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Direct and Element-Mediated Edaphic Effects Shape Resource-Conservative Leaf Trait Variation Across Serpentine and Limestone Soils

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ABSTRACT

Edaphically extreme substrates, such as serpentine and limestone, impose strong physiological constraints on plants and are frequently associated with resource-conservative leaf traits and narrow plant endemism. However, it remains unclear how edaphic selection shapes leaf traits and whether these shifts are driven primarily by direct soil-imposed constraints or indirectly by changes in leaf elemental composition. Here, we examined soil–leaf elemental relationships, leaf functional traits, and CSR strategies (Competitive, Stress-tolerant, and Ruderal strategy) using piecewise structural equation modeling across 16 populations of five closely related *Epimedium* taxa distributed on serpentine, limestone, and other substrates in western Japan. Despite marked differences in soil and leaf elemental composition between serpentine and limestone sites, populations on both substrates converged toward resource-conservative leaf traits, including lower specific leaf area (SLA) and a shift toward thicker leaves and higher leaf dry matter content (LDMC), even after accounting for species identity. Piecewise structural equation modeling revealed two pathways consistent with the proposed hypotheses: A strong direct association between soil elemental composition and leaf trait variation and a weaker indirect association involving leaf elemental composition. Together, our results suggest that edaphic mosaics are linked to convergent resource-conservative phenotypes through two partly overlapping pathways across serpentine and limestone soils: strong direct associations with leaf functional traits and weaker associations involving leaf elemental composition.

1 | Introduction

Edaphic heterogeneity across landscapes shapes plant diversity and can act as a selective force that promotes ecological divergence and speciation (Fine et al. 2005; Rajakaruna 2018; Hulshof and Spasojevic 2020). Certain soil types with distinct chemical compositions can impose strong selective pressures and are often associated with high levels of endemism, including taxa that have diverged from closely related lineages within the same region (Whittaker 1954; Brady et al. 2005; Sakaguchi et al. 2017; Sianta and Kay 2021). These patterns suggest that adaptation and ecological divergence can occur over comparatively short evolutionary timescales.

Edaphic extremes, such as serpentine and limestone/karst substrates, are repeatedly associated with narrow endemism and have long been framed as natural laboratories for ecological divergence and, at times, speciation driven by strong soil-mediated selection (Kruckeberg 1951; Antonovics and Bradshaw 1970; Rajakaruna 2004; Brady et al. 2005; Clements et al. 2006; Anacker 2014). Serpentine soils typically have elevated Ni and Cr, high Mg, and low Ca:Mg ratios, often with low nutrient availability, and can act as strong environmental filters for plant communities (Kazakou et al. 2008). Elevated Ni can reduce photosynthetic capacity (Chen et al. 2009), and low Ca:Mg ratios can cause Ca–Mg antagonism that constrains plant growth and survival (Tang and Luan 2017). In

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contrast, limestone soils are typically characterized by high Ca availability and alkaline pH, which are often associated with reduced availability and acquisition of micronutrients such as Fe and can contribute to environmental filtering in plant communities (Cross and Lambers 2021). Although Ca is an essential nutrient, excessive Ca supply can be toxic to plants (White and Broadley 2003), and shallow soils interspersed with exposed bedrock can intensify near-surface drought stress (Nardini et al. 2021; Liu et al. 2024). Consequently, both soil types are widely regarded as challenging environments for plant growth and often harbor specialized plant communities adapted to these conditions.

Gradients in soil conditions associated with chronic resource shortages are major components of environmental stress and are expected to be associated with coordinated shifts in leaf functional traits, such as specific leaf area (SLA) and leaf dry matter content (LDMC) (Grime 1974; Hodgson et al. 2011; Zhang et al. 2024), although the degree of environmental sensitivity may vary among trait categories (Zhang et al. 2024). For example, serpentine soils impose a suite of constraints that tend to favor reduced leaf size, small stature, and well-developed root systems, all of which are associated with stress tolerance (Brady et al. 2005). Plant responses to edaphic extremes often involve tougher, more resource-conservative leaves, including lower SLA, on both serpentine (Adamidis et al. 2014; Samojedny et al. 2022; Hidalgo-Triana et al. 2023) and limestone soils (Liu et al. 2024). Although such edaphic specialization and associated trait syndromes have been documented, the mechanisms generating these patterns remain less well resolved. In particular, the relative importance of direct edaphic effects versus indirect effects mediated by shifts in leaf elemental composition remains poorly understood.

The genus *Epimedium* comprises perennial herbaceous species that are widely but patchily distributed from East Asia to North Africa and Europe. Evolutionary radiation has been reported in *Epimedium*. The section *Diphyllon* diverged at approximately 2.11 Mya (Guo et al. 2022), whereas in the section *Macroceras*, diversification in the Japanese Archipelago began around 0.95 Mya with the colonization of various habitats (Kusatake et al. 2026). In the section *Macroceras*, species richness is high in western Japan, where populations occur on serpentine and limestone substrates. In this region, *Epimedium trifoliatobinatum* var. *trifoliatobinatum* is predominantly found on serpentine, whereas *Epimedium* × *setosum* is mainly restricted, but not limited to, limestone substrates. *Epimedium grandiflorum* var. *grandiflorum* is the only variety of the species that occurs in both serpentine and limestone habitats, whereas the other varieties of *E. grandiflorum* have geographically distinct distributions in northern Japan and the mountainous areas of Kyushu Island. *Epimedium diphyllum* and *Epimedium sempervirens* are not associated with any specific substrates. This recent divergence, coupled with repeated colonization of contrasting edaphic substrates, provides an excellent system for examining how soil chemistry and plant functional strategies are associated during recent ecological diversification.

In this study, we aimed to elucidate (i) whether edaphic effects appear even when accounting for species differences and (ii)

whether leaf trait strategies associated with stress tolerance respond to soil chemistry directly or primarily through leaf elemental composition. To address these questions, we used field data from five perennial *Epimedium* taxa on serpentine, limestone, and other soils in western Japan and applied piecewise structural equation modeling to examine how soil and leaf elemental compositions were associated with leaf functional traits and stress-related CSR (Competitive, Stress-tolerant, and Ruderal strategy) scores while accounting for species identity.

2 | Materials and Methods

2.1 | Field Collection and Soil Substrates

We collected leaf and soil samples from 16 populations representing five *Epimedium* taxa distributed across western Japan (Table 1; Figure 1): *E. diphyllum*, *E. trifoliatobinatum* var. *trifoliatobinatum*, *E. grandiflorum* var. *grandiflorum*, *E. × setosum* and *E. sempervirens*. *Epimedium* is taxonomically complex, and some phylogenetic relationships remain unresolved (Kusatake et al. 2026). Among these species, *E. trifoliatobinatum* var. *trifoliatobinatum* and *E. grandiflorum* var. *grandiflorum* belong to the same clade, and the other three taxa belong to other clades with traces of past introgression events. These taxa are generally morphologically distinguishable and were identified based on their vegetative and floral characteristics.

Based on the Seamless Digital Geological Map of Japan (<https://gbank.gsj.jp/seamless/>), six of the 16 populations occurred on serpentine substrates, and three occurred on limestone (Table 1). For each population, all leaflets from one compound leaf were collected from each of 10 individuals. To minimize variation in leaf age and developmental stage, we collected a single compound leaf from reproductive shoots that had emerged and flowered earlier in the same growing season. Fully expanded, mature leaves were collected from late July to early August 2025. All sampled shoots were at least 2 m apart from each other. Approximately 100 cm³ of soil was collected from each of three to five points within each site, depending on population size, after removing approximately 5 cm of surface soil and litter. The subsamples were homogenized and combined into a single composite soil sample for each population.

2.2 | Leaf Functional Traits and CSR Strategies

Leaf functional traits, including saturated fresh leaf mass, leaf area, leaf thickness, and dry leaf mass, were measured. For each individual, two to nine leaflets (depending on availability and interspecific differences in leaflet number) were placed in sealed plastic bags with reverse osmosis water, rehydrated, and washed at approximately 7°C for 24 h to obtain saturated fresh mass. After rehydration, the leaflets were gently blotted dry and weighed. Leaflet area was quantified from scanned images using ImageJ (Schneider et al. 2012). Leaflet thickness was measured using a thickness gauge while avoiding major veins. Leaflets were then oven-dried at 70°C for 72 h and reweighed to obtain dry mass. From these measurements,

TABLE 1 | Sampling locations and soil pH and elemental composition of the 16 *Epimedium* populations (Pop) analyzed in this study.

Species	Pop	Substrate	Latitude	Longitude	pH	Ca (mg/g)	Cd (μg/g)	Co (μg/g)	Cr (μg/g)	Fe (mg/g)	Mg (mg/g)	Mn (mg/g)	Ni (μg/g)
<i>E. diphylum</i>	IOK	Others	33.492	133.937	5.62	1.94	0.249	1.444	<0.001	0.369	0.252	0.656	0.569
	KRT	Others	33.511	133.896	4.70	0.66	0.246	1.415	0.493	0.897	0.256	0.152	0.594
	SRI	Others	33.583	133.716	6.12	4.28	0.282	1.594	<0.001	0.145	0.308	0.245	0.042
<i>E. sempervirens</i>	OTN	Others	34.765	133.999	5.16	2.10	0.728	6.202	3.426	1.386	1.460	0.324	1.999
	TGR	Others	35.263	133.798	4.61	0.35	0.247	0.646	<0.001	0.603	0.144	0.125	0.512
<i>E. × setosum</i>	FGO	Limestone	34.789	133.441	5.39	3.02	0.423	4.111	0.187	0.618	0.228	0.523	0.143
	HNK	Limestone	34.930	133.466	6.13	7.90	1.395	1.817	0.070	0.188	0.168	0.195	0.125
	YSD	Others	34.542	133.533	5.06	3.49	0.939	6.538	0.765	0.525	1.044	0.901	2.460
<i>E. trifoliatobinatum</i> var. <i>trifoliatobinatum</i>	ENG	Serpentine	33.586	133.512	6.03	0.85	0.257	36.310	3.709	0.235	2.308	0.338	175.405
	OKO	Serpentine	33.597	133.621	6.26	1.09	0.241	68.369	7.190	0.447	2.048	0.687	238.494
	OSK	Serpentine	33.590	133.586	6.23	1.36	0.233	66.856	10.484	0.982	4.080	0.712	329.854
	RND	Serpentine	33.581	133.492	6.50	0.98	0.172	47.474	4.449	0.408	2.528	0.491	223.562
<i>E. grandiflorum</i> var. <i>grandiflorum</i>	SGK	Others	33.611	133.555	5.21	1.40	0.257	5.943	0.493	0.745	0.356	0.390	2.428
	KIM	Limestone	33.629	133.453	7.03	6.96	1.495	6.545	2.465	0.487	1.276	0.378	3.374
	NSK	Serpentine	33.546	133.348	5.95	1.40	0.248	46.473	19.647	0.966	3.908	0.559	223.927
	SMB	Serpentine	33.546	133.375	6.50	2.21	0.228	36.444	19.177	2.386	9.048	0.589	346.321

Note: For each population, we report the concentrations of eight soil elements (Ca, Cd Co, Cr, Fe, Mg, Mn, and Ni; units as indicated). Soil values represent a single population-level composite sample, prepared by homogenizing subsamples collected from multiple points within each site.

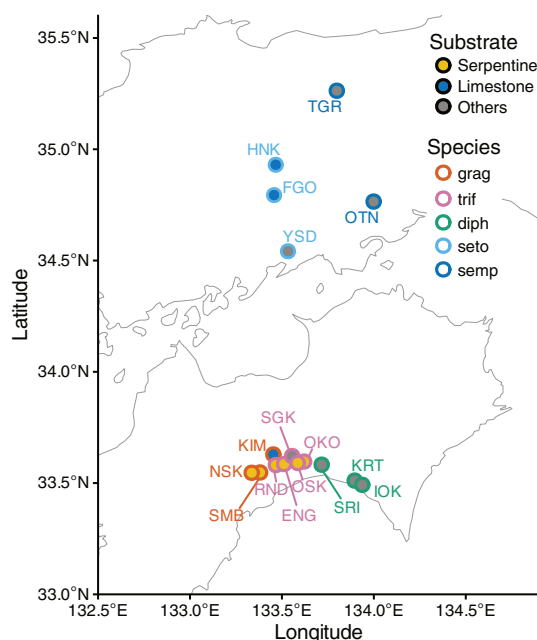


FIGURE 1 | Sampling locations of 16 populations of five *Epimedium* taxa in western Japan. Points are colored by substrate type (serpentine, limestone, and others) and shaped by species. diph, *E. diphylum*; grag, *E. grandiflorum* var. *grandiflorum*; seto, *E. × setosum*; semp, *E. semper-virens*; trif, *E. trifoliatobinatum* var. *trifoliatobinatum*.

we calculated specific leaf area (SLA = area/dry mass) and leaf dry matter content (LDMC = dry mass/saturated fresh mass). CSR strategy (C, S, and R proportions) was estimated from leaf area, SLA, and LDMC using Stratefy, following Grime's CSR scheme (Pierce et al. 2017). This method is highly reliable for identifying plant competitive (C), stress-tolerant (S), and ruderal (R) strategies, and has been widely adopted to assess strategic differences in CSR among plant communities in various regions (Li and Shipley 2017). The CSR component values were displayed on a ternary plot using the MultiTraits package in R.

2.3 | Soil and Leaf Elemental Composition

Soil samples were sieved through a 2 mm sieve to remove litter and coarse fragments and to homogenize the soil samples. After drying at 70°C, 5 g of the soil sample, without complete digestion, was mixed with 50 mL of 0.43 M HNO₃ and shaken for 1 h. After standing for 10 min, the suspensions were filtered through 0.45 μm syringe filters. The use of 0.43 M HNO₃ followed previous studies targeting geochemically reactive soil elemental fractions (Groenenberg et al. 2017). The resulting weak-acid extractable fraction was used to compare relative geochemical differences among soils rather than to estimate total or strictly plant-available concentrations. To measure the elemental composition of the leaves, dried leaf samples used for morphological trait measurements were crushed and digested using a microwave digestion system (ETHOS, Milestone, Italy) with a mixture of 20–30 mg of sample, 1 mL of 60% nitric acid, and 6 mL of Milli-Q water. Sample handling was performed using gloves and plastic tools to minimize potential contamination. For both

soil and leaf samples, the concentrations of eight elements (Ca, Cd, Co, Cr, Fe, Mg, Mn, and Ni) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent 5800, Agilent Technologies, USA).

In addition, soil pH for each population was measured by adding 25 mL of Milli-Q water to 10 g of soil on a dry-mass basis. The suspension was shaken for 30 min, allowed to stand for 60 min, and mixed again immediately before pH was measured using a Twin pH Meter II (HORIBA, Kyoto, Japan).

2.4 | Multivariate and Univariate Statistical Analyses

We performed principal component analysis (PCA) to reduce the multivariate datasets describing the soil and leaf elemental compositions and functional traits. Separate PCAs were performed for the eight soil elemental variables, the eight leaf elemental variables, and the four leaf functional traits (leaf area, leaf thickness, SLA, and LDMC). All variables were centered and scaled prior to PCA, and we retained the first two components (PC1 and PC2) from each PCA as orthogonal axes summarizing major gradients.

Leaf traits and PC scores derived from leaf traits and leaf elemental composition were compared among species or substrate types using linear mixed-effects models, with species identity or substrate type as the fixed effect, respectively, and population as a random intercept. Leaf traits were log-transformed when necessary to improve normality. Fixed effects were evaluated using ANOVA, and significant effects were followed by pairwise comparisons of estimated marginal means with Tukey adjustment, as implemented in emmeans. Because both leaf and soil elemental concentrations often violated normality assumptions, population-level values of each element (population means for leaf elements and single composite measurements for soil elements) were compared among species or substrate types using Kruskal-Wallis tests, followed by Dunn's post hoc tests with multiple-testing correction. Soil elemental PC scores were likewise analyzed at the population level using linear models, followed by Tukey-adjusted pairwise comparisons when significant.

To aid in the interpretation of the leaf trait PCA axes in terms of CSR strategy, we additionally fitted linear mixed-effects models with the S component as the response and leaf trait PC1 and PC2 as predictors, including species identity as a fixed effect and population as a random intercept. Because CSR scores are calculated from leaf area, SLA, and LDMC, these models were used primarily for axis interpretation rather than as independent hypothesis tests.

We then used partial redundancy analysis (partial RDA) to examine the relationships among soil elemental composition, leaf elemental composition, leaf functional traits (leaf area, SLA, leaf thickness, and LDMC), and CSR strategy using the vegan package in R. As multivariate response matrices, we used (i) a leaf elemental matrix (eight elements), (ii) a leaf functional trait matrix (leaf area, SLA, leaf thickness, and LDMC),

and (iii) a CSR matrix containing C, S, and R scores. Variables in each response matrix were centered and standardized to unit variance before analysis. Because soil elemental composition was measured at the population level, soil elemental PC1 and PC2 were used to explain variation in population means of leaf elemental composition, leaf functional traits, and CSR scores. In contrast, when leaf elemental composition was used as the predictor, analyses were conducted at the individual level to explain variation in individual leaf functional traits and CSR scores. Species identity was included as a conditioning variable in all models to account for average differences among species. For each model, we calculated the proportion of constrained variance (R^2) and adjusted R^2 , and evaluated the global model significance using permutation tests (999 permutations). When the global test was significant, we further examined the marginal effects of individual predictors using permutation tests.

In addition, to examine the potential effects of climatic variation among populations and species, which can also influence leaf functional traits (Gong and Gao 2019), we performed partial RDA using climate variables to explain population means of leaf traits and CSR strategy with species identity included as a conditioning variable. Seventeen climate variables listed in Table S1 were obtained from ClimateAP (Wang et al. 2017). Because the climate variables were highly correlated, we conducted PCA and used Climate PC1–PC3 as predictors in the RDA. Although Climate PC3 explained only a small proportion of the total variance (4.7%), it was included because it represented additional climatic variation independent of the major axes.

2.5 | Piecewise Structural Equation Modeling

We evaluated the hypothesized pathways linking soil and leaf elemental compositions with (i) leaf traits or (ii) S scores in CSR strategy using piecewise structural equation modeling (SEM). Because the S scores were calculated from the same set of leaf functional traits, we analyzed leaf traits and S scores in separate SEMs. In both SEMs, leaf elemental PC1 and PC2 were first modeled as response variables predicted by soil elemental PC1 and PC2. We then fitted either (a) two additional mixed-effects models in which leaf trait PC1 and PC2 were treated as separate response variables and predicted by soil elemental PC1 and PC2 and leaf elemental PC1 and PC2, or (b) one mixed-effects model in which the CSR S score was treated as the response variable and predicted by the same four predictors. The soil-to-trait paths represented direct soil–trait associations, whereas the paths involving leaf elemental PCs represented associations potentially mediated by leaf elemental composition. In all component models, species identity was included as a fixed effect, and population identity was included as a random intercept to account for the non-independence of individual plants within populations. Because soil elemental composition was represented by a single composite sample for each population, paths involving soil elemental PCs represent associations based on among-population variation and should therefore be interpreted at the population level. Component models were fitted using the lmer function and combined into a single piecewise SEM using psem in R. All continuous PC

variables used in the piecewise SEM and linear mixed-effects models were z-standardized prior to analysis to facilitate the comparison of effect sizes among predictors.

3 | Results

3.1 | Soil and Leaf Elemental Compositions

Most soil and leaf elemental variables differed significantly among the substrate types (Table S2). Serpentine soils showed markedly higher Ni, Cr, and Mg contents than limestone and other substrates, whereas limestone soils were characterized by higher Ca and lower Mg contents, and consequently higher Ca:Mg ratios. In contrast, soil pH broadly overlapped between serpentine and limestone soils: pH 5.95–6.50 in serpentine and pH 5.39–7.03 in limestone soils (Table 1). Leaf elemental composition generally showed parallel patterns across substrate types. Leaf Ca and Mg concentrations were generally higher than the corresponding soil concentrations, whereas Ni concentrations in serpentine populations tended to be lower in leaves than in soils. Species-level differences were less pronounced than substrate-type differences (Table S3).

PCA of the soil elemental composition showed that PC1 and PC2 explained 55.4% and 19.6% of the variance, respectively (Figure 2a). Soil elemental PC1 was characterized by negative loadings of Ni, Mg, Cr, and Co, indicating a serpentine-associated gradient, whereas soil elemental PC2 was driven mainly by positive loadings of Ca and Cd (Table S4). PCA of leaf elemental composition explained 29.9% (PC1) and 21.5% (PC2) of the variance (Figure 2b). Leaf elemental PC1 increased with Ca (and to a lesser extent Cd, Fe, and Mn) and decreased with Ni and Mg, representing a Ca-rich (higher Ca:Mg) versus serpentine-type elemental contrast. Leaf elemental PC2 primarily reflected the variation in Cd, Cr, Fe, Mg, and Mn (Table S4). Using population-level soil measurements, we found that soil elemental PC1 differed significantly among substrate types (one-way ANOVA; $F=25.97$, $p<0.001$) but not among species (Tables S2 and S3). In contrast, soil PC2 differed among species ($F=4.81$, $p=0.017$) and substrate types ($F=3.87$, $p=0.048$). For leaf elemental composition, mixed-effects ANOVA (population as a random intercept) showed that leaf PC1 varied among species ($F=5.53$, $p=0.011$) and substrate types ($F=51.26$, $p<0.001$). In contrast, leaf PC2 did not differ among species or substrate types.

3.2 | Leaf Functional Trait and CSR Strategy

We found that SLA was lower in limestone and serpentine than in other substrates (Table S2). Leaf area, leaf thickness, and LDMC did not differ significantly among the soil substrates. SLA was also lower in *E. grandiflorum*, *E. trifoliatobinatum*, and *E. × setosum*, which often occur on serpentine and limestone substrates, than in *E. diphyllum* and *E. sempervirens*, but the differences were not significant (Table S3). Leaf area and leaf thickness differed significantly among taxa, with *E. × setosum* and *E. sempervirens* having larger leaves than *E. diphyllum*, while *E. diphyllum* tended to have thinner leaves than the other taxa (Table S3). PCA of leaf

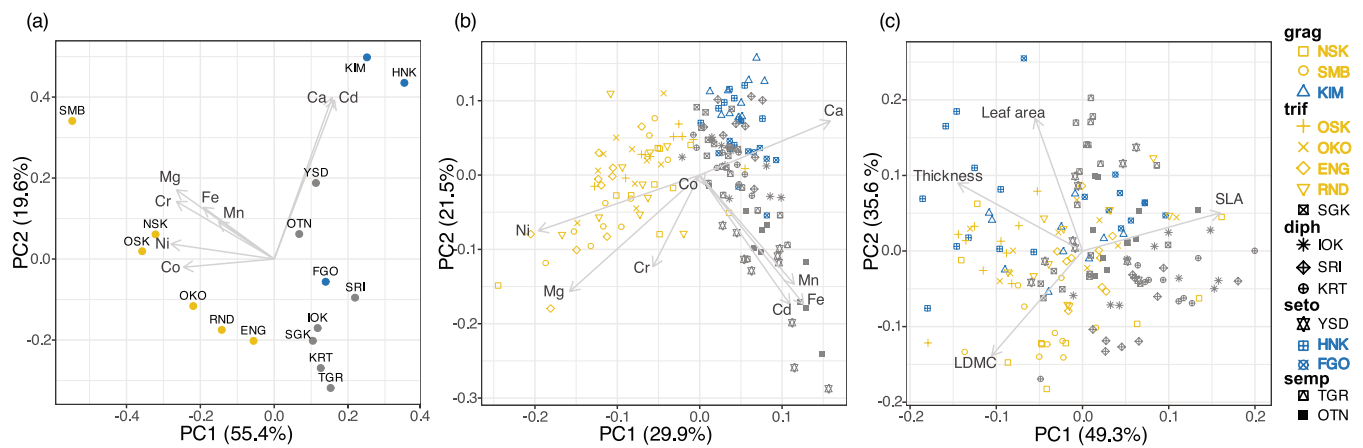


FIGURE 2 | Principal component analyses (PCA) of soil elemental composition, leaf elemental composition, and leaf functional traits of *Epimedii* populations in western Japan. (a) PCA of soil elemental concentrations (Ca, Mg, Fe, Mn, Ni, Cr, Co, and Cd). Each point represents a population and is labeled with its population code. (b) PCA of individual-level leaf elemental concentrations for the same elements. (c) PCA of individual-level leaf traits: Leaf area, specific leaf area (SLA), leaf thickness, and leaf dry matter content (LDMC). In all panels, colors indicate substrate type (yellow, serpentine; blue, limestone; gray, other substrates). In (b) and (c), symbols indicate taxa. diph, *E. diphyllum*; grag, *E. grandiflorum* var. *grandiflorum*; seto, *E. setosum*; trif, *E. trifoliatobinatum* var. *trifoliatobinatum*.

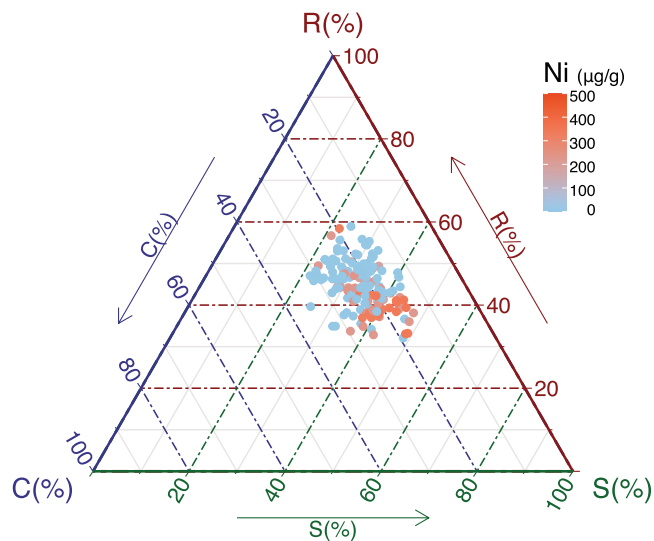


FIGURE 3 | Ternary CSR plot of individuals from *Epimedii* populations. Each point represents an individual's position along the competitive (C), stress-tolerant (S), and ruderal (R) axes (%). The color of the points indicates the soil Ni concentration (μg/g dry soil).

traits indicated that PC1 and PC2 explained 49.3% and 35.6% of the total variance, respectively (Figure 2c). Leaf trait PC1 described a trade-off between higher SLA and greater leaf thickness and LDMC, whereas higher leaf trait PC2 was mainly associated with larger leaf area and lower LDMC (Table S4). Mixed-effects ANOVA with population as a random intercept indicated that leaf trait PC1 differed among substrate types ($F = 5.46$, $p = 0.019$), whereas the effect of species was only marginal ($F = 3.04$, $p = 0.065$). In contrast, leaf trait PC2 differed significantly among species ($F = 6.67$, $p = 0.006$), but not among substrate types.

For the CSR strategies (Pierce et al. 2017), the focal taxa showed a trade-off between the stress-tolerant (S) and ruderal (R) strategies (Figures 3 and S1). When soil Ni concentrations were

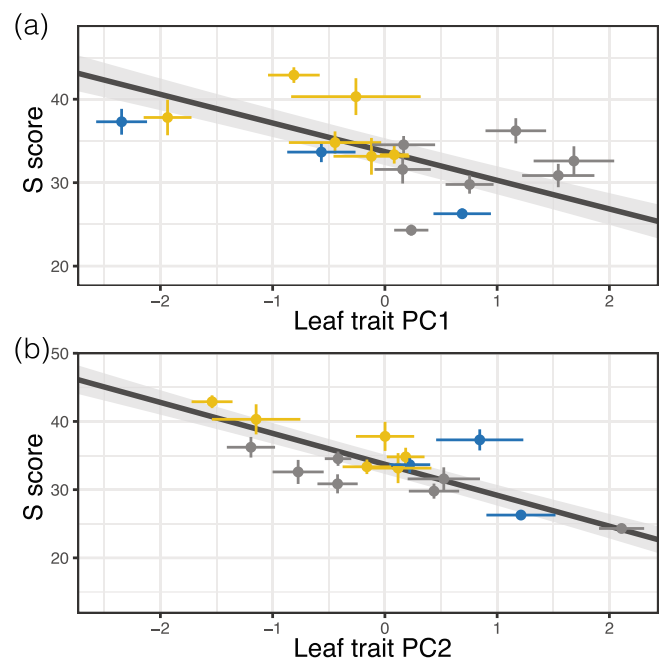


FIGURE 4 | Relationships between leaf trait principal components (PCs) and stress-tolerant (S) strategy across *Epimedii* populations. (a) S scores as a function of leaf trait PC1 and (b) PC2. Points show population means, with horizontal and vertical error bars indicating the standard errors of the leaf trait PC scores and S scores, respectively. Colors denote the substrate type (yellow, serpentine; blue, limestone; gray, other substrates). Black lines and dark gray ribbons show marginal predictions and their 95% confidence intervals from linear mixed-effects models fitted to individual-level data, with S as the response and leaf trait PC1 (a) or PC2 (b) and species identity as fixed effects and population as a random effect.

overlaid on the CSR triangle, populations occupying the stress-tolerant region generally occurred on soils with higher Ni concentrations (Figure 3). The linear mixed-effects models, which included species identity as a fixed effect and population as a

TABLE 2 | Summary statistics for partial redundancy analyses (RDA) examining relationships among soil elemental composition principal components (soil PCs), leaf elemental composition, leaf functional traits, and CSR (Competitive, Stress-tolerant, and Ruderal) strategy.

Response matrix	Predictors	<i>n</i>	No. of response variables	No. of predictors	<i>R</i> ²	Adj. <i>R</i> ²	<i>F</i> _{global}	<i>p</i> _{global}	Main predictors (marginal tests)
Leaf elements	Soil PCs	16	8	2	0.18	0.13	3.32	0.022	Soil PC1
Leaf traits	Soil PCs	16	4	2	0.18	0.17	3.41	0.026	Soil PC2 (Soil PC1)
Leaf traits	Leaf elements	160	4	8	0.09	0.06	2.67	0.001	Mg, Ca, Fe
CSR (C, S, R)	Soil PCs	16	3	2	0.17	0.16	3.25	0.043	Soil PC2 (Soil PC1)
CSR (C, S, R)	Leaf elements	160	3	8	0.07	0.03	1.99	0.017	Mg, Ca

Note: For each model, the table reports the sample size (*n*), number of response and predictor variables, total and adjusted *R*², global *F* statistics, and permutation-based *p* values for the global test. When the global test was significant, marginal (term-wise) permutation tests were used to identify the main predictors; elements in parentheses indicate predictors with weaker support ($0.05 < p < 0.10$).

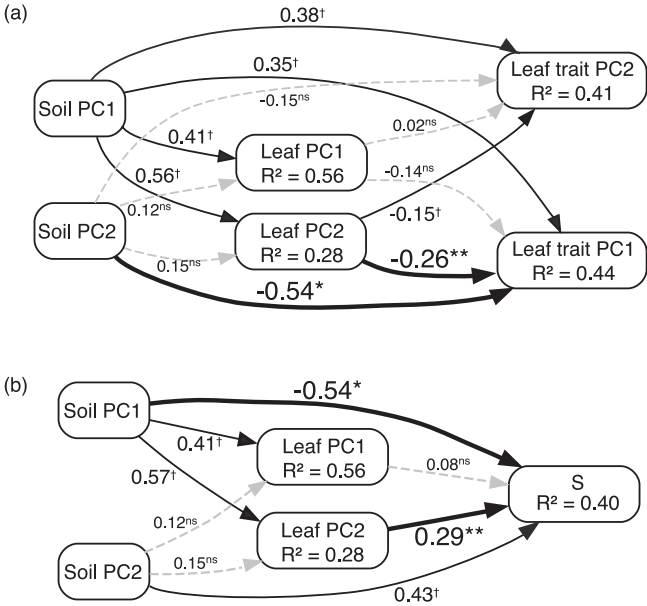


FIGURE 5 | Piecewise structural equation models (SEMs) describing associations among soil elemental composition, leaf elemental composition, leaf traits, and S scores. PCs represent principal components. (a) Soil elemental principal components (PCs) predict leaf elemental PCs, while soil and leaf elemental PCs jointly predict leaf trait PCs, with residual covariance allowed between the two leaf trait axes. (b) Soil elemental PCs and leaf elemental PCs jointly predict S scores. Numbers on arrows are standardized regression coefficients (β) from the component linear mixed-effects models, which included species identity as a fixed effect and population as a random effect. *R*² values shown next to the response variables indicate the marginal *R*² from these mixed-effects models. Significance codes: **, $p < 0.01$; *, $p < 0.05$; †, $0.05 \leq p < 0.10$; ns, $p \geq 0.10$.

random intercept, showed significant negative associations between each leaf trait PC axis and S scores (PC1: slope = -3.43 , $p < 0.001$; PC2: slope = -4.53 , $p < 0.001$; Figure 4).

3.3 | Partial Redundancy Analysis

Partial redundancy analysis (RDA) showed that the soil elemental PCs were strongly associated with the leaf elemental composition (Table 2). Soil elemental PCs explained a moderate proportion of the variation in the leaf elemental matrix (adjusted $R^2 = 0.13$, permutation ANOVA, $p = 0.022$). They also explained a significant proportion of variation in leaf traits (adjusted $R^2 = 0.17$, $p = 0.026$). The leaf elemental matrix also explained a significant but smaller proportion of variation in the leaf traits. The CSR scores were significantly associated with both soil elemental PCs and leaf elemental composition. Soil elemental PCs explained moderate variation in CSR scores (adjusted $R^2 = 0.16$, $p = 0.043$), whereas leaf elemental predictors explained a significant but smaller fraction of variation in CSR scores (adjusted $R^2 = 0.03$; $p = 0.017$). In addition, marginal tests indicated that soil elemental PC2 was associated with both leaf traits and CSR scores, whereas soil elemental PC1 showed weaker associations with these response variables.

Climate variables (Climate PC1–PC3; Figure S2) also explained significant variation in leaf traits and CSR scores (Tables S5 and S6), with adjusted R^2 values of 0.04 and 0.05, respectively.

3.4 | Piecewise Structural Equation Modeling

The piecewise SEMs using *z*-standardized variables showed an adequate overall fit to the data (Fisher's $C = 1.288$, $p = 0.525$; Figure 5). Soil elemental variation was only weakly related to leaf elemental composition: Soil elemental PC1 tended to be associated with leaf elemental PC1 and PC2, but these effects were marginal (leaf elemental PC1: $p = 0.059$; leaf elemental PC2: $p = 0.060$), whereas soil elemental PC2 showed no detectable association with either leaf elemental axis. Leaf elemental PC1 and PC2 were moderately explained overall (marginal $R^2 = 0.56$ and 0.28 , respectively).

In terms of leaf trait SEM, leaf trait PC1 was strongly associated with both soil elemental PC2 ($\beta = -0.54$, $p = 0.025$) and leaf elemental PC2 ($\beta = -0.26$, $p = 0.002$; Figure 5a, Table S7). Species identity remained significant for both leaf trait PCs ($p < 0.05$, Table S7). The residual covariance between leaf trait PC1 and PC2 was significant ($\beta = 0.18$, $p = 0.012$), supporting the inclusion of a correlated residual covariance between leaf trait PC1 and PC2. Leaf trait PCs were moderately explained (PC1 and PC2 marginal $R^2 = 0.44$ and 0.41 , respectively). The separate S-score SEM showed a broadly consistent result (Figure 5b, Table S8). S score was negatively related to soil elemental PC1 ($\beta = -0.54$, $p = 0.014$) and positively related to leaf elemental PC2 ($\beta = 0.29$, $p = 0.001$), with a weaker positive trend for soil elemental PC2 ($p = 0.074$). The model explained a comparable proportion of the variance in S scores (marginal $R^2 = 0.40$). The indirect effects estimated as the product of standardized path coefficients were -0.15 (0.56×-0.26) in the leaf-trait SEM and 0.17 (0.57×0.29) in the S-score SEM, respectively, both smaller than the corresponding direct soil-to-trait paths. As a robustness check, we fitted alternative models that included the soil and leaf elemental PC3. Including PC3 in alternative SEM formulations led to substantially poorer model fit (higher AIC and convergence issues), and these more complex models were not considered.

4 | Discussion

Edaphically extreme substrates, such as serpentine and limestone, have long been recognized as arenas where strong, spatially structured environmental stress generates opportunities for ecological divergence and speciation and thereby contributes to endemism (e.g., Hulshof and Spasojevic 2020). In our cross-substrate analysis within *Epimedium*, we showed that both soil and leaf elemental compositions were associated with leaf functional traits and CSR strategies even after accounting for species identity. Moreover, despite the contrasting edaphic conditions of serpentine and limestone substrates, populations on both converged toward resource-conservative leaf traits, including lower SLA and a tendency toward thicker leaves and higher LDMC, and exhibited higher S scores. Although climatic conditions also showed significant associations with leaf traits and CSR strategies, climate explained a relatively small proportion of variation in the separate RDA analyses. Climatic and edaphic variables were not evaluated jointly; therefore, their relative contributions cannot be directly compared in this study. Previous work has suggested that climatic modulation of the leaf economics spectrum is relatively modest (Wright et al. 2004). Taken together, these results suggest that edaphic conditions contribute to the convergence of resource-conservative trait combinations associated with stress tolerance across substrates, while climatic variation may play a role.

The substrate geology largely explained the weak-acid extractable soil elemental composition in our study system: Serpentine-derived soils were characterized by elevated Ni and Mg, whereas limestone soils had higher Ca concentrations. Because the 0.43 M HNO_3 extraction reflects a geochemically reactive rather than strictly plant-available fraction, the measured concentrations may not directly represent elemental availability to plants. Our analyses also did not quantify major

nutrients such as N, P, and K, which should be incorporated in future studies to better assess effects on plant growth and fitness. Nonetheless, the measured elemental composition appears to capture general edaphic differences among substrates. Soil and leaf elemental compositions varied among species and substrate types. Ca, Mg, and Cd occurred at relatively high concentrations in leaves, whereas Ni occurred at much lower concentrations in leaves than in the soil of serpentine populations. In plants inhabiting edaphic extremes, such patterns can result from several processes, including selective nutrient uptake and reduced translocation of potentially toxic metals from roots to shoots (Baker 1981; Lazzaro et al. 2021; Gonnelli et al. 2025). The modest soil–leaf relationship observed in our SEM analysis is also consistent with the possibility that elemental concentrations in leaves are not determined solely by soil concentrations. Other unmeasured factors, including interactions with rhizosphere microorganisms (Dijoux et al. 2025), may also influence elemental uptake.

Epimedium populations tended to converge on leaf traits across the species, with thicker leaves, higher LDMC, and lower SLA (representing leaf trait PC1) in both serpentine and limestone. Both leaf trait PC1 and PC2 were significantly associated with the S score of CSR, whereas leaf trait PC1 also captured edaphic differentiation, suggesting a shift toward more stress-tolerant leaf traits across extreme soil conditions even after accounting for species differences. This consistent shift indicates convergence toward resource-conservative leaf traits among the *Epimedium* taxa across contrasting edaphic extremes.

Our piecewise SEM analysis with z-standardized data suggests that edaphic influences on leaf functional traits are consistent with two complementary, partially decoupled pathways. First, soil elemental composition showed a direct association with leaf functional traits and S scores, indicating that soil-mediated constraints are not fully captured by leaf ionomic variation alone. Plant elemental composition and inter-element relationships can also vary among soil types (Mizuno et al. 2024). Edaphically extreme habitats impose not only chemical constraints but also physical and biotic constraints (Tsiripidis et al. 2010; Nardini et al. 2021; Bontpart et al. 2024; Dijoux et al. 2025). These constraints can influence leaf functional traits through pathways beyond ionomic mediation.

Second, we detected an indirect association between soil elemental composition and leaf traits that was consistent with potential mediation by leaf elemental composition. Specifically, soil elemental PC1 tended to be positively associated with leaf elemental PC2, which in turn was strongly associated with both leaf functional trait PC1 and S scores. This pattern suggests that variation in elemental accumulation and partitioning, particularly along the axis represented by leaf elemental PC2 (higher Mg and multiple metals including Fe, Mn, and Cr), may be associated with functional differences among individuals and populations. Tolerance to edaphic extremes often depends on active regulation of elemental uptake, transport, and detoxification through transporter control, chelation and vacuolar sequestration, antioxidant defenses, and the maintenance of Ca–Mg homeostasis under edaphic stoichiometric imbalance (Hall 2002; Maestri et al. 2010;

Tang and Luan 2017). Such regulation can entail metabolic and allocation costs (Maestri et al. 2010). These mechanisms could contribute to both direct and indirect associations with leaf traits and S scores in our SEM framework. Thus, the direct and ionome-mediated pathways identified here may partially overlap, rather than be mechanistically separable. Consistent with this interpretation, direct edaphic associations with leaf functional traits were stronger than the corresponding ionome-mediated associations. However, because soil elemental composition was characterized using a single composite sample per population, inferences regarding soil-related associations are based on among-population variation and do not account for within-population heterogeneity in soil conditions. Nevertheless, the overall pattern suggests that *Epimedium* responses were more strongly linked to direct edaphic variation in leaf economics, whereas ionic shifts accounted for additional but comparatively weaker variation in leaf functional traits.

Edaphic extremes can act as strong physical and chemical environmental filters that shape adaptive divergence (Konečná et al. 2020) and contribute to endemism and diversification in many plant lineages (Rajakaruna 2018). In *Epimedium*, we found parallel shifts in leaf trait space across serpentine and limestone habitats, consistent with convergence toward similar stress-tolerant trait combinations, although common garden or reciprocal transplant experiments will be needed to separate plasticity from heritable differentiation and to test whether similar phenotypes arise via replicated evolution or distinct mechanistic routes. Overall, our study shows that within a young radiation, populations across contrasting edaphic extremes can converge on resource-conservative leaf traits, with soil elemental composition and ionic covariation contributing to patterns of trait variation beyond species identity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study, including soil and leaf elemental composition data, leaf functional trait measurements, and CSR strategy data, are available in Zenodo at <https://doi.org/10.5281/zenodo.22127932>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Climate variables for each population site obtained from ClimateAP. **Table S2:** Soil and leaf elemental concentrations and leaf functional traits across substrate types. **Table S3:** Soil and leaf elemental concentrations and leaf functional traits across species. **Table S4:** Eigenvalues and variable loadings for the first four PCA axes (PC1–PC4) based on soil elemental composition, leaf elemental composition, and leaf functional traits. **Table S5:** Eigenvalues and variable loadings for the first three PCA axes (PC1–PC3) based on climatic variables. **Table S6:** Summary statistics for partial redundancy analyses (RDA) of climate variables. **Table S7:** Results of the piecewise structural equation model (SEM) linking soil elemental composition (PC1–PC2), leaf elemental composition (PC1–PC2), and leaf morphological traits (PC1–PC2). **Table S8:** Results of the piecewise structural equation model (SEM) linking soil elemental composition (PC1–PC2), leaf elemental composition (PC1–PC2), and S score in CSR scheme. **Figure S1:** Ternary CSR plot colored by soil Cr concentration. **Figure S2:** PCA of climate variables at the sampling sites.