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SOIL-DRIVEN INTRASPECIFIC LEAF TRAIT PLASTICITY IN HIMALAYAN SHRUB *RUBUS ELLIPTICUS* L.: A MULTISCALE ASSESSMENT FROM AZAD JAMMU AND KASHMIR

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Abstract

Understanding the impact of edaphic factors on leaf functional traits is crucial for predicting plant responses to global climate change; however, substantial knowledge gaps remain regarding how leaf trait respond to soil factors under changing climatic conditions. This study examined leaf functional trait variation in a Himalayan shrub *Rubus ellipticus* across soil gradients using multiple linear mixed effect model and generalized additive mixed model approaches. Among morphological traits plant height and leaf area responded positively to soil pH, calcium (Ca) and total nitrogen (TN) but declined consistently with potassium (K) and electrical conductivity (EC) demonstrating soil fertility promotes biomass accumulation whereas ionic imbalance constrain growth. Anatomical traits exhibited sclerenchyma thickness and trichome density enhanced with TOC and stomatal density with TN whereas metaxylem vessel area and stomatal density significantly reduced with TN and Ca respectively reflecting shifts in structural defense and allocation patterns. Physiological traits i.e., proline, total soluble sugars (TSS), chlorophylls and carotenoids were positively associated with pH, EC and TOC but declined strongly with K suggesting nutrient and ion-mediated regulation of osmotic adjustment and pigment investment. Correlation analysis revealed that anatomical traits were more tightly coordinated than physiological traits, while several trait groups responded independently, indicating partial decoupling across functional dimensions. Multivariate analysis showed distinct trait-soil association patterns, reflecting divergent resource use strategies. The study suggests that high variance in different sets of leaf functional traits in *Rubus ellipticus* differ greatly in adaptability to soil conditions and decoupling of leaf functional traits suggests that plant traits can vary self-sufficiently for more possible plant trait assemblages.

Key words: Soil gradient; Functional traits; Intraspecific; Himalaya; Linear mixed effects; Trait coupling

Introduction

Understanding how edaphic factors influence leaf functional traits is essential for predicting plant responses to global environmental changes (Iqbal *et al*., 2024), informing biological conservation efforts, and developing forest management strategies aimed at optimizing plant functions and services (Yuan *et al*., 2020). Despite significant alterations in soil ecosystems due to global change, the impact of soil properties on plant traits remains poorly elucidated (Irshad *et al.,* 2024), leaving a substantial gap in our understanding of how leaf traits adjust their internal structure and function in response to soil factors (Ahmad *et al.,* 2020). Several studies have investigated the variability in leaf traits and uncovered consistent relationships between these traits and soil properties demonstrating that soil conditions directly influence internal photosynthetic CO_2 regulation and leaf functional responses (Cornwell *et al.,* 2018).

The Western Himalaya, recognized as a global biodiversity hotspot, supports a high diversity of endemic and rare species that are especially vulnerable to environmental change (Tewari *et al*., 2017; Pandey *et al*., 2024). Soil gradients encompass substantial environmental variability and thus provide effective natural frameworks for examining functional trait responses and adaptive strategies across landscapes (Ma *et al*., 2026). There is a strong likelihood that soil properties are influenced by functional traits related to acquiring and utilizing soil resources, such as nutrients and water (Jager *et al*., 2015). There are often species with rapidly growing leaves, which have high nitrogen and phosphorus concentrations, high specific leaf area (SLA), low leaf dry matter content (LDMC), and thin leaves in soils with high nutrient availability (Ordonez *et al*., 2009). A large amount of research has been done to understand how soil gradients affect both plant trait expression and ecosystem function independently (Buzzard *et al*., 2016; Fyllas *et al*., 2017; Singh & Verma, 2025).

Plant leaf characteristics at different levels, i.e., cellular, tissue, and organ, offer valuable insights into their interaction with environmental factors such as soil properties. These traits, encompassing stomatal features at the cellular level, anatomical characteristics at the tissue level, and morphological attributes at the organ level, are crucial for understanding carbon assimilation, water utilization, and gas exchange dynamics (Baillie & Fleming, 2020; Liu *et al*., 2019). At the cellular level, stomata play a pivotal role in gas

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exchange between the leaf and the atmosphere (Lee & Bergmann, 2019). The opening and closing mechanism of stomata directly modulates the equilibrium between carbon uptake through photosynthesis and water loss via transpiration (Ainsworth & Rogers, 2007). At the tissue level, the thickness of the palisade mesophyll is intricately linked with light absorption capacity and the photosynthetic efficiency of the leaf (Terashima *et al*., 2011). Furthermore, increased thickness of the spongy mesophyll enhances plant resilience to drought and cold conditions in temperate regions, facilitating adaptation to low temperatures and moisture levels (Liu *et al*., 2019).

A plant's relative growth rate is positively correlated with SLA at the organ level (Turnbull *et al*., 2016). In comparison to a resource-acquisitive approach, a resource-conservative approach signifies a higher LDMC. It is crucial to understand how leaf traits vary and coordinate across different scales – cell, tissue, and organ in order to predict plant responses to environmental factors (Wigley *et al*., 2016).

Plant traits have been emphasized heavily in recent research (Faucon *et al.,* 2017; Kimball *et al*., 2016) in order to drive ecosystem processes. There is evidence that leaf traits are associated with soil carbon (C) storage, belowground carbon inputs (Guyonnet *et al*., 2018), and soil physical properties (Gould *et al*., 2016), suggesting broader implications for soil management. Plant-soil relationships can provide insights into processes such as net ecosystem exchange and ecosystem respiration, which ultimately affect carbon dynamics within ecosystems (De Long *et al*., 2019; Luo *et al*., 2020). Plant communities' leaf functional traits are expected to converge under extreme environmental gradients, with SLA increasing (Carreno-Rocabado *et al*., 2016) and LDMC decreasing in response to higher soil nutrient availability (Jager *et al*., 2015). Yet how these patterns manifest within species along edaphic gradients remain poorly understood.

Soil resources play a pivotal role in shaping leaf functional traits, with variations observed across different ecosystems. These differences in soil composition can significantly impact the relative significance of foliar traits within the Leaf Economics Spectrum (LES). For instance, N often acts as a primary driver of resource allocation (Craine *et al*., 2002), while soil phosphorus (P) commonly imposes limitations on plant growth (Baker *et al*., 2003), thereby exerting a substantial influence on resource allocation patterns. Consequently, variations in phosphorus concentrations are anticipated to exert a more pronounced effect on leaf traits within plant communities characterized by distinct phosphorus gradients, compared to broader global patterns (De-Walt *et al*., 2006). In Himalayan shrub species, variation in soil properties is expected to drive intraspecific differences in leaf traits, underscoring the need to understand how edaphic gradients shape plant functional strategies. *Rubus ellipticus* is a common shrub native to the Himalaya, occurring in both open grasslands and forests at an elevation of 1200-2100 meter above sea level. Due to its invasive nature, it is well adapted in open fields and in canopies of moist forest in western parts of Himalaya. Despite its wide ecological amplitude, intraspecific, multi-scale leaf trait responses to soil gradients remain poorly explored in this species. We hypothesized that key edaphic factors including TOC, N and P are major drivers of leaf trait variations in *Rubus ellipticus*. The focus of this study was to investigate three primary research questions: 1. How do leaf functional traits change in response to different soil conditions? 2. Which specific soil variables play the most significant role in explaining variations in these leaf traits? 3. What correlations can be observed between different leaf traits, and do they tend to converge or diverge under certain conditions?

To our knowledge, no prior research has examined leaf functional traits within co-existing plant functional types in Azad Jammu and Kashmir, particularly regarding their response to shifting soil conditions and its impact on functionality.

Materials and Methods

Study area: Field research was conducted in the Poonch district of the Western Himalayan forests located in Pakistan administrated Azad Jammu and Kashmir (33.8540° N, 73.7507° E). The area covers typical vegetation zones prevailing from sub-tropical pine forests to high altitude moist temperate forests. The lower elevation sites comprise mixed deciduous forests, dominated by *Quercus incana* and *Pinus roxburghii* at elevations between 800 to 1600 m a.s.l. In contrast, the higher elevation sites consist primarily of coniferous and broad-leaved temperate forests extending from 1,400 to 3,400 m a.s.l., and are dominated by *Pinus wallichiana*, *Cedrus deodara, Abies pindrow*, and *Picea smithiana*. *Cedrus deodara* is a highly valued widespread species that predominantly occurs in the western part of the Himalayan range at an elevation ranging from 1900 to 2700 m a.s.l. The climate is humid continental type which is often characterized by hot summers and mild winters with infrequent frost.

Plant and soil sampling: For the assessment of morphological, anatomical and physiological traits, fully expanded mature leaves were sampled from the uppermost canopy of individuals representing different populations across multiple locations. These locations vary in altitude, mean annual temperature and precipitation. About 4-5 leaves were collected from each twig with three replicates from each population at each sampling site. For physiological traits, leaves were wrapped in aluminum foils before they were treated with liquid nitrogen and stored at -80 ºC until processed. For soil predictors i.e., pH, electrical conductivity (EC), potassium (K), calcium (Ca), total organic carbon (TOC), and total nitrogen (TN), composite soil samples were used for analysis.

Morphological traits: Leaves were scanned, and their area was determined using 'Image J' software (Schneider *et al*., 2012). Leaf fresh weight was promptly measured upon collection, using an electronic balance with precision to 0.0001 g. For determination of leaf dry weight, samples were oven-dried at 37°C until a constant weight was attained and subsequently enclosed in airtight polyethylene bags (Wang *et al*., 2021). Plant height was measured for each specimen from the plant base to its maximum length (Ahmad *et al*., 2020).

Anatomical traits: Following field sampling, leaves were sectioned into small pieces (1.0 × 0.5 cm) along the mid-rib region. Subsequently, these pieces were immersed in a solution comprising formalin, acetic acid, and alcohol (50%; alcohol:formalin:glacial acetic acid:glycerin = 90:5:5:5) within 3–6 hours of collection, facilitating the examination of leaf anatomical traits (Liu *et al*., 2019). Transverse sections were prepared using a hands-free technique and sequentially treated with ethanol concentrations ranging from 30% to 100% (Ahmad *et al*., 2020). Safranin and fast green stains (1% aqueous safranin and 0.5% fast green in 95% ethanol) were employed in combination: safranin delineated lignified cell walls of xylem vessels and sclerenchyma tissues, while fast green highlighted primary walls.

The transverse leaf sections were mounted in Canada balsam and subsequently photographed using a camera-fitted microscope, specifically a Meiji light microscope from Tokyo, Japan. The acquired data underwent processing using ImageJ for Windows (version 1.38; National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/). For the assessment of stomata and trichome density, leaf samples (1 × 1 cm) underwent surface scraping using a razor blade (Liu *et al*., 2018; Ullah *et al*., 2018), followed by treatment with NaClO to eliminate mesophyll tissues. After careful separation of the upper and lower epidermis, these fragments were stained with safranin dye and mounted in glycerol. For each individual plant, three replicates were sampled and two photographs were captured from different fields of view for each replicate. The number of stomata and trichomes in each photograph was then counted (Sobrado, 2003).

Physiological traits: Using the calorimetric method outlined by Bates *et al*., (1973), the proline contents of leaf samples were determined. Using a Hermle Z326K centrifuge, frozen leaf tissues were pulverized in liquid nitrogen and thoroughly mixed with 4 ml of 3% aqueous sulpho-salicylic acid. Proline concentration was determined using the supernatant obtained after centrifugation at 10,000 rpm for 15 minutes at 4°C. In a sealed test tube, 2 ml of supernatant was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, and the mixture was heated at 95-100°C for one hour. It was then immediately cooled in an ice bath, followed by addition of 4 ml of toluene by vigorously stirring for 2 minutes. We measured the absorbance of the upper phase containing the chromophore in toluene at 520 nm with a microplate reader (Analytikjena SPECORD® 200 PLUS) after allowing the mixture to stand at room temperature for 30 minutes. We also recorded the absorbance of a blank containing only toluene. The proline concentration was calculated from a standard curve and calculated based on fresh weight as:

$$\mu\text{moles proline per gram tissue of fresh weight material} = \frac{[(\mu\text{g proline.ml}^{-1}) \times (\text{ml})\text{ toluene} \times (\text{ml})\text{ sulpho-salicylic acid}]}{(115.5\ \mu\text{g}\ \mu\text{mole} \times \text{sample (g)})}$$

We used a modified protocol of Yemm and Willis (1954) for estimating total soluble sugar (TSS). Frozen plant material (~50 mg) was homogenized in chilled 80% ethanol using a mortar and pestle. Afterwards, the ethanolic mixture was centrifuged at 3000 rpm for 20 minutes at 4°C. About 2 ml of chilled 0.2% anthrone reagent were then added to the supernatant and the mixture was vortexed vigorously for 20 seconds. Reaction tubes were heated in a water bath at 95°C for 10 minutes and subsequently cooled to room temperature. Following chilling, assay tubes were vigorously shaken for 15 to 20 seconds, and absorbance was measured at 620 nm using a microplate reader. TSS were quantified using a calibration curve prepared with glucose standards and expressed as mg. g^{-1} FW.

Carotene pigments were quantified using the protocol described by Berger (1953) while the total chlorophyll content was assessed spectrophotometrically following the method outlined by Arnon (1949). Plant material was crushed in 80% acetone with the help of a mortar and pestle. Then, the crude extract was kept overnight at 4°C in the dark. The extracts were then centrifuged for 5 minutes at 3000 rpm. The optical density of the supernatant was measured at 663 nm, 645 nm, and 480 nm using 80% acetone as a blank control, employing a SPECORD® 200 PLUS spectrophotometer (Analytikjena, Germany). The values were expressed as $mg \cdot g^{-1}$ DM.

Soil analysis: Approximately 2 kg of soil were collected from depths of 0-15 cm and 15-30 cm within a 1 m^2 area, following a Z-shaped pattern, utilizing a soil auger. These samples were amalgamated to create a composite soil specimen, which was then stored in labeled polythene bags for subsequent analysis. The soil was crushed and homogenized to obtain a fine powder and then air-dried for 2-3 days. Subsequently, the soil samples were enclosed in plastic bags and stored at 4 °C in a laboratory freezer, as per the method outlined by Ahmad *et al*., (2020). Soil pH and EC were measured utilizing a pH meter (WTW series InoLab pH) and an EC meter (Cond 720, USA), respectively. This was achieved by preparing soil-water suspensions in a ratio of 1:2.5 for pH measurement and 1:10 for EC measurement, followed by the insertion of the respective pH and EC meter electrodes. To ascertain the concentration of K and Ca cations, soil water extracts were obtained through a digestion process, with subsequent measurements conducted utilizing an atomic absorption spectrophotometer (Hitachi 220, Japan). The percentage of soil organic carbon was determined via the wet combustion method, following the Walkley & Black (1934) procedure. Available nitrogen content was estimated through Kjeldahl digestion, as outlined by Subbiah & Asiza (1956).

Statistical analysis: Linear mixed-effect models (LMM) were developed to examine the responses of plant functional traits to ecological factors and soil parameters. These models encompassed various dependent variables, including morphological, anatomical, stomatal, and physiological leaf traits (El Din *et al*., 2020). Edaphic factors such as soil pH, EC, K, Ca, TOC and TN were considered as fixed effects, while altitude was treated as a random effect (Li *et al*., 2020). The MuMIn package within R 4.0.5 (R Development Core Team, 2021; Barton, 2019) was utilized to orchestrate subgroup regression analyses.

Furthermore, to explore potential nonlinear relationships, generalized additive mixed-effect models (GAMM) were employed (Li *et al*., 2020; Wood, 2019). Although both LMM and GAMM models yielded essentially identical conclusions, the former excelled in representing the reliable influence of predictors on dependent variables, while the latter proved excellent at reflecting significant correlations between predictors and dependent variables (Zuur *et al*., 2009; Ali *et al*., 2019). Principal component analysis (PCA) was conducted using the "FactoMineR" and "factoextra" packages in R, facilitating further exploration and understanding of the data structure.

Results

Response of morphological traits to soil factor: In LMM model (Table 1, Fig. 1), plant height increased with pH, Ca and TOC (, while decreased with K and EC likely because these factors enhance nutrient availability and soil structure thereby promoting growth. (Fig. 1a). Leaf area increased with pH and TN but declined with K (Fig. 1b). Leaf fresh weights increased with TN, pH and Ca while declined significantly with increasing K and EC (Fig. 1c). Leaf dry weights increased in association with TOC and TN whereas decreased significantly with increasing K and EC (Fig. 1d). To further evaluate potential non-linear relationships and smooth response patterns, GAMM model (Fig. 2) was applied. GAMM results showed linear increasing trends of plant height with pH, Ca, and TOC and decreasing trends with EC, K and TN. Leaf area exhibited slight non-linear decreasing trend with K and TOC and linear increasing trend with pH and TN. Leaf fresh weight had strong linear increasing trend with pH, Ca, TOC and TN but linear decreasing trend with EC and K. Leaf dry weight had non-linear decreasing trend with K and linear decreasing trend with EC while linear increasing trend with TOC and TN (Fig. 2). Although GAMMs captured some non-linear trends, the LMM results are considered more reliable because most soil–trait relationships were approximately linear and LMMs provide clear significance estimates. Ecologically, these trends suggest that soil nutrient availability and fertility promote leaf expansion and biomass, whereas high K or EC may constrain growth and leaf development by disrupting nutrient balance and creating osmotic stress.

Response of leaf anatomical traits to soil factors: In LMM models (Table 2, Fig. 3), leaf thickness declined with TOC (Fig. 3a) that reflects nutrient rich, low stress conditions allowing plants to adopt a fast-growing competitive strategy. Sclerenchyma thickness showed positive associations with TOC and EC whereas negative associations with Ca and TN (Fig. 3b). Metaxylem vessel area significantly decreased with TN (Fig. 3c). Stomatal density declined with Ca but increased with TN (Fig. 3d). Trichome density was significantly enhanced in association with TOC, TN and pH but declined strongly with K suggesting enhanced structural defense and surface modification under nutrient enriched conditions (Fig. 3e). Other leaf traits showed no significant relationships with soil variables. Complementary GAMM analysis (Fig. 4) revealed a non-linear relationship of stomatal density with K and of metaxylem vessel area with TOC. Leaf thickness, sclerenchyma thickness and metaxylem vessel area showed linear increasing trend with EC and K, while stomatal density with pH and Ca. Rest of the predictors had negative impact on anatomical traits (Fig. 4). Despite some non-linear trends in GAMMs, the anatomical traits indicate that higher TOC and TN enhance leaf structural and protective traits, while elevated K and Ca constrain anatomical development, reflecting resource-driven trade-offs.

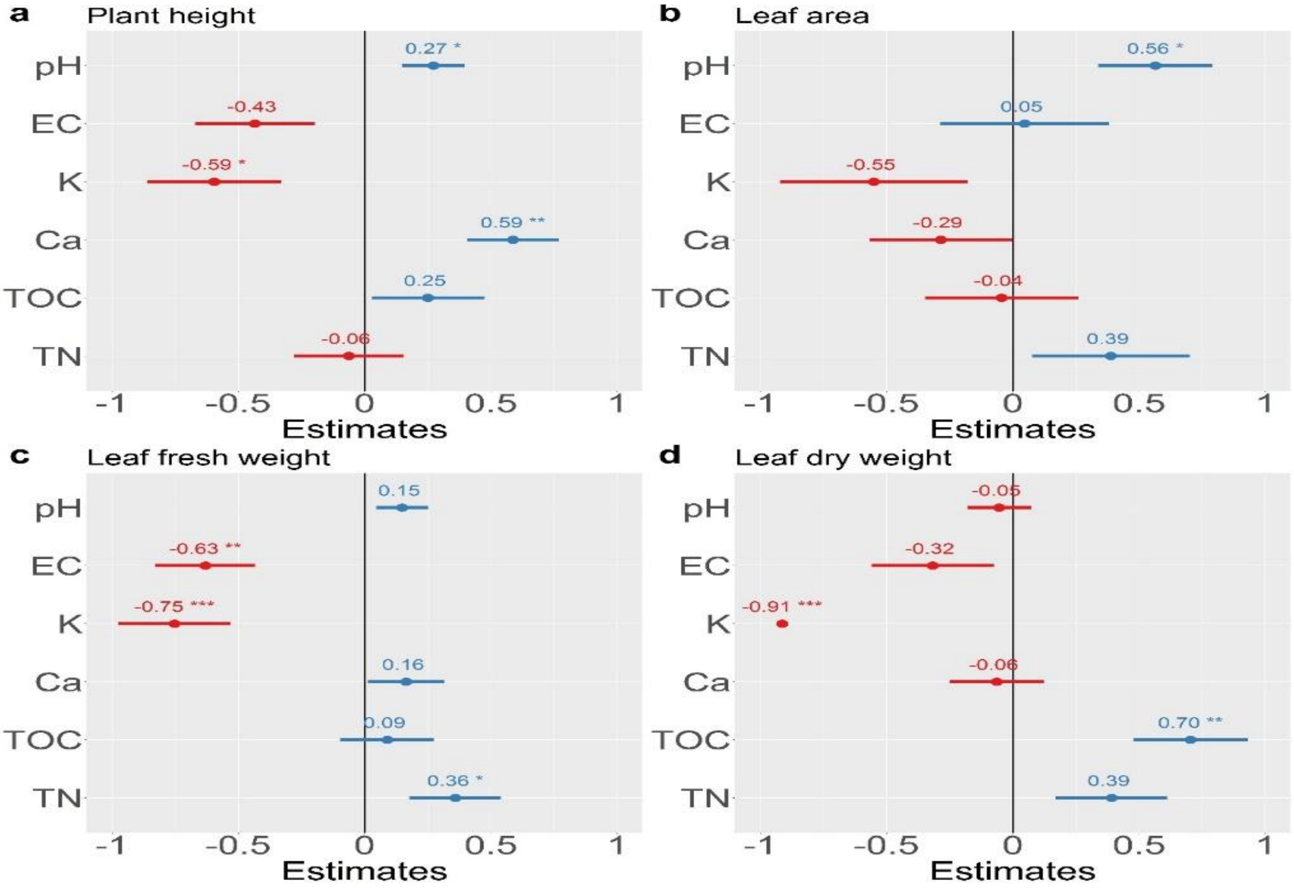

Fig. 1. Results derived from linear mixed effect model showing response of leaf morphological traits to soil factors. *$p<0.05$; **$p<0.01$.

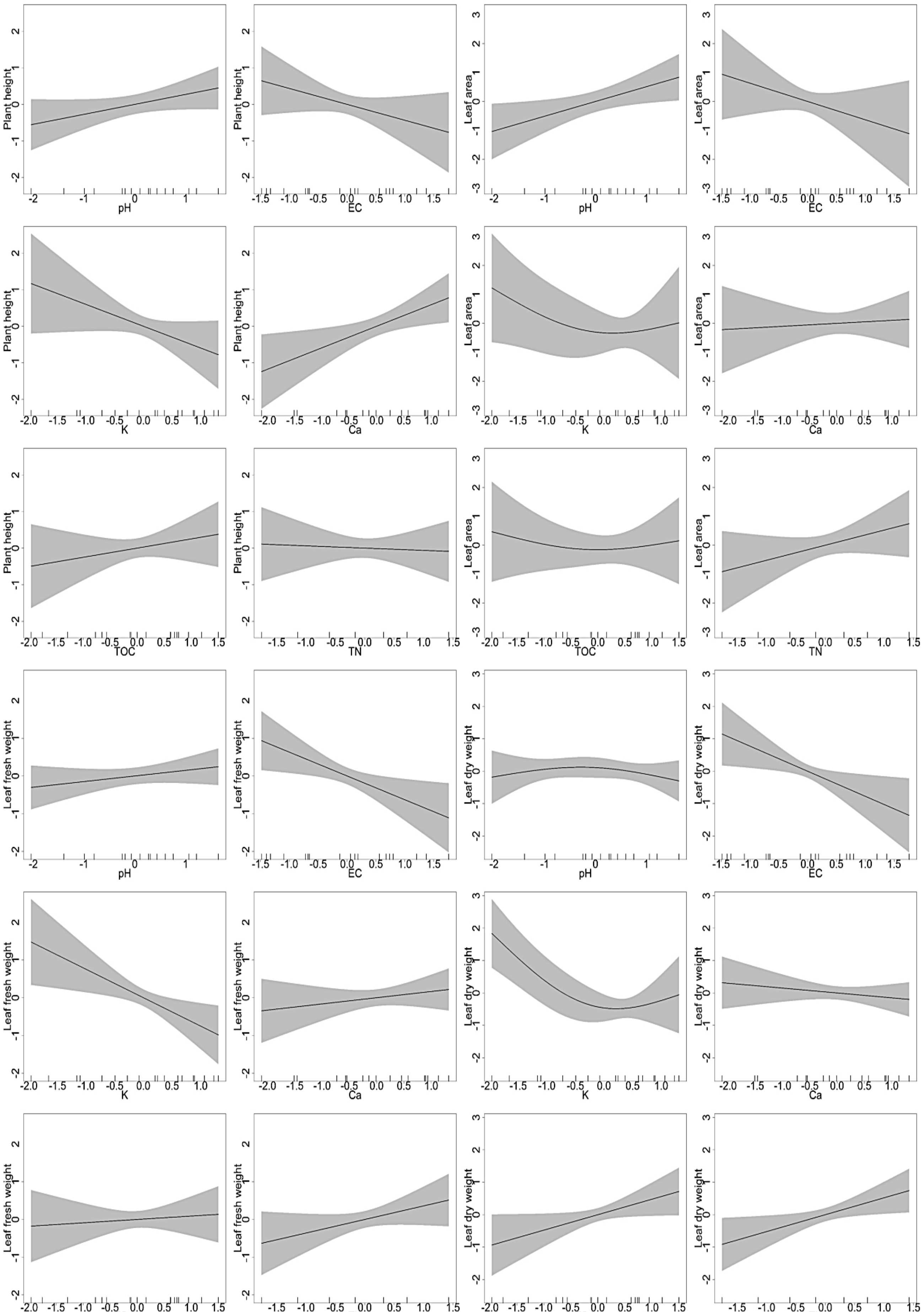

Fig. 2. Generalized additive mixed-effect model showing estimated responses of leaf morphological traits to soil factors.

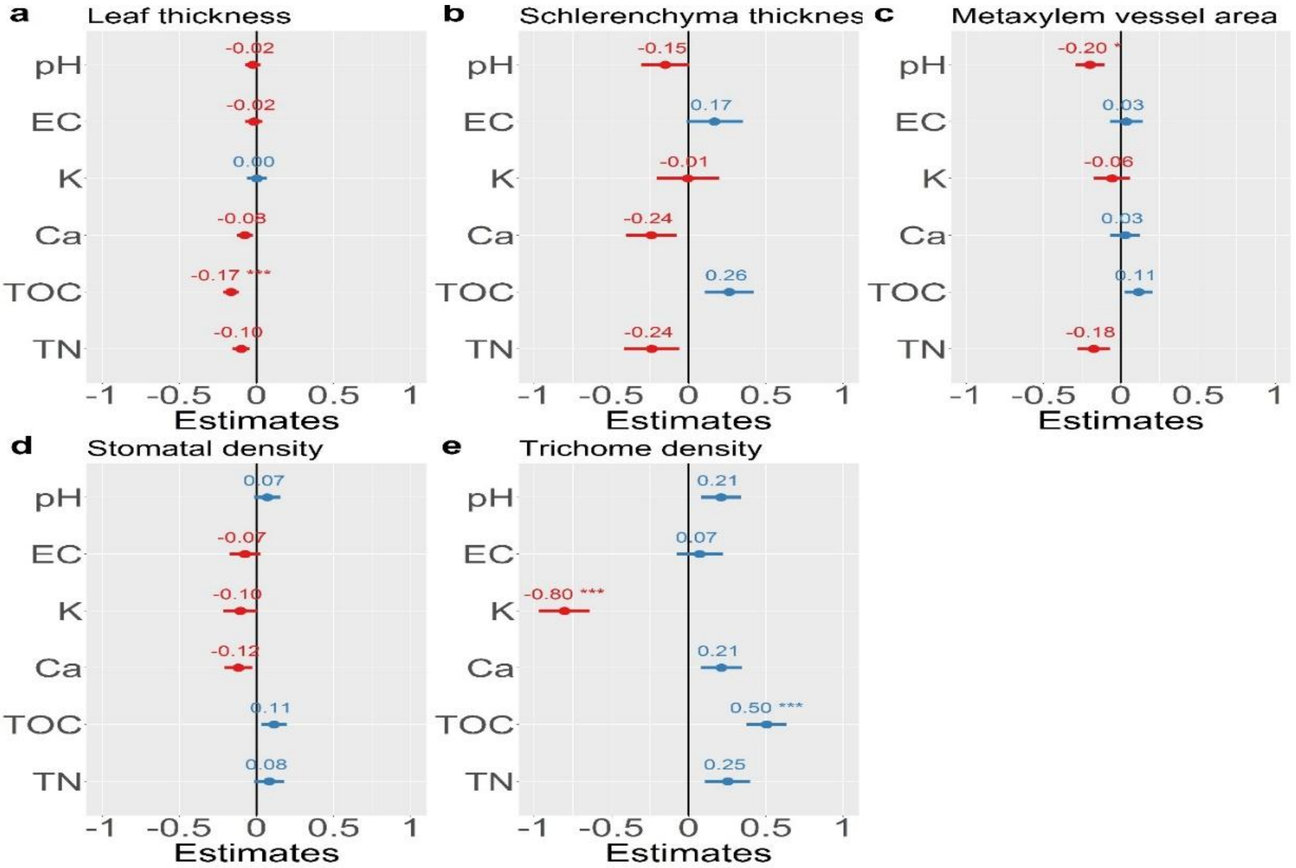

Fig. 3. Results derived from linear mixed effect model showing response of leaf anatomical traits to soil factors. *$p<0.05$; **$p<0.01$.

Response of physiology to soil factors: In LMM model (Table 3, Fig. 5), proline was positively associated with pH and EC but showed a strong negative association with K (Fig. 5a). TSS increased together with pH, Ca and TN while significantly decreased with K (Fig. 5b). Total chlorophyll enhanced in association with pH, EC and TOC; declined together with K and TN (Fig. 5c). Carotenoid content significantly declined with K while increased with pH, TOC, and TN (Fig. 5d) indicating nutrient and ion mediated regulation of photosynthetic pigment allocation. GAMM outputs confirmed and refined these patterns by revealing nonlinear responses of traits to edaphic gradients. The GAMM results (Fig. 6) indicated a slight non-linear response of proline to pH and a moderate non-linear response to K. TSS and total chlorophyll exhibited a slight non-linear trend with TOC and K respectively. Carotenoids showed linear but decreasing trends with K (Fig. 6). Overall, physiological traits responded positively to higher pH, EC, TOC, and TN, but were consistently suppressed by elevated K, indicating nutrient-driven shifts in stress tolerance and metabolic allocation, with minor non-linear patterns observed in GAMMs.

Correlation matrix: The correlation between leaf traits was not consistent (Fig. 7) indicating complex coordination patterns. Moderate positive correlation was observed between morphological traits. Strong positive correlation was found between anatomical traits except SD which showed strong negative correlation with other anatomical traits. In physiological traits, strong positive correlation was observed between PR and TSS and both traits except SD, showed strong positive correlation with anatomical traits and weak negative correlation with morphological traits (Fig. 7). The observed trait correlations indicate coordinated shifts between resource-acquisitive and stress-tolerant strategies, whereby structural reinforcement and osmotic regulation are enhanced under soil resource limitation, reflecting adaptive functional integration.

Principal component analysis: Principal component analysis (PCA) was used to test the associations between leaf morphological, anatomical and physiological traits (Fig. 8). For morphological traits (Fig. 8a), PCA axis 1 explained 37.42% of total variation with strong loadings of LFW, K, P, EC and TOC representing a nutrient biomass acquisition gradient. PCA axis 2 elucidated 23.48% variation driven by LDW, SLA, PHT, CA and pH indicating variation in leaf construction and allocation strategy. For anatomical traits (Fig. 8b), PCA axis 1 explained 41.23% of total variation with strong loading of MVA, TD, LT, SCT, N, K, P and EC reflecting a structural investment and nutrient response gradient. Whereas axis 2 revealed 29.83% variation with loading of pH, CA and SD revealed that epidermal gas-exchange traits respond more specifically to edaphic factors. For physiological traits (Fig. 8c), PCA axis 1 represented 36.96% of variance with loading of PR, TSS, TOC, K, P and EC indicating stress-metabolic response gradient. However, axis 2 accounted for 25.92 % total variation with dominance of Car, TCH, CA and pH representing variation in photosynthetic pigment investment. Overall, the PCA supports a soil-driven shift between acquisitive and conservative functional strategies. All the abbreviations used are presented in full form in Table 4.

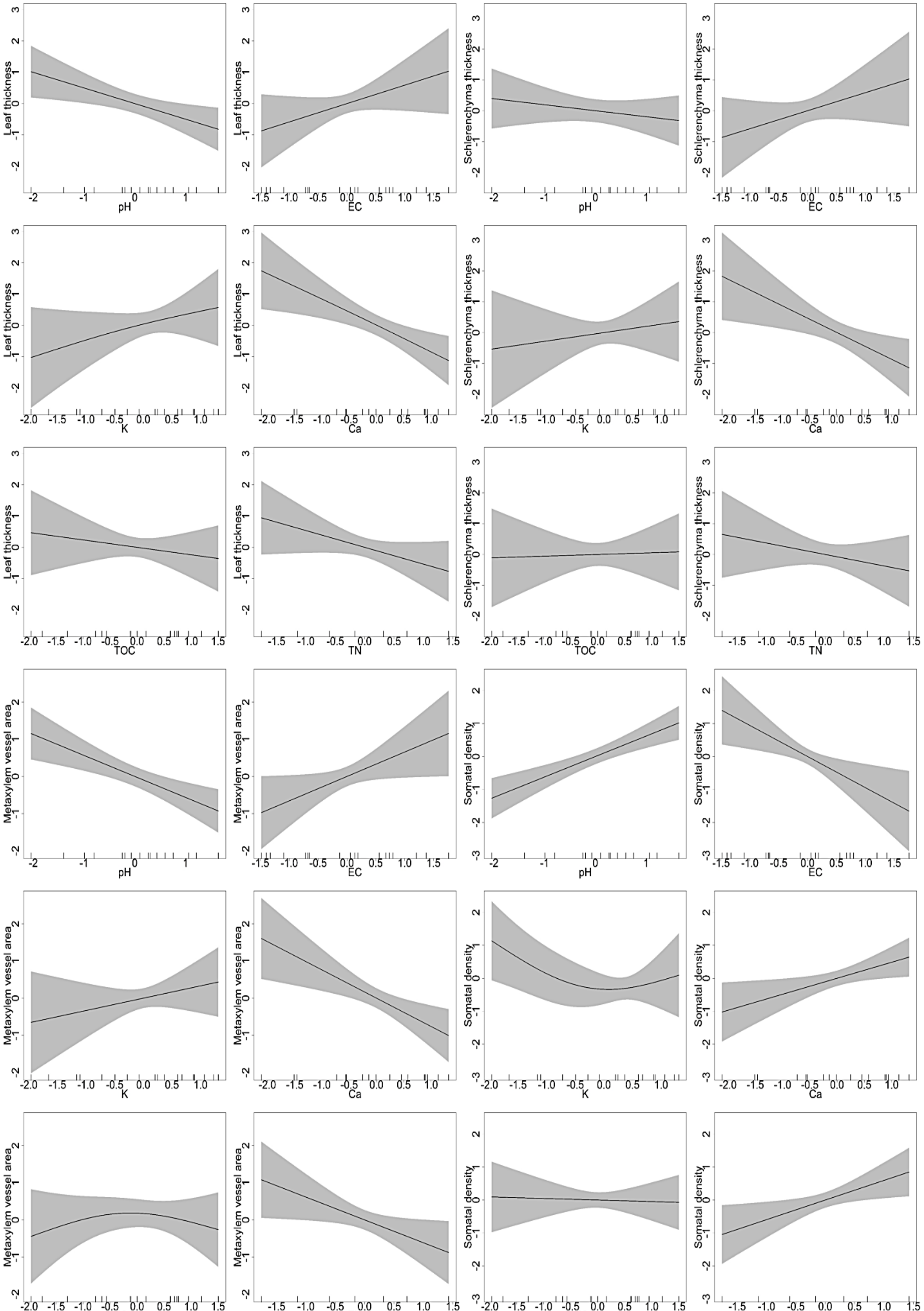

Fig. 4. Generalized additive mixed-effect model showing estimated responses of leaf anatomical traits to soil factors.

Table 4. Full form of the abbreviations used in the manuscript.

Abbreviation	Full form
Ca	Calcium
CAR	Carotenoids
EC	Electrical Conductivity
GAMM	Generalized Additive Mixed-effect Model
K	Potassium
LA	Leaf Area
LDMC	Leaf Dry Matter Content
LDW	Leaf Dry Weight
LFW	Leaf Fresh Weight
LMM	Linear Mixed-effect Model
LT	Leaf Thickness
LWC	Leaf Water Content
MVA	Metaxylem Vessel Area
P	Phosphorus
PCA	Principal Component Analysis
PHT	Plant Height
PR	Proline
SCT	Sclerenchyma Thickness
SD	Stomatal Density
SLA	Specific Leaf Area
TCH	Total Chlorophyll
TD	Trichome Density
TN	Total Nitrogen
TOC	Total Organic Carbon
TSS	Total Soluble Sugar

Discussion

The functional traits of leaves are greatly influenced by environmental conditions and seasonal variations found in mountainous ecosystems (Li *et al*., 2025). Examining intraspecific functional leaf trait variation is critical for understanding plant adaptive strategies under shifting environmental conditions (Kassout *et al*., 2026). These variations may lead to noticeable differences in the growth and development of plants (Guo *et al*., 2017; Wang *et al*., 2018). These changes frequently result in a smaller plant-environment contact area while increasing the plant's resilience (Kofidis *et al*., 2007; Reich *et al.,* 2014). As such, leaf economic and morphological attributes are important markers for evaluating the influence of the environment on the characteristics of plants as well as their tolerance level (Rai *et al.,* 2011). Research on soil nutrients and habitat response reveals that important influences on plant functional features are exerted by elements including pH, soil N and P (Guo *et al*., 2018; Pan *et al*., 2018). The study conducted by Luo *et al*. (2019) indicates that differences in leaf functional attributes are mostly driven by plant characteristics, such as life form or functional type, as suggested by changes in the C, N and P content of leaves.

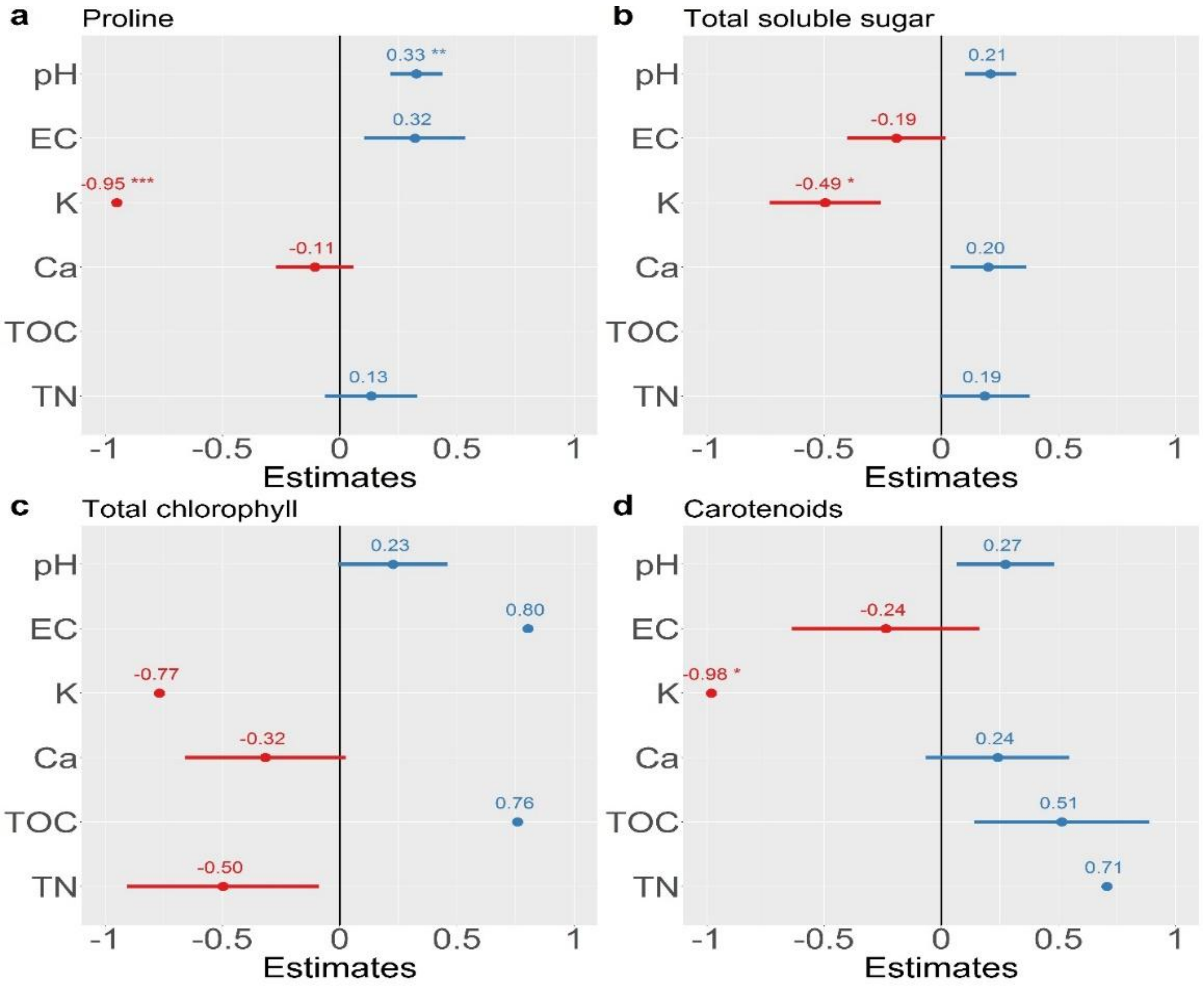

Fig. 5. Results derived from linear mixed effect model showing response of leaf physiological traits to soil factors. *$p<0.05$; **$p<0.01$.

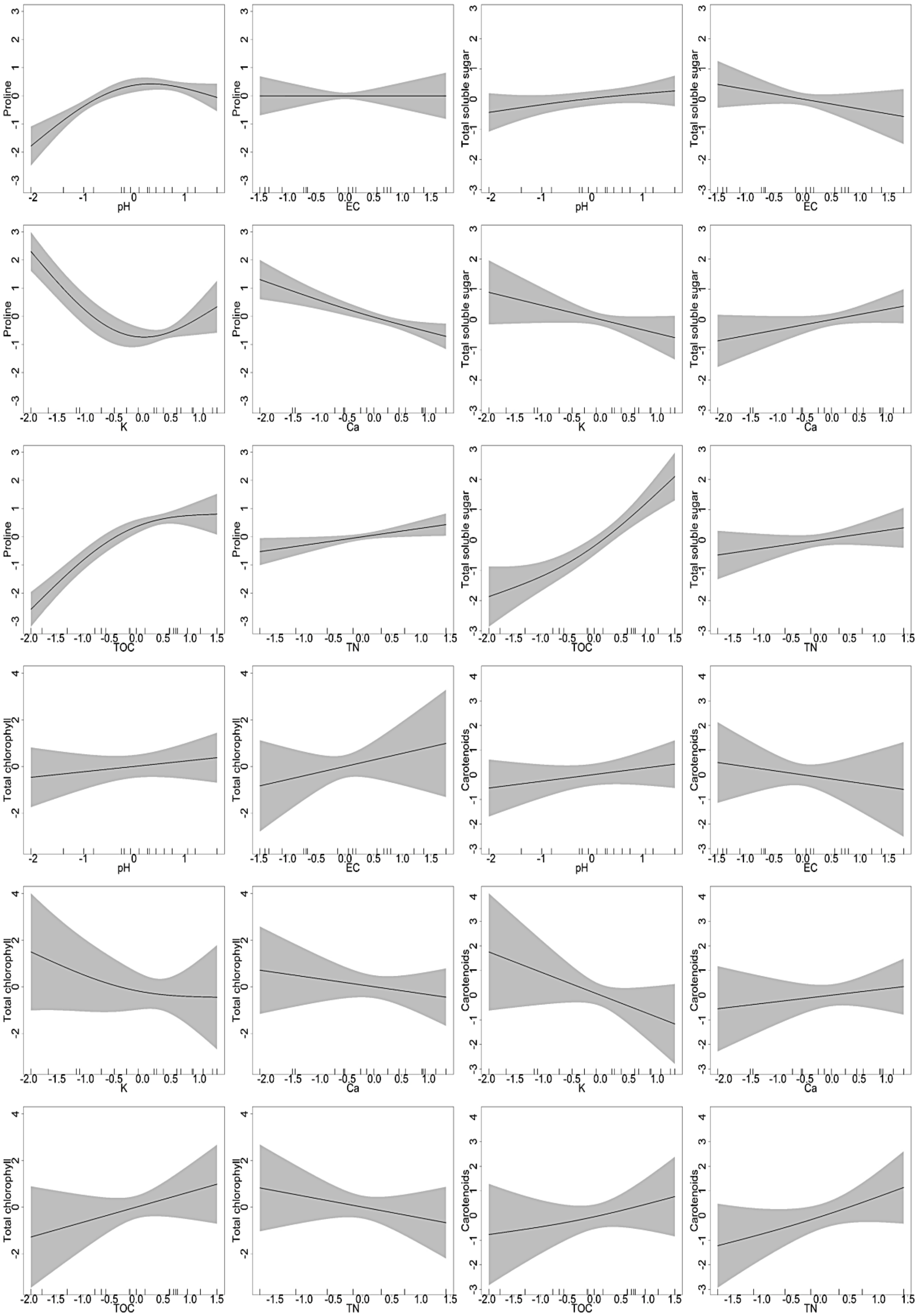

Fig. 6. Generalized additive mixed-effect model showing estimated responses of leaf physiological traits to soil factors.

Table 1. Results derived from multiple linear mixed-effect models showing impacts of soil factors on leaf morphological traits.

Predictors	PH			LA			LFW			LDW		
	Estimates	Statistic	p	Estimates	Statistic	p	Estimates	Statistic	p	Estimates	Statistic	p
pH	0.27	2.21	**0.027**	0.56	2.5	**0.012**	0.15	1.45	0.148	-0.05	-0.43	0.667
EC	-0.43	-1.83	0.067	0.05	0.14	0.89	-0.63	-3.19	**<0.001**	-0.32	-1.31	0.189
K	-0.59	-2.23	**0.026**	-0.55	-1.48	0.139	-0.75	-3.38	**<0.001**	-0.91	-3.35	**<0.001**
Ca	0.59	3.23	**<0.001**	-0.29	-1.01	0.312	0.16	1.08	0.278	-0.06	-0.34	0.731
TOC	0.25	1.13	0.258	-0.04	-0.15	0.883	0.09	0.49	0.628	0.7	3.11	**0.002**
TN	-0.06	-0.29	0.774	0.39	1.25	0.213	0.36	1.99	**0.047**	0.39	1.77	0.076

Table 2. Results derived from multiple linear mixed-effect models showing impacts of soil factors on leaf structural traits.

Predictors	LT			SCT			MVA	Statistic	p	SD	Statistic	p	TD		
	Estimates	Statistic	p	Estimates	Statistic	p	Estimates			Estimates			Estimates	Statistic	p
pH	-0.02	-0.47	0.638	-0.15	-0.98	0.326	-0.2	-2.15	**0.032**	0.07	0.8	0.422	0.21	1.61	0.108
EC	-0.02	-0.3	0.766	0.17	0.92	0.359	0.03	0.33	0.743	-0.07	-0.76	0.447	0.07	0.48	0.631
K	0	0.04	0.968	-0.01	-0.03	0.974	-0.06	-0.49	0.626	-0.1	-0.96	0.338	-0.8	-4.88	**<0.001**
Ca	-0.08	-1.47	0.142	-0.24	-1.48	0.139	0.03	0.29	0.771	-0.12	-1.33	0.185	0.21	1.58	0.113
TOC	-0.17	-3.36	**0.001**	0.26	1.67	0.096	0.11	1.26	0.208	0.11	1.35	0.178	0.5	3.96	**<0.001**
TN	-0.1	-1.75	0.081	-0.24	-1.35	0.178	-0.18	-1.68	0.093	0.08	0.86	0.393	0.25	1.73	0.083

Table 3. Results derived from multiple linear mixed-effect models showing impacts of soil factors on leaf physiological traits.

Predictors	PR			TSS			Tchl			Car		
	Estimates	Statistic	p	Estimates	Statistic	p	Estimates	Statistic	p	Estimates	Statistic	p
pH	0.33	2.92	**0.003**	0.21	1.92	0.055	0.23	0.98	0.328	0.27	1.32	0.188
EC	0.32	1.49	0.136	-0.19	-0.91	0.363	0.8	1.79	0.073	-0.24	-0.59	0.554
K	-0.95	-3.92	**<0.001**	-0.49	-2.09	**0.037**	-0.77	-1.52	0.128	-0.98	-2.18	**0.003**
Ca	-0.11	-0.64	0.523	0.2	1.24	0.213	-0.32	-0.92	0.357	0.24	0.79	0.432
TOC	1.28	6.37	**<0.001**	1.16	5.91	**<0.001**	0.76	1.81	0.071	0.51	1.37	0.169
TN	0.13	0.68	0.493	0.19	0.97	0.334	-0.5	-1.21	0.225	0.71	1.93	0.053

Plant economic features are significantly impacted by variations in the availability of nutrients in the soil (Fang *et al.*, 2019). Soil factors influence plant growth both directly and indirectly, with soil TN emerging as a significant driver of functional trait variation (Li *et al.*, 2025). It is closely linked to leaf functional trait expression, likely due to its role in regulating N use efficiency and resource acquisition strategies (Laughlin *et al.*, 2015). TN and TOC are essential for plant growth and are involved in succession dynamics, nutrient cycling, and ecosystem management (Tsukaya, 2018). Crucial factors and markers of plant development, acquisition, and conservation include SLA, which indicates leaf area per unit dry mass, and LDMC, which indicates leaf dry mass per unit water-saturated fresh leaf mass due to their close association with leaf functional traits, plant physiology, and growth dynamics (Poorter & Rozendaal, 2008). A smaller SLA corresponds to a larger light capture area for leaves, facilitating enhanced carbon assimilation (Wilson *et al.*, 1999). LDMC, SLA and leaf water content (LWC) exhibited the greatest intraspecific variability, indicating substantial phenotypic plasticity in leaf economic traits under resource-limited conditions (Ji *et al.*, 2020). Leaf dry weight demonstrates a significant increase in tandem with TOC and TN levels, indicative of plants' resource acquisition capabilities. As soil carbon and nitrogen levels escalate, the capacity for resource competition is bolstered (Yang *et al.*, 2018). Overall, soil properties underpin plant water and nutrient uptake, thereby shaping trait variability and adaptive responses.

In terms of morphological traits, N and TOC enhanced leaf biomass and specific leaf area because higher N availability supports protein synthesis, chlorophyll production and metabolic activity whereas TOC improves soil fertility and water retention providing the resources necessary for leaves to expand and allocate biomass efficiently. The observed increase in fresh and dry weights with TOC and TN suggests that leaf photosynthetic capacity might decrease with increasing N, despite the growth stimulation of plant functional types. These findings align with previous research by Wilson *et al.*, (1999). The limitation of leaf photosynthetic capacity due to N addition provides a reasonable explanation for the low SLA induced by K, Ca, and TOC. It reflects thicker or denser leaf construction; a trait commonly associated with conservative resource use strategies. Such structural investment increases leaf longevity and nutrient retention, which is advantageous under heterogeneous or nutrient limited soil conditions. The reduction in SLA could be an effective strategy of *Rubus ellipticus* for nutrient conservation and facilitating plant growth in nutrient-poor environments, as noted by Wilson *et al.*, (1999). Leaf morphology commonly shifts along elevation gradient and fluctuating nutrient conditions with reduced surface area and increased thickness (Wright *et al.*, 2017). Similarly, leaf size, biomass and plant height tend to decline in nutrient limited conditions (Halbritter *et al.*, 2018).

Soil TOC demonstrated a notable adverse influence on leaf anatomical traits (LT), stomatal traits (SL), and physiological traits (TSS, PR) likely because higher organic carbon enhances nutrient and water availability, reducing the need for structural and stress-protective investments and allowing plants to allocate more resources toward growth and metabolic activity. The contrasting responses of stomata and leaf thickness to the N or P indicate nutrient specific regulation of leaf structure and gas exchange capacity. P enrichment increased stomatal traits while reducing leaf thickness, likely because improved P availability enhances metabolic activity and cell division, promoting greater stomatal development and reducing structural investment in thicker tissues, thereby facilitating more efficient CO_2 diffusion and photosynthetic performance. Concurrently, leaf thickness exhibited an increase with higher soil N addition, suggesting a shift in nutrient-limiting conditions from N to P (Yan *et al.*, 2015).

Drought-stressed species often possess smaller and denser stomata, facilitating quicker adaptation (Wang *et al.*, 2016), thereby promoting photosynthetic rates. Furthermore, an increase in leaf thickness correlated with rising soil nitrogen levels, signifying a shift in nutrient limitation from nitrogen to phosphorus (Yan *et al.,* 2015). Soil TOC had a positive influence on sclerenchyma thickness likely because higher organic matter improves nutrient and water availability, allowing plants to invest in structural reinforcement and mechanical stability. Thicker leaves serve to protect plants from solar damage, while larger vessels facilitate water transport (Niinemets, 2001). Sclerification plays a crucial role in evaluating a plant's resilience to harsh environmental conditions like cold and frost (Ahmad *et al.*, 2016). Intensive sclerification in the cortical region and surrounding vascular bundle areas aids in safeguarding tissues from adverse external conditions, thereby assisting plants in minimizing water loss (Yujing & Yong, 2000).

The soil pH, TOC, and TN were found to have a positive influence on stomatal density because of improved nutrients and soil fertility that enhance plant metabolic activity during leaf development, promoting greater stomatal development and consequently, more efficient gas exchange and photosynthesis. This could potentially be attributed to the availability of sufficient carbon during the short growing season, which could explain why plants in the Himalayas at high altitudes tend to have small and densely packed stomata (Schreiber *et al.,* 1999). Conversely, a decrease in stomatal density might occur due to soil water deficiency, which is often caused by reduced root water absorption due to low soil temperatures (Krasensky *et al.*, 2012). Additionally, high levels of irradiation at high altitudes and the consequent reduction in CO_2 transport impedance facilitated by a larger mesophyll area under such conditions may lead to a decrease in stomatal numbers (Barros *et al.*, 2015).

Moreover, TOC exhibited a significant positive impact on trichome density, likely because higher soil organic matter enhances nutrient and water availability, enabling plants to invest more resources in protective leaf structures, which can reduce water loss, limit herbivory, and improve stress tolerance. However, soil K exhibited a significant negative effect, potentially reflecting a trade-off between nutrient-driven growth and protective structures. The high density of trichomes reflects the surrounding environmental conditions and the plant's ability to withstand harsh climates, including temperature fluctuations, light exposure, UV radiation, herbivore and pathogen attacks, excessive transpiration, as well as roles in seed dispersal and protection (Ahmad *et al.*, 2020; Ning *et al.*, 2016).

The primary role of proline is to enhance osmotic adjustment, thereby enabling plants to withstand cellular damage caused by reactive oxygen species (ROS) during periods of low-temperature stress (Chun *et al.*, 2018; Alvarez *et al.*, 2021). Additionally, proline, along with soluble sugars, aids in maintaining optimal cellular function, metabolism, and development by increasing cellular fluid concentration and preserving cell membranes (Arroyo *et al.,* 2003; Basu *et al*., 2007). In the current study, TN positively influenced the production of proline and TSS by providing essential elements for amino acid and carbon metabolism, which enhance osmotic adjustment and stress tolerance. In contrast, elevated soil K may negatively affect these osmoprotectants because excessive K can disrupt nutrient balance or resource allocation, directing energy toward growth and structural maintenance rather than stress-related metabolite synthesis. Proline, acting as a low-molecular-weight chaperone, is acquired by plants to mitigate water loss during stress, thus contributing to long-term growth maintenance (Hasanuzzaman *et al.,* 2019; Zandalinas *et al.,* 2018; Meletiou-Christou & Rhizopoulou, 2017).

Although proline levels continued to rise with increased P addition, the shift from nutrient limitations being predominantly phosphorus-limited to nitrogen-limited was observed (Yan *et al*., 2012). TSS showed an increase alongside soil nitrogen levels, suggesting that N treatment boosted antioxidant defense in leaves by elevating soluble sugar levels, thereby enhancing stress tolerance and plant growth capacity (Song *et al.,* 2019). According to Agren *et al*., (2012), once the N:P supply ratios achieve a nutrient-balanced state, augmenting either nutrient within a specific range would boost growth rates by allocating more resources to the limited element. Consequently, our findings indicate that the increase in soil nutrients primarily drives changes in leaf functional traits, rather than alterations in nutritional limitation status (Wang *et al*., 2021).

The relationships among leaf traits vary across herbaceous plants, shrubs, and trees, with some associations specific to certain plant functional groups. A study by Li *et al*., (2015) highlighted decoupling between leaf morphological and anatomical traits in subtropical forests of Guangdong and Yunnan provinces, indicating that functional traits operate independently, allowing for diverse combinations to regulate leaf temperature and optimize water and nutrient uptake. Liu *et al*. (2019) proposed that inconsistent correlations among leaf traits in different plant functional groups reflect diverse adaptive strategies for resource acquisition, while Yin *et al*., (2018) identified consistent trait relationships in the Loess Plateau, China. Discrepancies between these findings and those of Yin *et al*., (2018) are attributed to habitat differences, particularly variations in water availability. Together, these contrasting patterns indicate that leaf trait relationships are environment-dependent, underscoring the need for species-level and edaphic gradient based analyses to better resolve functional trait relationships.

Conclusion

The current investigation addresses a significant gap in our understanding concerning the impact of soil conditions on leaf functional traits at the species level in the Himalayan mountains. These regions are characterized by cold temperatures and summer droughts, which profoundly influence species' functional responses. Our findings suggest that soil variation plays a crucial role in shaping plant eco-physiology, leading to local adaptations aimed at maximizing fitness. Notably, we observed considerable variation in various traits, including plant height, leaf area, stomatal density, proline, and soluble sugars across soil gradients. This variability implies their involvement in resource utilization strategies and osmotic adjustment. Moreover, the uncoupling of leaf functional traits suggests that plants can independently vary these traits, potentially resulting in diverse plant trait assemblages. Our study highlights the resilience of *Rubus ellipticus* to edaphic factors, possibly due to the local proliferation of soil-adapted species.

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