environment (R Core Team, 2023). Model diagnostics included inspection of residuals versus fitted values and Q–Q plots to verify homoscedasticity and approximate normality of residuals. Statistical significance was assessed at $\alpha = 0.05$. For models with significant results, marginal (R^2m) and conditional (R^2c) coefficients were calculated using the performance package (Lüdecke *et al.*, 2019). All figures were generated using the R packages *ggplot2* (Wickham, 2016) and *patchwork* (Pedersen, 2024).

Results

Species-specific soil nutrients and trait characterization

Soil nutrient concentrations and plant functional traits varied among the three *Leiothrix* species (Tables 1–2). Soils associated with *L. spiralis* exhibited the highest concentrations of several micronutrients, particularly Fe (347.23 ± 322.72 $mg.dm^{-3}$), together with relatively elevated Zn (0.31 ± 0.09 $mg.dm^{-3}$) and Cu (0.33 ± 0.77 $mg.dm^{-3}$). *Leiothrix mucronata* occurred in soils with intermediate Fe availability (134.22 ± 109.26 $mg.dm^{-3}$) and comparatively higher variability in Cu concentrations (0.52 ± 1.73 $mg.dm^{-3}$). In contrast, soils associated with *L. crassifolia* showed lower Fe concentrations (90.03 ± 75.70 $mg.dm^{-3}$). On the other hand, Zn (0.22 ± 0.14 $mg.dm^{-3}$), Mn (0.40 ± 0.13 $mg.dm^{-3}$), and B (0.09 ± 0.13 $mg.dm^{-3}$) were similar across species.

Morphological traits and biomass allocation patterns varied across the three *Leiothrix* species (Table 2). *Leiothrix crassifolia* was characterized by relatively high values of plant diameter (5.30 ± 1.42 cm), leaf length (2.87 ± 0.73 cm), and total biomass (1.24 ± 0.64 cm). *Leiothrix spiralis* exhibited intermediate values for several vegetative traits, while also presenting relatively high root length (3.83 ± 1.23 cm) and substantial biomass allocation to belowground structures, reflected by elevated RSRatio (0.73 ± 1.28) and SSRatio (3.85 ± 3.37). In contrast, *L. mucronata* was characterized by comparatively reduced values for traits such as leaf width (0.05 ± 0.01 cm), root biomass (0.20 ± 0.16 g), and total biomass (0.39 ± 0.22 g), whereas biomass allocation ratios remained within the range observed across the studied species. Overall, these patterns reveal substantial variation in morphological traits, biomass allocation, and soil nutrient associations across the studied *Leiothrix* species.

Soil nutrient effects on plant morphology and biomass allocation

Plant diameter and leaf and root traits were the most related traits to soil nutrients in *Leiothrix crassifolia* (Figure 2, Table 3). Plant diameter decreased significantly with increasing Fe, while Cu and K showed positive effects (Table 3, Figure 2A). Leaf length was negatively associated with Fe and positively associated with K (Table 3, Figure 2B). Leaf width also declined with increasing Fe and increased with K (Table 3, Figure 2C). Leaf dry mass in *L. crassifolia* was significantly influenced by micronutrients (Table 3, Figure 2D). Specifically, Fe showed a negative association, whereas Cu and B had positive effects on leaf dry mass. Additionally, the root dry mass declined with increasing Zn (Table 3, Figure 2D). In contrast, root length (χ^2 = 4.41, df = 6, p = 0.62), rhizome dry mass (χ^2 = 4.44, df = 6, p = 0.61) did not differ significantly from null models. Dry mass allocation ratios in *L. crassifolia* responded differently to nutrients. While the root:shoot ratio did not differ significantly from null models (x^2 = 12.51, df = 6, p = 0.051) the belowground:aboveground dry mass ratio decreased with Zn and Cu and increased with Fe (Table 3, Figure 2F).

In *Leiothrix spiralis*, soil nutrient effects were less broad and restricted to a subset of traits (Table 3, Figure 3). Leaf width increased with Fe and decreased with Zn and Mn (Table 3, Figure 3A). Leaf dry mass showed a similar pattern, increasing with Fe and declining with Zn and Mn (Table 3, Figure 3B). No significant soil nutrient effects were detected for plant diameter (χ^2 = 11.73, df = 6, p = 0.06), leaf length (χ^2 = 12.06, df

= 6, p = 0.06), root length (χ^2 = 4.14, df = 6, p = 0.65), root dry mass (χ^2 = 7.82, df = 6, p = 0.25), rhizome dry mass (χ^2 = 3.32, df = 6, p = 0.76), or dry mass allocation ratios (Root:Shoot Ratio - χ^2 = 8.27, df = 6, p = 0.22; Belowground:Aboveground Dry Mass Ratio - χ^2 = 11.10, d f = 6, p = 0.08).

In *Leiothrix mucronata*, soil nutrient effects were found only for one measured trait (Table 3, Figure 4). No significant associations were detected for plant diameter (χ^2 = 6.58, df = 6, p = 0.36), leaf dimensions (Length - χ^2 = 6.20, df = 6, p = 0.40; Width - χ^2 = 7.20, d f.= 6, p = 0.30), root length (χ^2 = 4.76 d f = 6, p = 0.57), leaf dry mass (χ^2 = 11.26, d f = 6, p = 0.08), root dry mass (χ^2 = 2.93, df = 6, p = 0.81), or allocation ratios (Root:Shoot Ratio - χ^2 = 11.25, df = 6, p = 0.08). Rhizome dry mass was the only trait significantly related to nutrients, declining with increasing Mn and Cu (Table 3, Figure 4A). Belowground:Aboveground Dry Mass Ratio decreased with Mn and Fe and increased with K (Table 3, Figure 4B).

Interspecific variation in soil nutrient degree of responsiveness

The magnitude, breadth, and consistency of soil nutrient effects differed among the three *Leiothrix* species (Figures 1–3). *Leiothrix crassifolia* exhibited the broader responsiveness degree to soil nutrient variation, with significant relationships detected across multiple vegetative morphological traits (Figure 2A–C), leaf dry mass (Figure 2D), and dry mass allocation ratios (Figure 2E–F). In this species, all five micronutrients were significantly associated with at least one trait.

Leiothrix spiralis showed a more restricted pattern of responsiveness, with significant soil nutrient effects limited to leaf width and leaf dry mass (Figure 3A–B). No significant effects were detected for other vegetative traits, belowground biomass components, or allocation ratios.

In contrast, *L. mucronata* displayed minimal responsiveness to micronutrient variation, with significant effects detected only for rhizome dry mass (Figure 4A), indicating a markedly narrower range of responsiveness relative to the other species.

Discussion

Soil nutrients as significant correlates of plant morphology and biomass allocation

Our results demonstrate that micronutrients exert species-specific and trait-specific effects on plant morphology and biomass allocation in *campos rupestres*, revealing an important but still underexplored dimension of plant-nutrient

relationships in rock outcrop ecosystems. The dominant role of macronutrients, particularly phosphorus, as drivers of plant responses in these environments is well established (Lambers *et al.*, 2008; Silveira *et al.*, 2016), it represents only part of the edaphic filter. For instance, recent evidence from *Leiothrix* populations in the same study system demonstrated that soil phosphorus availability influences biomass allocation patterns and allocation relationships among vegetative organs, particularly affecting belowground investment and storage structures (Demetrio *et al.*, 2026). However, our findings show that fine-scale variation in other nutrient availability, especially micronutrients content, can also shape vegetative traits and allocation patterns, even among closely related endemic species, with some species being more responsive to nutrient availability than others. This evidence expands current ecological frameworks for understanding plant strategies in oligotrophic systems, reinforcing calls to move beyond macronutrient-centered perspectives and to explicitly incorporate micronutrients into analyses of plant–soil relationships in rock outcrop vegetation (Hopper, 2009; Lambers *et al.*, 2014).

For each *Leiothrix* species, soil nutrients influenced both absolute trait values and biomass partitioning among plant organs. Variation in leaf size and leaf biomass has direct consequences for light interception and carbon gain, while shifts in belowground allocation affect anchorage, storage capacity, and the acquisition of limiting resources such as water and nutrients (Poorter *et al.*, 2012; Reich, 2014; Poorter & Ryser, 2015). In environmentally harsh systems, even relatively subtle nutrient-driven adjustments in morphology and allocation may have disproportionate effects on plant performance, as growth and survival operate close to physiological and resource-use limits (Grime, 2001; Lambers *et al.*, 2008). By simultaneously influencing multiple components of plant form and allocation, nutrients thus emerge as functionally relevant drivers of plant strategies rather than background variables in rock outcrop ecosystems.

Importantly, these effects would likely remain obscured if soil chemistry were treated only through coarse fertility classes or single-nutrient frameworks. Previous work in nutrient-poor and geochemically heterogeneous systems has shown that broad descriptors of soil fertility can mask biologically meaningful variation in individual elements and their effects on plant traits (Hopper, 2009; Silveira *et al.*, 2016). Our results reinforce the idea that resolving soil chemistry into its constituent elements provides critical insights into how fine-scale edaphic heterogeneity translates into plant

morphological and allocational variation in *campos rupestres*, particularly in environments where multiple chemical constraints operate simultaneously (Lambers *et al.*, 2014; Zappi *et al.*, 2017).

Interspecific variation in responsiveness to soil nutrients

Species differ markedly in the number of traits associated with soil nutrient variation, consistent with our hypothesis that species may differ in their ecological responses to edaphic heterogeneity. In nutrient-poor and heterogeneous environments, species often differ in how their traits and allocation patterns are associated with soil nutrient variation, reflecting divergent positions along gradients of resource acquisition and stress tolerance (Grime, 2001; Reich, 2014). The three *Leiothrix* species examined here exhibited a clear gradient of responsiveness, ranging from broader responsiveness, with multiple traits varying in response to soil variation, to very narrow responsiveness patterns, indicating contrasting patterns of association with soil chemical variation within the same habitat. Such interspecific differences are consistent with evidence that fine-scale edaphic heterogeneity can promote strategy differentiation even among closely related taxa in oligotrophic systems (Hopper, 2009; Silveira *et al.*, 2016).

Leiothrix crassifolia exhibited the strongest and most consistent morphological responses to nutrient concentration, which could reflect a more dynamic response and a comparatively nutrient-acquisitive strategy. Species with acquisitive strategies typically show strong growth responses to variation in nutrient availability but may also be more sensitive to chemical imbalances (Grime, 2001; Reich, 2014). In our study, soil iron showed negative effects on vegetative dimensions and leaf biomass, potentially consistent with negative effects of elevated Fe concentrations on vegetative growth (Connolly & Guerinot, 2002; Briat *et al.*, 2010). In contrast, copper and boron concentrations were positively associated with leaf growth and biomass accumulation, which may be related to the known physiological importance of these nutrients for plant metabolism and structural development (Hänsch & Mendel, 2009). Together, these patterns suggest that *L. crassifolia* benefits from certain nutrients, exhibiting positive associations between them and vegetative traits, but may also present stronger negative associations under elevated micronutrient concentrations.

Leiothrix spiralis displayed an intermediate pattern of responsiveness, with both positive and negative effects depending on the micronutrient and trait considered, but

with a narrower breadth of affected traits compared to *L. crassifolia*. Iron enhanced leaf width and leaf biomass, suggesting positive associations between Fe availability and leaf traits within the sampled conditions, whereas zinc and manganese were associated with reduced leaf size and biomass, indicating that higher nutrient concentrations were associated with reduced trait values (Hänsch & Mendel, 2009). Such contrasting responses are consistent with previous studies that have shown that nutrients may exert both positive and negative effects depending on concentration and species context (Broadley *et al.*, 2012). In our study, this specific context is shown by the divergent responses to Fe, which, while acting as a possible stressor for *L. crassifolia*, potentially functioned as a growth-enhancing resource for L. spiralis. This mixed response may be consistent with trait patterns linked to an intermediate ecological strategy, allowing growth under favorable chemical conditions while maintaining comparatively moderate allocation responses across nutrient gradients. Intermediate strategies of this type are expected to be particularly advantageous in spatially heterogeneous substrates, where nutrient availability varies sharply over short distances, as is typical of quartzitic rock outcrops (Grime, 2001; Hopper, 2009).

In contrast, *Leiothrix mucronata* showed minimal responsiveness to nutrient variation across most traits, with significant effects detected only for manganese and copper on rhizome dry biomass. The general absence of micronutrient effects on leaf and root traits is compatible with physiological regulation and limited plasticity in response to soil chemical variation, a pattern commonly associated with conservative, stress-tolerant strategies (Grime, 2001; Reich, 2014). This potentially conservative growth pattern is fully consistent with the species' distinctly vegetative size and lower total ramet biomass compared to the other species studied. Species adopting such strategies tend to buffer growth rates and allocation patterns against environmental heterogeneity, thereby reducing sensitivity to short-term fluctuations in resource availability (Poorter & Ryser, 2015). By limiting responsiveness to nutrient variation, plants may be associated with greater stability under chemically heterogeneous conditions, maintaining stable performance under persistently harsh edaphic conditions characteristic of nutrient-poor, metal-influenced substrates (Lambers *et al.*, 2008; Hopper, 2009).

Taken together, these contrasting responses reveal a gradient of micronutrient responsiveness within the genus *Leiothrix*, ranging from highly responsive species to

species exhibiting comparatively limited responsiveness to nutrient variation. Such strategy continuums are well documented in plant ecology and reflect coordinated variation in growth rates, nutrient use, and tolerance to environmental stress (Grime, 2001; Reich, 2014). Importantly, this gradient is expressed not only in overall growth responses but also in how different biomass compartments respond to nutrient variation, consistent with the view that allocation patterns are central components of plant ecological strategies (Poorter *et al.*, 2012). By shaping trait expression and allocation even among closely related species occupying the same habitat, micronutrients and macronutrients emerge as relevant drivers of functional differentiation within narrowly defined ecological contexts (Violle *et al.*, 2007; Hopper, 2009).

Implications for coexistence, conservation, and rock outcrops ecology

In chemically heterogeneous environments such as *campos rupestres*, differential responsiveness to soil nutrients can contribute to niche partitioning and facilitate species coexistence by reducing direct competition for uniformly scarce macronutrients (Hutchinson, 1957; Tilman, 1982). In old infertile landscapes, fine-scale variation in soil chemistry is expected to promote coexistence by allowing species with contrasting patterns of nutrient association and ecological responses to occupy distinct edaphic niches (Hopper, 2009; Silveira *et al.*, 2016). By influencing how species allocate biomass and respond to soil chemical variation, micronutrients may therefore help stabilize coexistence among closely related taxa within the fine-scale mosaics of edaphic conditions characteristic of rock outcrop vegetation (Violle *et al.*, 2007; Zappi *et al.*, 2017).

One limitation of our study is its correlative nature, which prevents direct inference of causal relationships between soil nutrients and plant trait variation. Although we detected significant associations between specific soil nutrients and biometric and biomass allocation traits in *Leiothrix* species, these relationships may also be influenced by additional environmental factors not explicitly incorporated into our analyses. In particular, variables such as soil phosphorus availability (Demetrio & Coelho, 2018), pH, and soil moisture (Coelho *et al.*, 2006, 2008a;b) are known to influence plant performance and nutrient dynamics in *campos rupestres* and may interact with the nutrients evaluated here. Previous analyses conducted using the same

study system demonstrated that soil phosphorus availability influences biomass allocation patterns and allocation trade-offs among vegetative organs in *Leiothrix*, particularly affecting belowground investment and storage structures (Demetrio *et al.*, 2026). However, phosphorus was not correlated with the other nutrients analyzed in the present study, suggesting that the nutrient–trait relationships reported here are unlikely to simply reflect indirect phosphorus effects. Additionally, Ca and Mg were initially evaluated but excluded from the final models due to strong collinearity with Mn and Zn. Nevertheless, nutrient interactions and synergistic effects among soil components may also contribute to the observed patterns. Future studies integrating broader edaphic characterization and experimental manipulations will therefore be important to disentangle the relative and interactive effects of multiple soil variables on plant functional strategies in rock outcrop ecosystems.

From a conservation perspective, recognizing the ecological role of soil nutrients, especially micronutrients, is particularly relevant for the *campos rupestres* of Serra do Cipó, where endemic species are increasingly exposed to anthropogenic pressures. Atmospheric deposition, mining activities, tourism, and soil disturbance are threats to Brazilian *campos rupestres* (Silveira *et al.*, 2016) and can alter micronutrient availability and metal concentrations, potentially affecting species differentially depending on their nutrient responsiveness (Zappi *et al.*, 2017). Species with acquisitive strategies may respond strongly to such changes, whereas conservative species may be more resilient but less capable of exploiting altered conditions. Incorporating soil chemical composition, including micronutrients, into conservation planning could therefore improve predictions of species vulnerability and community responses to environmental change.

Synthesis and Conclusions

In conclusion, our study demonstrates that micronutrients are active drivers of plant morphological and biomass allocation strategies in nutrient-poor rock outcrop ecosystems. By revealing strong interspecific differences in micronutrient responsiveness within an endemic genus, we expand the conceptual framework of plant-nutrient relationships in *campos rupestres* beyond its traditional macronutrient focus. These findings deepen our understanding of the ecological processes structuring

rock outcrop vegetation and highlight new avenues for research on plant adaptation, coexistence, and conservation in these singular ecosystems.

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Author's Contributions

Guilherme Ramos Demetrio: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing.

Luziene Seixas: Formal analysis, Visualization, Writing – review & editing.

Grazielle Sales Teodoro: Validation, Writing – review & editing.

Flavia de Freitas Coelho: Conceptualization, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The datasets generated and analyzed during the current study, along with the R scripts used for data analysis, are available in the "Leiothrix_Micronutrients" GitHub repository. [https://github.com/grdemetrio/Leiothrix_Micronutrients].

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Supplementary Material

The following online material is available for this article:

Table S1. Pairwise correlations among soil nutrient concentrations (Fe, Zn, Mn, Cu, B, K, Ca, Mg and P were evaluated using Pearson correlation coefficients to assess potential collinearity among predictors. Correlations were computed using the cor() function from the *Hmisc* package in R, and the statistical significance of correlations was assessed using the cor.mtest() function, which provides associated *p* values and confidence intervals (Lower CI and Upper CI). Significant p values (α = 0.05) are presented in bold.

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Table and Figure Captions

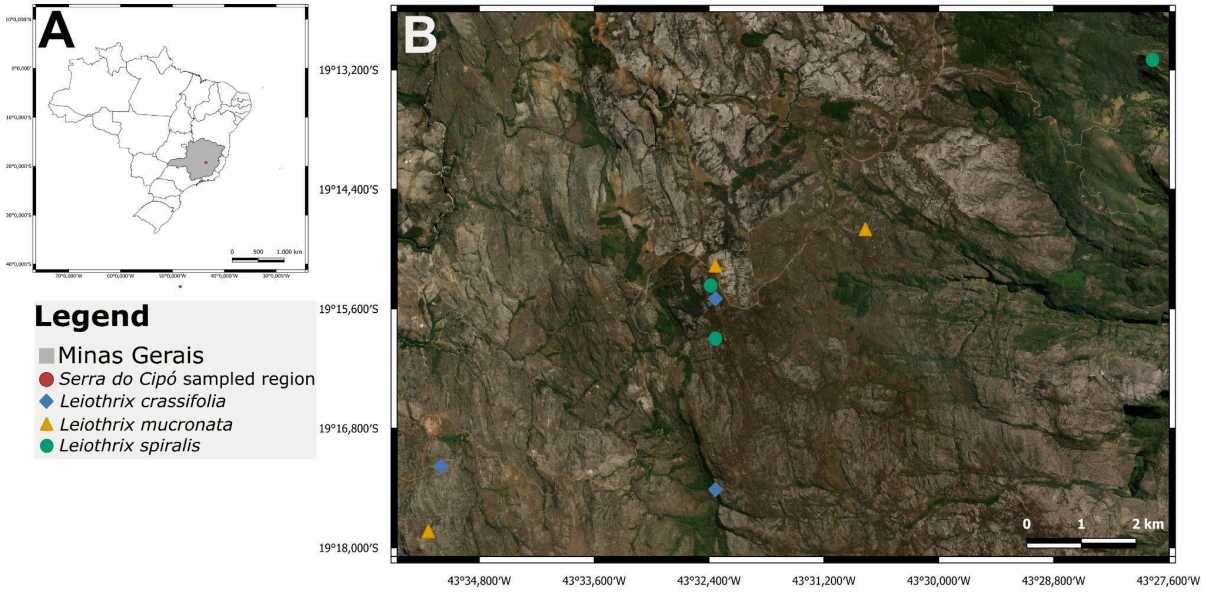

Figure 1. Study area and sampling locations of three *Leiothrix* species in the Serra do Cipó region, Minas Gerais, Brazil. (A) Location of the sampled region within the state of Minas Gerais. (B) Distribution of sampling sites for *Leiothrix crassifolia* (blue diamonds), *L. mucronata* (yellow triangles), and *L. spiralis* (green circles) over a satellite image of the study area. The map illustrates the spatial arrangement of populations used for soil and plant trait sampling in Brazilian rocky outcrop ecosystems (*campos rupestres*).

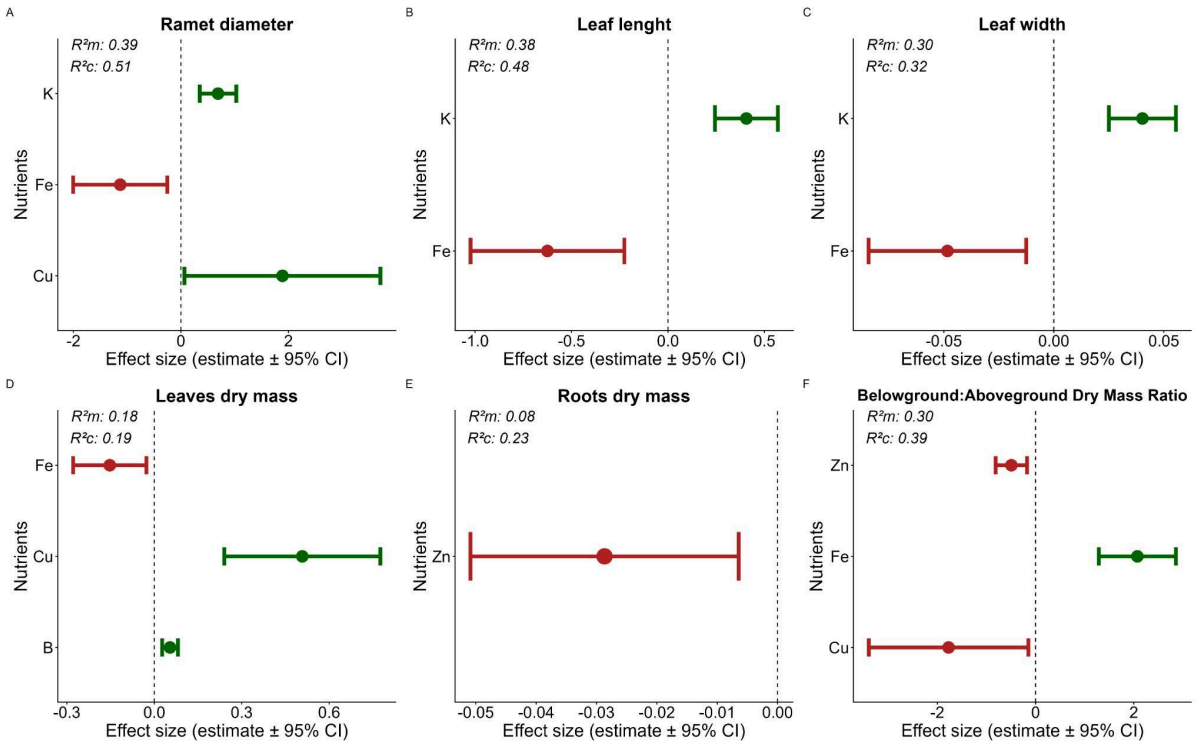

Figure 2. Standardized effect sizes (model estimates ± 95% confidence intervals) from Gaussian mixed-effects models relating plant traits to soil nutrient concentrations in *Leiothrix crassifolia*. Panels show effects on (A) ramet diameter, (B) leaf length, (C) leaf width, (D) leaf dry mass, (E) root:shoot ratio, and (F) belowground:aboveground dry mass ratio. Points represent fixed-effect estimates, and horizontal bars indicate 95% confidence intervals. Vertical dashed lines denote zero effect. Green symbols indicate positive effects, and red symbols indicate negative effects. The R^2m represents the variance explained by the fixed effects (soil nutrients), while the R^2c accounts for the variance explained by fixed and random effects.

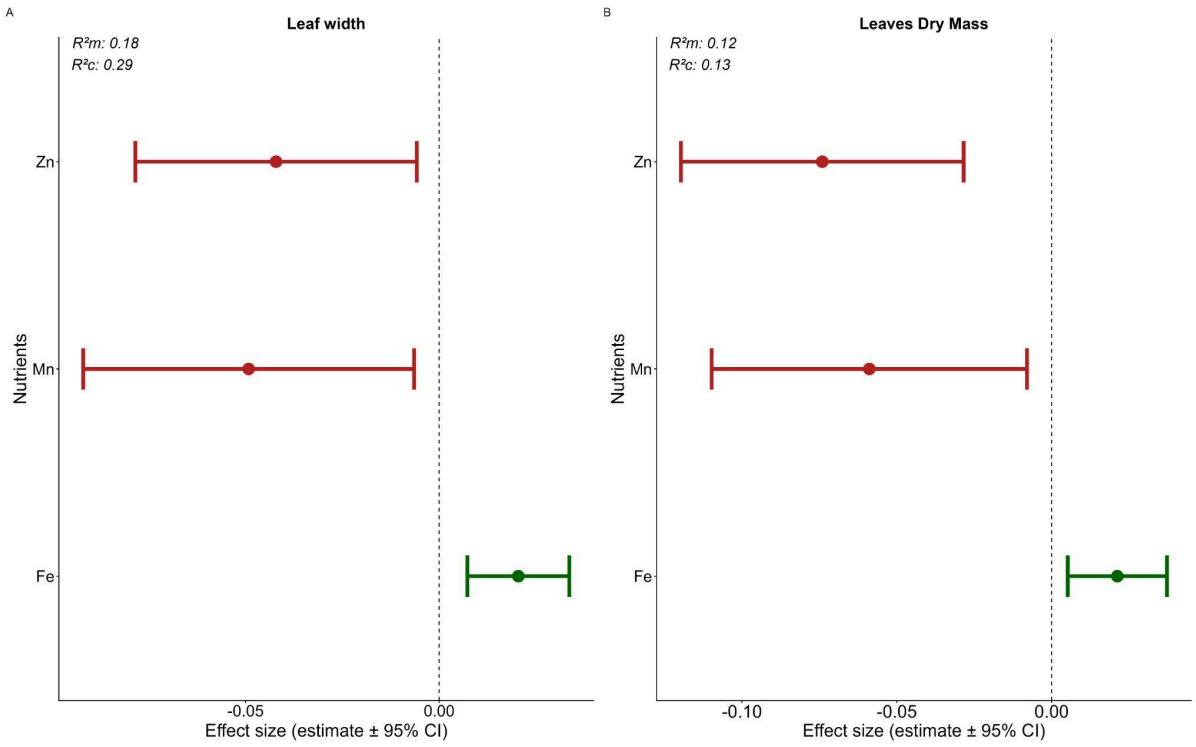

Figure 3. Standardized effect sizes (model estimates ± 95% confidence intervals) from Gaussian mixed-effects models relating soil nutrient concentrations to leaf traits in *Leiothrix spiralis*. Panels show effects on (A) leaf width and (B) leaf dry mass. Points represent fixed-effect estimates, and horizontal bars indicate 95% confidence intervals. Vertical dashed lines denote zero effect. Green symbols indicate positive effects, and red symbols indicate negative effects. The R^2m represents the variance explained by the fixed effects (soil nutrients), while the R^2c accounts for the variance explained by fixed and random effects.

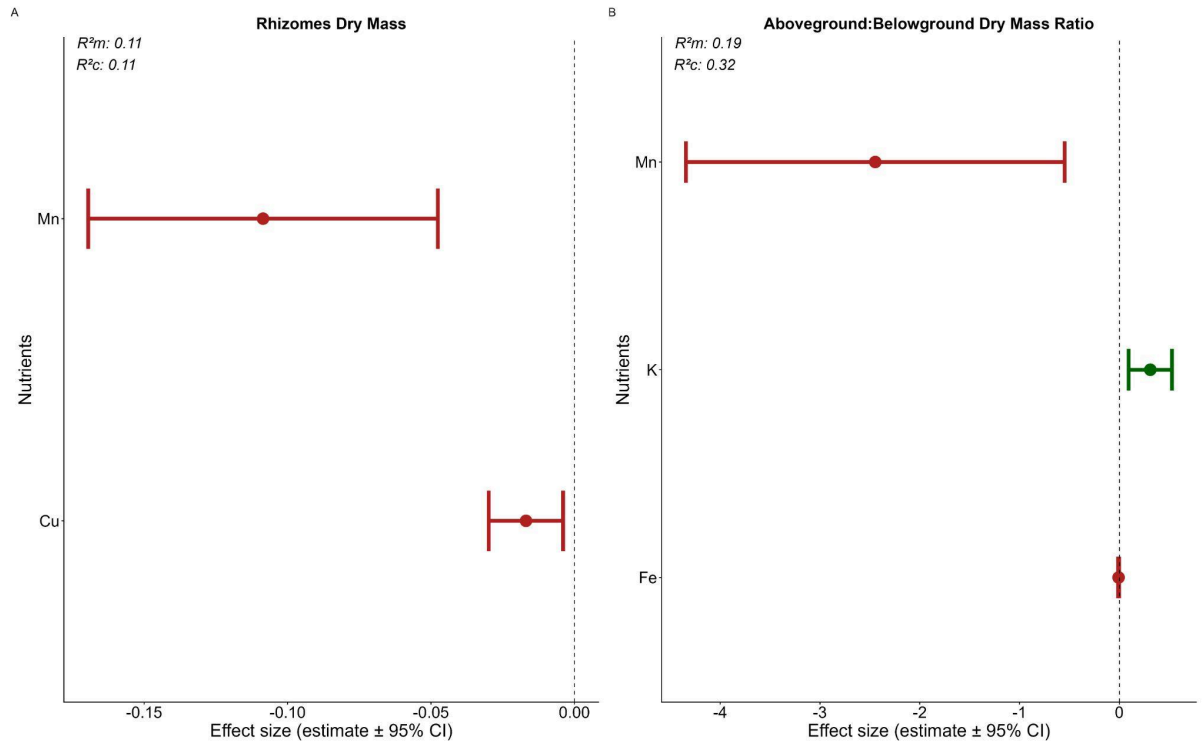

Figure 4. Standardized effect sizes (model estimates ± 95% confidence intervals) from Gaussian mixed-effects models relating soil nutrient concentrations to rhizome dry mass in *Leiothrix mucronata*. Points represent fixed-effect estimates, and horizontal bars indicate 95% confidence intervals. Vertical dashed lines denote zero effect. Green symbols indicate positive effects, and red symbols indicate negative effects. The R^2m represents the variance explained by the fixed effects (soil nutrients), while the R^2c accounts for the variance explained by fixed and random effects.

Table 1. Mean (± SD) soil nutrient concentrations ($mg.dm^{-3}$) associated with populations of *Leiothrix crassifolia*, *L. mucronata*, and *L. spiralis*.

	Species		
Micronutrient	***L. crassifolia***	***L. mucronata***	***L. spiralis***
Zn	0.22 ± 0.14	0.29 ± 0.23	0.31 ± 0.09
Fe	90.03 ± 75.70	134.22 ± 109.26	347.23 ± 322.72
Mn	0.40 ± 0.13	0.40 ± 0.37	0.41 ± 0.10
Cu	0.11 ± 0.17	0.52 ± 1.73	0.33 ± 0.77

B	0.09 ± 0.13	0.06 ± 0.04	0.08 ± 0.05
Mg	0.1 ± 0	0.11 ± 0.03	0.1 ± 0
Ca	0.1 ± 0	0.25 ± 0.22	0.29 ± 0.09
K	12.44 ± 2.87	11.11 ± 2.93	12.55 ± 3.94

Table 2. Mean (± SD) morphological traits, biomass components, and allocation ratios measured for individuals of *Leiothrix crassifolia*, *L. mucronata*, and *L. spiralis*.

Trait	Species		
	L. crassifolia	***L. mucronata***	***L. spiralis***
Diameter (cm)	5.30 ± 1.42	2.42 ± 0.57	4.25 ± 1.35
Leaf length (cm)	2.87 ± 0.73	1.29 ± 0.31	2.31 ± 0.71
Leaf width (cm)	0.41 ± 0.08	0.05 ± 0.01	0.38 ± 0.09
Root length (cm)	3.47 ± 0.86	2.87 ± 1.02	3.83 ± 1.23
Leaf mass (g)	0.46 ± 0.30	0.14 ± 0.12	0.20 ± 0.14
Root mass (g)	0.11 ± 0.08	0.05 ± 0.04	0.11 ± 0.15
Rhizome mass (g)	0.67 ± 0.40	0.20 ± 0.16	0.54 ± 0.65
Belowground mass (g)	0.78 ± 0.44	0.25 ± 0.17	0.65 ± 0.69
Total ramet mass (g)	1.24 ± 0.64	0.39 ± 0.22	0.85 ± 0.75
Root:shoot ratio	0.29 ± 0.21	0.52 ± 0.45	0.73 ± 1.28
Belowground:Aboveground ratio	2.11 ± 1.37	2.84 ± 2.78	3.85 ± 3.37

Table 3. Results of Gaussian mixed-effects models evaluating the relationships between soil nutrient concentrations and morphological traits, biomass components, and biomass allocation ratios in *Leiothrix crassifolia*, *L. spiralis*, and *L. mucronata*. For each species and response variable, the table reports fixed-effect estimates, standard errors, z values, and associated p values for intercepts and significant nutrient predictors. Only

predictors retained in the final models are shown. Random effects accounted for the hierarchical sampling structure (quadrats nested within areas). The variance explained by the models is indicated by marginal (R^2m) and conditional (R^2c) R-squared values. The R^2m represents the variance explained by the fixed effects (soil nutrients), while the R^2c accounts for the variance explained by fixed and random effects. Significant p-values (α = 0.05) are presented in bold.

Species	Response variable	Source of variation	Estimate	Std Error	z value	p value	R^2c	R^2m
Leiothrix crassifolia	Ramet diameter	Intercept	5.08	0.29	17.19	**< 0.001**	0.51	0.39
		Fe	-1.12	0.44	-2.52	**< 0.01**		
		Cu	1.88	0.92	2.03	**< 0.05**		
		K	0.68	0.17	3.98	**< 0.01**		
	Leaf length	Intercept	2.54	0.11	21.55	**< 0.001**	0.48	0.38
		Fe	-0.62	0.20	-3.07	**< 0.001**		
		K	0.40	0.08	4.88	**< 0.01**		
	Leaf width	Intercept	0.38	0.009	41.18	**< 0.001**	0.32	0.30
		Fe	-0.05	0.01	-2.83	**< 0.01**		
		K	0.04	0.006	6.31	**< 0.001**		
	Leaves dry mass	Intercept	0.48	0.04	11.00	**< 0.001**	0.19	0.18
		Fe	-0.15	0.06	-2.38	**< 0.05**		
		Cu	0.50	0.13	3.71	**< 0.01**		
		B	0.05	0.01	3.90	**< 0.001**		
	Root dry mass	Intercept	0.10	0.01	8.89	**<0.001**	0.23	0.08
		Zn	-0.02	0.01	-2.52	**<0.01**		
	Belowground:Aboveground Dry Mass Ratio	Intercept	2.51	0.26	9.38	**< 0.001**	0.39	0.30
		Zn	-0.49	0.16	-3.01	**< 0.01**		
		Fe	2.07	0.40	5.17	**< 0.001**		
		Cu	-1.77	0.82	-2.13	**< 0.05**		
Leiothrix spiralis	Leaf width	Intercept	0.38	0.009	38.56	**< 0.001**	0.29	0.18
		Zn	-0.04	0.01	-2.27	**< 0.05**		
		Fe	0.02	0.006	3.04	**< 0.01**		
		Mn	-0.04	0.02	-2.26	**< 0.05**		

	Leaves dry mass	Intercept	0.19	0.01	16.18	**< 0.001**	0.13	0.12
		Zn	-0.07	0.02	-3.18	**< 0.01**		
		Fe	0.02	0.008	2.59	**< 0.01**		
		Mn	-0.05	0.02	-2.26	**< 0.05**		
Leiothrix mucronata	Rhizome dry mass	Intercept	0.20	0.01	18.39	**< 0.001**	0.11	0.11
		Mn	-0.02	0.007	-3.49	**< 0.001**		
		Cu	-0.01	0.007	-2.54	**< 0.05**		
	Belowground:Aboveground Dry Mass Ratio	Intercept	2.70	0.40	6.68	**<0.001**	0.32	0.19
		Fe	-1.69	0.63	-2.66	**<0.01**		
		Mn	-0.56	0.22	-2.52	**<0.05**		
		K	1.03	0.37	2.80	**<0.01**		

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