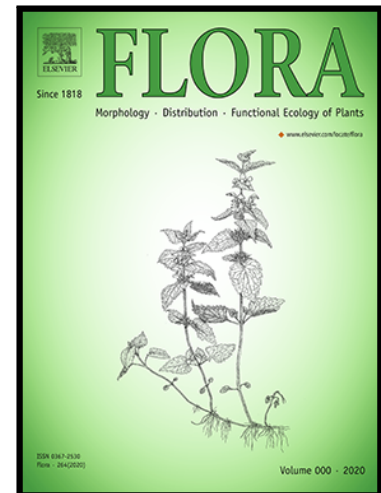


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Species identity and resource availability explain variation among above and below-ground functional traits in Himalayan temperate forests

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## Highlights

- Increased trait divergence with spatial and temporal variation.
- Trait variances are maximally distributed at the species level (~48%).
- Broadleaved showed increased trait coordination with varying environments.
- Coniferous species showed increased trait adaptability to changing environments.
- Trait analysis with soil nutrients showed maximum variance with Root>Stem>Leaf.

Journal Pre-proof

**Species identity and resource availability explain variation among above and below-ground functional traits in Himalayan temperate forests**

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**Abstract**

Trait variation across spatial and temporal scales allows plants to adapt to changing environments. Understanding the mechanisms driving trait variability, it is important to unravel how plant functional traits adapt to changing climates. Plant chemical traits (C, N, P, K, S, Ca, Mg, Mn, Zn, Fe, Cu and Na) for leaf, stem, and root were studied for ten dominant evergreen tree species along increasing elevation (1500-3000 m asl) for three seasons (summer, monsoon, and winter) in temperate forests of western Himalaya, India. We also collected five soil samples from each plot to analyze chemical trait variation with respective soil nutrient concentrations. Our results suggested that temperature significantly ( $P < 0.05$ ) affected leaf traits, whereas stem and root traits were maximally driven by light and water availability ( $P < 0.05$ ). Maximum trait variance was observed at species level (~48%) followed by individual level (~45%). Coniferous species displayed strong trait coordination among organs, whereas broad-leaved species showed strong correlation with environmental resources. Trait correlation with respective soil nutrient concentration was found strongest for the root, followed by stem, and ultimately got lost for leaf traits (except Ca, Mg and Mn). Weaker plant-soil interaction for leaf traits showed external factors such as lopping alter plant-soil relationship. We provide evidence of varying degrees of trait conservatism within broad-leaved and coniferous trees that leads to increased trait variability. We also emphasize the importance of considering major abiotic and biotic stresses exerted on different plant organs when investigating trait variation along environmental gradients.

**Keywords:** Broad-leaved species; Coniferous species; Fine roots; Trait coordination; Variance partitioning.

## Introduction

Environmental gradients such as increasing elevation that leads to variation in temperature and precipitation act as a natural laboratory in analyzing species' response to changing climate (Spitzer et al., 2023). Plant functional traits play an important role in providing shreds of evidence regarding the interaction of plant species with their surrounding environment (Silva et al., 2018). For instance, species at higher elevations (cooler climate) tend to exhibit increased leaf thickness and nitrogen content per unit area as compared to species at lower elevations (Read et al., 2014). There is also known to be a great variation in light intensity with changing elevation which results in a decrease in leaf area or plant height with increasing elevation as an adaptation to cooler climates (Poorter et al., 2009). However, such studies have focused explicitly on above-ground or below-ground traits, and we know little about the effect of environmental variation on both above and below-ground plant traits.

Together, leaf, stem, and root form the most important and dynamic components of plant functions which are known to be tightly coupled with each other (Record et al., 2016). The plant economic spectrum suggests that trait variation along resource gradient (light, water, and nutrient) might be coordinated to survive in the environment with the goal that cheap or expensive tissues consistently occur across the leaf, stem, and root organs (Flores-Moreno et al., 2019; Reich, 2014a). A few studies reported co-ordination among leaf, stem, and root traits (Fortunel et al., 2012) along edaphic and climatic gradients (Fortunel et al., 2009; Lourenço et al., 2021) and suggested synchrony in the construction cost of plant components. Opposing this statement, a few other studies have reported the traits to be independent of each other, suggesting independent trade-offs among traits at the organ level (Wright et al., 2007). The inconsistency in results could be primarily attributed to different ecosystems selected for the study along with the types of traits chosen. Therefore, to acquire a comprehensive understanding of leaf, stem, and root trait coordination, it is essential to

conduct research within, between and among different vegetation types considering important traits that are essential for the functioning of each plant component.

Around 30 plant chemical traits are known to be associated with plant growth and metabolism (Marschner, 2011). The interaction of such chemical traits in an ecosystem provides an integrative approach to explore plant nutrient concentration among different organs (leaf, stem, and root). The importance lies especially in the upward intake of nutrients from the soil by roots that are essential to plant growth and development (Spitzer et al., 2023). Studies have found that nitrogen and phosphorous content in the leaf, stem, and root are excellent indicators of soil nutrient availability (Parkinson and Allen, 1975). Also, in mountain ecosystems, seasonal variability due to differences in water availability like the drought in summer, wetness in monsoon, and snow cover in winter season have a significant effect on plant traits such as carbon and resource allocation to above and below-ground parts (Cornelissen et al., 2003). This exerts a direct influence on nutrient accessibility to plants, modulated by interactions with factors such as topography, climatic conditions, forest types, etc. (Kumar et al., 2021a). Broad-leaved and coniferous species in the Himalayan region are characterized by distinct seasonality of precipitation and temperature that affects the nutrient productivity (Ralhan and Singh, 1987). Coniferous species such as chir pine exhibit a higher proportion of nutrient (N, P and K) retranslocation compared to broad-leaved species such as oak (Chapin et al., 1980). This difference in nutrient retranslocation plays an important role in facilitating the expansion of pine in areas that were dominated by oak (Singh et al., 1994a). Hence, the uptake and assimilation of resources and nutrients via roots demand rapid acquisition of nutrient via leaves (Reich, 2014). A study in temperate ecosystems reported that nitrogen and phosphorus content in belowground components (roots) were more than double-fold higher than in aboveground components suggesting great variation in nutrient turnover rates with water availability (Meier et al., 1985). However, in tropical Amazon

Forest, environmental association with fine root traits was very weak in comparison to leaf traits that showed stronger relation with soil fertility. The increase in nutrient content requirement by leaf tissues could be a possible reason for increased correlation between leaf and soil nutrient content (Vleminckx et al., 2021). Among all the environmental factors, soil nutrient availability has known to be the main factor significantly affecting plant chemical (nutrient) trait variation (Pang et al., 2021).

A whole-plant resource economic spectrum has never been successfully explored in Himalayan region, despite some strong shreds of evidence which show that these components typically capture vital trade-offs that determine the species' ecological role in its community (Garkoti, 2012; Rawat and Singh, 1988). Additionally, with changing environments from lower to higher elevations, there occurs a huge variation in anthropogenic activities such as logging, grazing, etc. People residing in forested regions are greatly dependent on forest resources for their livelihood and survival (Dhyani et al., 2018) which significantly affects the overall plant functioning. For instance, tree foliage is highly used for feeding livestock since time immemorial in the Himalayan region (Verma and Mishra, 1998) which ultimately impacts the leaf life span and longevity. This study aims to assess how plant chemical traits are coordinated among different plant organs along the environmental gradient. The following questions were addressed: 1). How do plant chemical traits vary with changing environments (spatially i.e., as a proxy to change in elevation and associated resource gradient) and changing season (temporally)? 2). How do traits vary across different ecological scales? 3). How are traits coordinated among different plant organs and along resource gradient i.e., light, water, and nutrient availability? We hypothesized that plant chemical traits would strongly be driven by the resource availability (light, water, and soil nutrient) across topographical variation and the traits would vary significantly among species due to functional differences between broad-leaved and coniferous species. It was also

hypothesised that trait coordination among organs would vary among broad-leaved and coniferous species due to differences in adaptation strategies with increasing environmental heterogeneity.

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## Materials and methods

### *Study Area*

The present study was carried out in Swana Reserved Forest (SRF) ( $30^{\circ} 86'N$  -  $31^{\circ}01' N$  and  $77^{\circ} 38' E$  and  $77^{\circ}49' E$ ), a protected area that lies in Rajgarh range of Sirmour district, Himachal Pradesh (Fig. S1). The climate varies from moderate and cold to very cold at higher elevations. Temperature ranges from  $2^{\circ}C$  in winter season (minimum) to  $30^{\circ}C$  in summer season (maximum). The average annual rainfall is about 125cm (District Survey Report, 2016). The altitude varies from around 1500m to 3100m asl (Fig. S1). In elevation zone less than 2000m, the parent rock material comprises phyllite, schist and quartzite with soil types varying from clayey to sandy loam whereas, in an elevation zone greater than 2000m, granite, gneiss, micaceous and schist are dominant rock types with soil type varying from shallow to moderately deep clay loam. In the higher elevation zone, brown and podzol types of soils usually occur (Rawat et al., 2010). The soils are generally acidic in nature. SRF was classified under Group 12- Himalayan moist-temperate forest (Champion and Seth, 1968). These forests occur in pure as well as mixed forms.

### *Sampling Design*

A stratified sampling approach was used to analyze trait variation along an environmental gradient in Swana Reserved Forest (SRF) (Fig. 1). Having an elevation range of 1500 m to 3000 m asl, the study site was divided into three elevational sites i.e., 1500m-2000m (Lower elevation), 2000m-2500m (Middle elevation) and 2500m-3000m (Higher elevation). Two plots of 0.1 ha ( $\sim 31.6m \times 31.6m$ ) were established along increasing elevational scale of 150 m, altogether forming six plots within each site. The slope angle varied in the range of  $14^{\circ}$  to  $40^{\circ}$ . Elevation and geographical coordinates were determined using GPS-Garmin. Slope aspect and aspect azimuth were calculated using a Magnetic compass. Slope angle was

measured using a clinometer. Plot-level temperature was measured using a soil thermometer which was calculated as monthly average soil temperature. Relative radiation intensity (hereafter light intensity, RI) was calculated for each plot following Paudel and Vetaas (2014) using the equation,  $RI = \cos(180^\circ - \Omega) \times \sin(\beta) \times \sin(\phi) + \cos\beta \times \cos\phi$

Where,  $\Omega$ =aspect (slope azimuth),  $\phi$ =latitude and  $\beta$ =slope inclination. In the study site, anthropogenic disturbances are minimal due to low population density. However, local communities rely on the forest for fuel and fodder purposes. Lopping, defined as cutting off branches from the main stem of the tree, could be seen as the most common anthropogenic activity in the forest. Lopping intensity was calculated in each plot by counting the number of lopped tree individuals against the total tree individuals (Rawal et al., 2012) (Table S1).

After a reconnaissance survey of the study area, ten dominant tree species (five broad-leaved and five coniferous evergreen) were selected for trait analysis based on abundance. Broad-leaved evergreen tree species comprised individuals of *Quercus floribunda*, *Q. leucotrichophora*, *Q. semecarpifolia*, *Neolitsea pallens* and *Rhododendron arboreum* whereas coniferous evergreen tree species included *Pinus roxburghii*, *Cedrus deodara*, *P. wallichiana*, *Picea smithiana* and *Abies pindrow*. The dominant vegetation in each plot is mentioned in Table S1. In each of the eighteen plots (6 plots  $\times$  3 elevation sites = 18 plots), tree individuals were classified based on their DBH classes (Diameter at Breast Height, 10-20cm, 20-30cm, 30-40cm, >40cm) and three individuals from each DBH class were sampled to capture differences in age and stand development within plots.

#### *Plant Functional Trait sampling and analysis*

Leaf, stem and fine root samples were collected for 287 individuals for three different seasons i.e., summer (May and June 2019), monsoon (July-August 2019) and winter (Jan-Feb 2020). To facilitate sampling in the subsequent seasons, each selected individual was tagged with a unique ID. From each target tree ( $\geq 10$  cm DBH), twig samples were collected with the

help of local people by tree climbing or by cutting from the ground with branch cutters. Twig samples were collected with fully expanded leaves with little or no damage by herbivores or pathogens. Twenty leaves were sampled from each tagged tree. Stem samples were extracted by coring 20 cm large cores at 1.37 m using an increment borer of 6mm internal diameter. Fine roots (<2 mm) were sampled by loosening the soil near the tree individuals and extracting root samples using fine root corers of 8 cm internal diameter. The number of individuals sampled from each species are mentioned in Table S3. Overall, we sampled 287 leaf samples, 287 stem samples and 90 root samples from each season. In the laboratory, selected samples (leaf, stem, and root) were cleaned to remove any dust or soil particles. Fine root samples were aggregated at plot level because root identification at species level was not possible. After cleaning, the samples were dried in an oven at 60 °C for 72 hours to achieve constant weight. The leaf samples were finely grinded using a mixer grinder whereas stem and fine root samples were grinded and passed through 0.5 mm sieve using Willey mill before estimating the nutrient concentrations.

Carbon, Nitrogen and Sulphur content for the finely grinded samples were determined following the Dumas combustion method using an elemental analyzer CHNS (Euro, EA-3000). Plant samples were digested using tri-acid mixture of conc.  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{HClO}_4$  in the ratio of 1:5:1 on a hotplate having 90 °C temperature till milky white slurry type formation occurred (Chapman, 1976) and washed with 5 ml of 1:1 HCl with distilled water. Phosphorous was analyzed using diacid mixture of Nitric acid ( $\text{HNO}_3$ ) and Perchloric acid ( $\text{HClO}_4$ ) in a ratio of 3:1 and the color intensity was read after 10 min using spectrophotometer at 420 nm (blue filter) (John 1970) Calcium, Potassium and Sodium were analyzed using flame photometer (766 nm) (Okalebo et al., 2002) whereas, Magnesium, Copper, Iron, Manganese, and Zinc were analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, model Ultima 2 Jobin Yvon, France).

### *Soil nutrient analysis*

Five randomly located soil cores at depths of 0–10, 10–20, and 20–30 cm were collected from each plot using a soil corer of 5 cm internal diameter. In total, 80 soil samples were packed in airtight zip bags and brought to the laboratory for further analysis. Soil samples were collected for the three different seasons to analyze variations in soil moisture content (MC). Here soil MC was used as a proxy for variation in soil water availability. Soil MC was estimated following Mishra (1968). Samples were air-dried for 48 hours and sieved through 2mm. The Bouyoucos hydrometer method of silt and clay measurement was followed for soil texture (soil type) analysis using 10% Calgon's solution (sodium hexametaphosphate) (Okalebo et al., 2002). Samples were grinded to 200 mesh size (~0.075 mm) using an agate mortar and pastel. 0.5 g of the very finely grinded soil sample were used for digesting the samples following Shapiro and Brannock (1962) using conc. Hydrogen fluoride (HF), conc. Nitric acid (HNO<sub>3</sub>) and HClO<sub>4</sub> in a Teflon crucible. The nutrient analysis was done using spectrophotometer, flame photometer, CHNS elemental analyzer and ICP-OES like plant tissues.

### *Statistical analysis*

Data normality was assessed based on Shapiro–Wilk's test and homogeneity of variance were tested by Levene's test. Data were transformed to log-normal if required. To answer question 1, we used two-way ANOVA (Analysis of Variance) to assess significant trait variation among organs along changing elevation (spatial) and season (temporal) and the data were visualized using the ggplot2 package. Variations in environmental factors (light, water, soil type and temperature) with increasing elevation were analyzed using correlation diagram (corrplot package). Further, we applied multiple regression model to analyze the important environmental factors driving plant chemical trait variation. Variance inflation Factor (vif)

was used to check the multicollinearity among the environmental variables ( $vif < 5$ ) using car package. To answer question 2, we used variance partitioning across ecological scales using nlme and ape packages (Messier et al., 2010). The study design accounted for nested ecological scales i.e., site level (nested in a transect along elevation), plot level (nested in sites), species level (nested in plots) and individual level (nested in species). Values for root traits were removed from further analysis since the identification of root samples at species level was not possible. Overall trait variations between broad-leaved and coniferous tree species were further assessed using t-test to analyze differences in trait means irrespective of spatial variation. The differences in trait values were visualised using boxplot diagram. Further, trait variation at the species level was analyzed using one-way ANOVA followed by Tukey's post hoc test using agricolae package. To answer question 3, we used Principal Component Analysis (PCA) plot to analyze coordination between leaf and stem organs. We further used Pearson correlation analysis among organs to determine the relationship between plant chemical traits and environmental factors using Hmisc package. Environmental factors having a correlation greater than 0.5 were removed from the analysis. In the end, plant chemical traits at the organ level (leaf, stem, and root) were regressed against their respective soil nutrient concentration aggregated at plot level (18 plots) using ggplot2 package. Trait values were averaged for all three seasons to answer questions 2 and 3. All statistical analyses were conducted using R version 3.5.0 (R Core Team, 2018).

## Results

### *Spatial and temporal variation of plant functional traits*

Plant chemical traits varied with increasing elevation and changing seasons for different organs (Figure 2, Table S2). Among leaf traits, C and Mn were found significantly varying with elevation ( $P<0.05$ ) whereas, among stem traits, P, K, Ca, Mg, Mn, Zn, and Na were found significantly varying with elevation ( $P<0.05$ ). For root traits, P, K, Ca, Mg, Mn, Zn, Fe, and Cu were found significantly varying with elevation ( $P<0.05$ ). Considering seasonal variation, leaf traits showed significant variation for P, K, Mn, Zn, and Cu ( $P<0.05$ ). Among stem traits, K, Ca, Mg, Mn, Zn, Fe, and Na were found significantly varying with changing seasons ( $P<0.05$ ). Among root traits, P, K, Mg, Mn, Zn, Cu, and Na were found significantly varying with season ( $P<0.05$ ) (Fig. 2, Table S2). Root traits showed maximum values in winter season (N, P, K, S, Ca, and Cu) while, stem traits showed maximum values in monsoon season (C, Mg, and Mn). Similarly, leaf traits also showed maximum values in monsoon season (Zn, Fe and Cu). Overall, the trait values for stem and root organs (C, P, S, Zn, Fe and Cu) decreased with elevation while leaf traits were found increasing with elevation, however, the differences were not significant throughout. Correlation plot among environmental parameters showed that elevation was negatively correlated with temperature ( $r=-0.51$ ,  $P<0.01$ ) and positively correlated with moisture content ( $r=0.45$ ,  $P<0.05$ ) (Fig. 3). Further, exploring the underlying environmental drivers that cause trait variation, multiple linear regression suggested that plant chemical traits at the leaf level were maximally affected by temperature followed by water availability (Table 1). However, for stem traits, we found that light availability followed by water availability significantly governed trait variation ( $P<0.05$ ). Among root traits, light availability and temperature were major drivers regulating trait variation in the study sites (Table 1).

*Trait variation across ecological scales*

Trait variation across ecological scales (Sites/Plots/Species/Individuals) revealed maximum variation occurring at the species level (~48%) followed by the individual level (~45%) (Fig. 4). Overall, species and individual levels together explained the total trait variance, irrespective of the traits considered because the contribution of plot level and site level variances were very small (observed only in Cu and S, respectively). The maximum variation at species level was observed for Ca (~78%) followed by Mg (~71%), K (60%), Mn (57%), Zn (~50%) and Fe (~50%). Further trait variation among plant types i.e., broad-leaved, and coniferous species showed that chemical traits significantly differed for leaf and stem organs except for N, S and Zn (Fig. 5). The average maximum trait values were found significantly greater ( $P < 0.05$ ) for broad-leaved tree species for N, K, S, Ca, Mg, Mn, and Zn in comparison to coniferous tree species (Fig. 5, Table S3). However, no significant differences were observed for C and P. Also, Fe, Cu and Na were reported significantly greater ( $P < 0.05$ ) in coniferous leaves.

*Trait coordination among plant organs and along resource gradient*

PCA plot representing trait coordination between leaf and stem traits for broad-leaved species showed less coordination as compared to coniferous species that showed relatively stronger trait coordination between organs (Fig. 6). Further, leaf and stem traits were correlated with the available resource gradient and was found that RI (Radiation Intensity) was negatively correlated with SCC, LCaC, LMgC, SPC, SKC, SCaC and SMgC and LMnC ( $P < 0.05$ ), whereas it was positively correlated with LCC, LKC, SNC, SSC and SZnC ( $P < 0.05$ ) for broad-leaved tree species. The MC (Moisture Content) was found negatively correlated with SMgC, SKC, SPC, SCaC, LNaC, LCaC and SCC ( $P < 0.05$ ) and positively correlated with LKC, LMgC and LZnC ( $P < 0.05$ ) (Table. 2). Among coniferous species, the correlation plot

depicted a stronger correlation among chemical traits (Fig. 6, Table 3). For instance, LNC was found positively correlated with LSC ( $r= 0.78$ ,  $P<0.01$ ). SSC was found positively correlated with SMgC ( $r= 0.59$ ,  $P<0.05$ ) and SNC ( $r= 0.58$ ,  $P<0.05$ ). The correlation plot revealed that broad-leaved traits were strongly coupled along with resource gradient whereas, coniferous traits displayed strong correlation between leaf and stem trait values (Fig. 6, Table 2, 3).

Soil nutrients such as C, Ca, Mg, Mn, Zn, Cu and Na were found to increase with elevation whereas, Fe showed a decreasing trend with elevation (Fig. S2). At the organ level, chemical traits were regressed against their respective soil nutrients (Fig. 7). Stem and root traits were strongly correlated with soil nutrient concentration, however, leaf traits (except Ca, Mg and Mn) failed to show significant relationships with soil nutrients implying a weaker degree of plant-soil coupling. For instance, regression analysis between soil carbon content and root carbon content showed a significant proportion of variance of 49% ( $P<0.05$ ) that reduced to 30% at stem level ( $P<0.05$ ) and was completely lost at leaf level. Similarly, soil nitrogen content and root nitrogen content indicated negative relation ( $r^2 = -0.29$ ,  $P<0.05$ ) which increased at stem level ( $r^2=0.35$ ,  $P<0.01$ ) and ultimately got weakened at leaf level. Soil Ca content showed a positive proportion of variance (77%) at root level ( $P<0.001$ ) which got reduced to only 13% with leaf traits. Overall, root chemical traits (C, N, P, K, Ca, Mg, Fe and Cu) were found to show a strong coupling with soil nutrient concentrations which was followed by stem traits (C, N, Zn and Na) and reduced significantly at leaf level.



## Discussion

We found stem and root traits to decrease with elevation while the opposite was observed for leaf traits. Considering seasonal variation, we found maximum values for root traits in winter season whereas leaf and stem traits showed maximum values in monsoon season. Leaf traits (Zn, Fe, Cu, and Na) were majorly driven by temperature whereas stem traits (Zn, Mg, and Mn) and root traits (P, Ca, Mg, Mn, and Fe) were majorly driven by light availability. This was in agreement with the initial hypothesis that plant chemical traits are strongly driven by topographical heterogeneity and associated resource availability. Trait variation across the ecological scales showed maximum variation at the species level (~48%) suggesting species variability to be an important factor causing trait variations. In the end, we found that trait coordination among organs was relatively stronger in coniferous species suggesting stronger adaptability to varied environmental conditions (Song et al., 2022) as compared to broad-leaved species. The study also suggested that trait variance with soil nutrients was maximum for root, followed by stem, which ultimately got diminished for the leaf. These differences indicate the variation in adaptation strategies used for resource utilization by the broad-leaved and coniferous species (Hikosaka et al., 2021).

*Trait variability among plant organs is majorly driven by light availability and temperature*

Trait values were generally found decreasing with an increase in elevation for stem and root, whereas the opposite was observed for leaf. The reported results agreed with studies that suggested a decrease in nutrient content with an increase in elevation. This concluded that species succession along the elevation decreases due to a reduction in the ability to assimilate C with an increase in environmental stress (Müller et al., 2016a, 2016b). Also, a decrease in temperature with increasing elevation limits the availability of resources for plant growth. Few studies have also reported an increase in leaf N and P content with increasing elevation

as an adaptation to enhance metabolic activity and growth rates with reduced temperature (Han et al., 2005). This was in line with our study that suggests leaf traits to be majorly driven by temperature and their trait values increased with elevation. We observed increased leaf N content during summer season, which could be attributed to higher rates of mineralization and nitrification driven by high temperature at lower elevation. Another contribution factor could be the enhanced nitrogen-fixing microbial activity and higher litter accumulation (Kumar et al., 2021; Zhang et al., 2013). Conversely lowest value in the winter season could be due to lower rates of mineralization and nitrification processes (Singh and Singh, 1986). Our study showed increased micronutrient concentrations during monsoon season, attributed to the availability of optimal temperature and moisture content during monsoon season (Kumar et al., 2021). We observed significant variation in micro-nutrients (Mn, Zn, Fe, Cu and Na) along elevation and season irrespective of organs while the opposite was observed for macronutrients. This showed that macro-nutrients required in higher amount experience less change and are impervious to modifications in the environment (Han et al., 2011). The nutrient uptake was greatly affected by the change in season suggesting the species to be very sensitive to changes in temperature, light, and water availability. Root traits were maximally affected by light and water availability indicating that species allocate a higher amount of nutrients in uptake machinery compared to photosynthetic machinery (Garkoti, 2012; Verma et al., 2021). This supports that light and water variability leads to increased nutrient allocation to belowground parts such as fine roots compared to above-ground organs (leaves and stems) (Verma et al. 2021). The statement holds true for our study since water availability was comparatively lower at lower elevations as compared to higher elevations. Light and water availability are directly linked to temperature in any region and thus growth rates of any plant species are dependent on the ability of the species to tolerate a range of stress (Singh and Verma, 2019). For instance, species growing in low temperatures (higher

elevations) expand their cell producing many smaller cells with increasing cell wall material per unit area and cell layers offering increased protection against cold stress (Poorter et al., 2009). Thus, plants at higher elevations have increased leaf nutrient content suggesting a greater construction cost in defence mechanisms against cold stress. However, there remains an inconsistency in the pattern of trait variations with increased topographical heterogeneity (Nambiar 1987) because of differences in micro-climatic conditions and resource availability.

*Species variability causes significant variation in plant chemical traits*

An inconsistency in the pattern of chemical trait variations along spatial and temporal scales led to the exploration of other factors such as forest/species types that causes trait variations. Variance partitioning clearly suggested that trait variation was maximum at the species level. Similar results were observed by (Vilà-Cabrera et al., 2015) for Mediterranean forests. They found maximum trait variability at the species and family followed by the population (individual) level, but a few traits were fairly distributed across all the organisational scales. High amount of trait variation at the species scale explains high trait conservatism among the species thus providing shreds of evidence for trait divergence in the region (Brodribb et al., 2012; Carnicer et al., 2013). We found a high proportion of intra-specific variation which could result from varied management activities such as lopping at lower and middle elevation zones especially because local people are dependent on forest resources for fuel and fodder purposes. Species such as *Quercus leucotrichophora*, *Pinus roxburghii* and *Cedrus deodara* abundant in lower and middle elevation sites are heavily lopped for livelihood purposes in the region. Therefore, variance partitioning for the chemical traits showed increased differentiation at the population level for C, N, P, S and Cu suggesting that external factors (lopping) are significantly affecting the trait variation across ecological scales. We found increased trait values for broad-leaved species in comparison to coniferous species. This

discrepancy might be attributed to the slower decomposition rate of coniferous leaves like chir pine having higher carbon-to-nitrogen ratio leading to slower release of nutrients (Ralhan and Singh, 1987). This high ratio also leads to the immobilization of available nitrogen from the soil. Since, broad-leaved species such as *Q. leucotrichophora* is a high nutrient demanding species, it fails to establish itself in sites having lower nutrient ((Singh et al., 1994). Furthermore, variation in trait values at species level is also governed by species abundance at the community level and associated trade-offs (Rawat et al., 2019; Tang et al., 2019). Such species are also known to be characterized by higher leaf construction costs and slower nutrient returns because of their over-utilisation for fuel and fodder purposes. This holds in alignment with our study showing increased leaf nutrient concentration in broad-leaved as compared to coniferous species. Broad-leaved species are known to possess conservative growth strategies promoting resistance of species to environmental stresses. In contrast, coniferous species such as *Picea smithiana* and *Abies pindrow* are known to have fast-growing and resource-acquisitive traits which indicate increased competitive ability (Singh, 2021). Thus, our results strongly support the inclusion of management activities while analyzing trait adaptability to environmental variability.

#### *Coniferous species showed relatively stronger trait coordination than broad-leaved*

Our results showed that trait coordination among coniferous species is relatively stronger than broad-leaved species. Moreover, we found that traits were strongly coupled along resource gradients for broad-leaved species. Such relation was not found for coniferous species indicating weaker coordination with resource availability implicating stronger adaptability and highly competitive nature in coniferous as compared to broad-leaved species (Zhang et al., 2022). Increased coordination among organs in coniferous species showed increased plasticity with topographical heterogeneity (Huo et al., 2021). For broad-leaved species, a strong correlation with soil water availability (MC) suggested increased nutrient

allocation in belowground parts, especially fine roots as a mechanism to fight against drought and resource scarcity (Körner, 2021; Sergeeva et al., 2021). Though this allocation might come at the cost of reduced biomass allotment to reproductive parts and photosynthetic organs (Ma et al., 2017). This was true in our case since we found reduced micro-nutrient contents in leaf organs as compared to root and stem organs.

Nutrients in plants primarily originate from the soil and senesced leaves. Soil nutrient concentration in this study strongly indicated a degree of soil nutrient transfer to the plant organs (Zhang et al., 2022). Trait-based trade-offs are likely to occur both within and across organs along a continuum ranging from (carbon and nutrient) acquisitive to conservative strategies (Wright et al., 2004). We found strong correlation with root traits followed by stem traits with their respective soil nutrient concentration. Our results were in line with a study conducted in subarctic flora integrating leaf, stem and root traits analyzing their coordination which revealed that traits exhibited a strong correlation in the region (Freschet et al., 2010). Another study conducted in Mediterranean region suggested traits among the organs to be coordinated along the conservative-acquisitive strategy scale (de la Riva et al., 2016). However, we found a weak correlation between environmental resources and leaf traits. This might be due to increased dependence on forest species for fuel and fodder purposes, especially *Q. leucotrochiphora* which is known to be heavily lopped for livestock feeding purposes (Verma and Mishra., 1999). Thus, along with the fine-scale topographical heterogeneity and varied resource availability, our results suggested strong coordination among organs in coniferous suggesting coniferous possess resource-acquisitive traits. However, broad-leaved showed a stronger correlation with the available resources suggesting the resource-conservative strategy possessed by them (Kawai and Okada, 2019). While at the organ level, our study suggests that topographical heterogeneity and varying environmental

resources cause strong trait variance at the root level which ultimately gets diminished at the leaf level due to external disturbances such as lopping.

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## Conclusion

The study concluded that increased chemical trait variation along elevational gradient was majorly driven due to light availability for stem (P, K, S, Ca, Mg, Mn, Zn, and Cu) and root traits (P, Ca, Mg, Mn, Zn, Fe and Cu) and due to temperature variability for leaf traits (S, Zn, Fe, Cu and Na). Root trait values were found highest in winter season, while leaf and stem traits were found maximum in monsoon season. Trait variability was found maximum at species followed by population/individual level. We found strong trait coordination among leaf and stem organs for coniferous whereas broad-leaved showed strong correlation with environmental resources. Thus, the maximum variation in chemical traits was majorly governed due to species variability, seasonal differences, and light and water availability. Trait correlation with respective soil nutrient concentration was found strongest for the root traits, followed by stem traits, which further got diminished at leaf level (except Ca, Mg and Mn) thus implying a weaker degree of plant-soil coupling. Weaker plant-soil interaction for leaf traits was majorly due to anthropogenic activities such as lopping in the lower and middle elevation zones since local people are dependent on forest resources for fuel and fodder purposes. We confirm that species variability and the associated resource availability cause considerable variation in above- and below-ground traits along the Himalayan mountains. The study also emphasizes inclusion of plant morphological and physiological traits along to facilitate the overall understanding of plant functional dynamics in such regions. The implications of such study could be beneficial in conserving the fragile mountain ecosystems in the Himalayas.

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## Figures

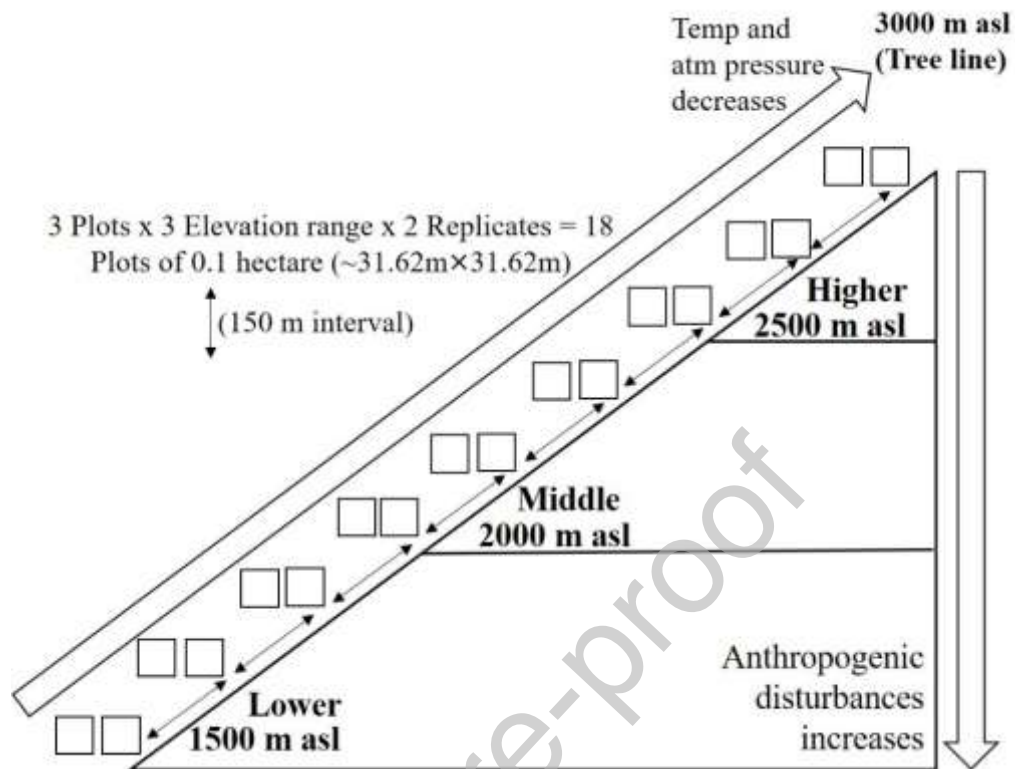
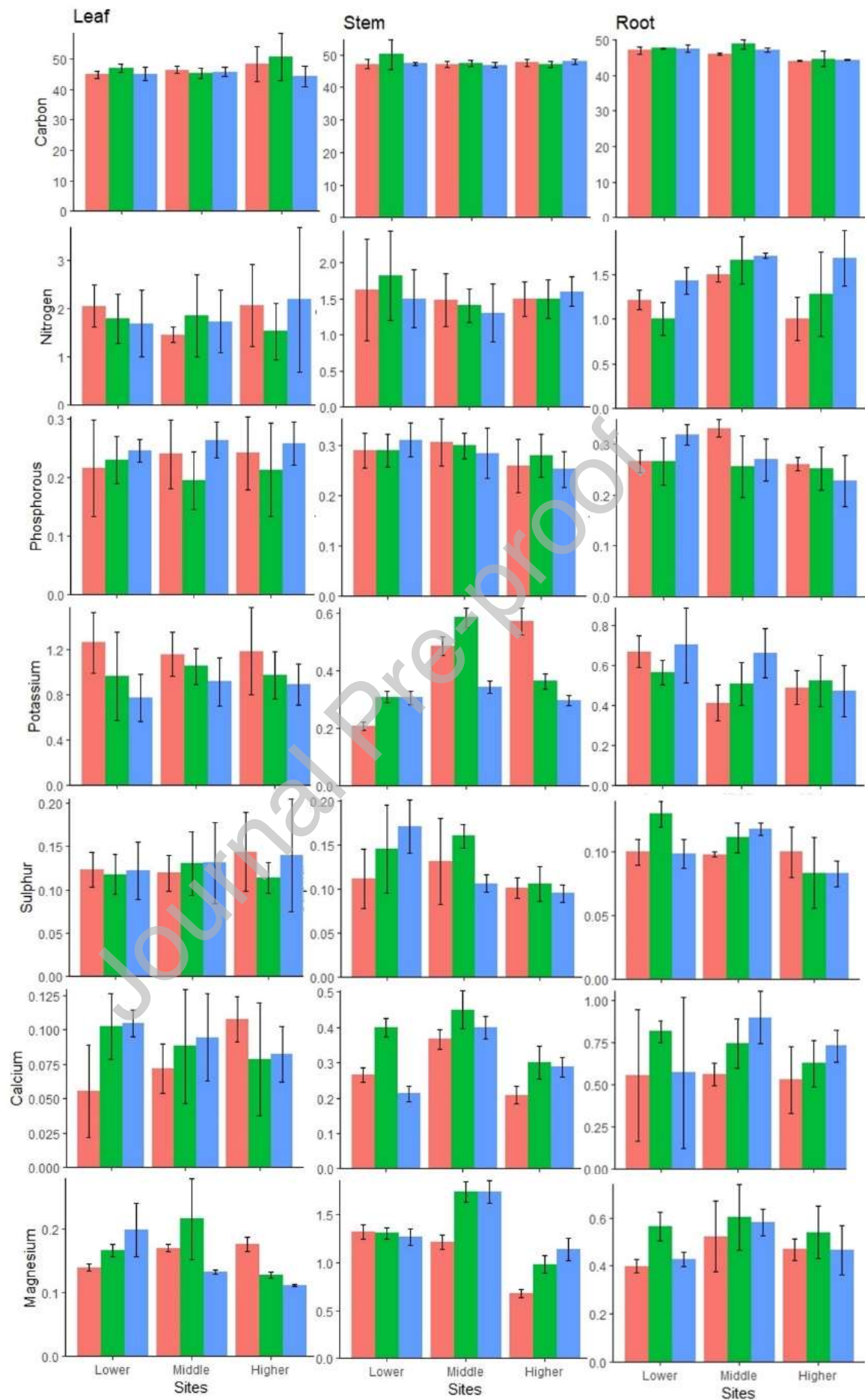


Fig.1. Schematic representation of sampling strategy in the study site. Temp refers to monthly average temperature and atm refers to atmospheric pressure.





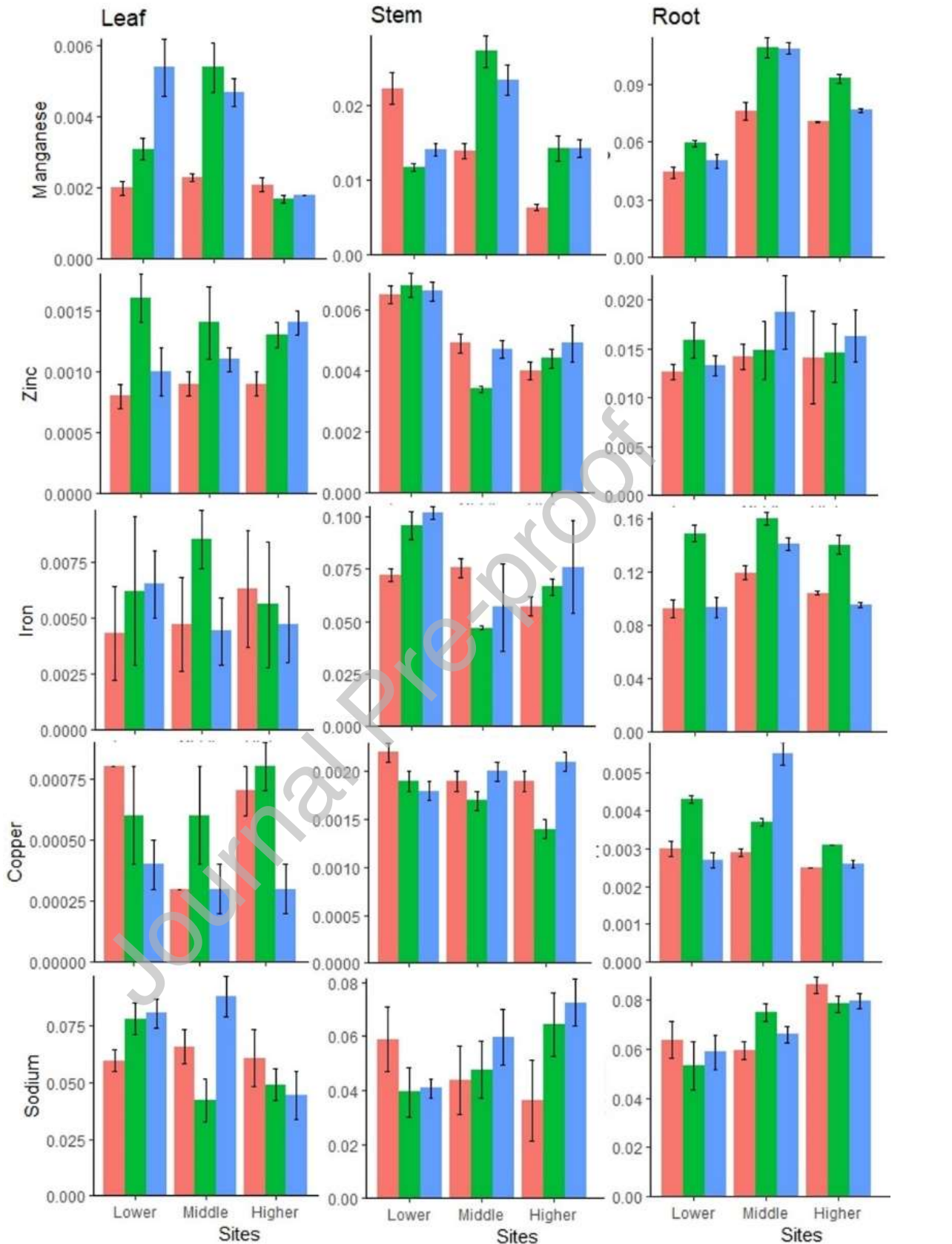
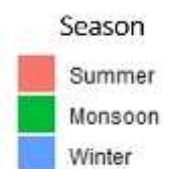


Fig. 2. Spatial and temporal variability of plant functional traits ( $\pm$ S.D.) Carbon (C, %), Nitrogen (N, %), Phosphorous (P, %), Potassium (K, %), Sulphur (S, %),



Calcium (Ca, %), Magnesium (Mg, %), Iron (Fe, %), Copper (Cu, %), Manganese (Mn, %), Zinc (Zn, %) and Sodium (Na, %) among organs of tree species with increasing elevation (Lower:1500-2000 m asl, Middle: 2000-2500 m asl and Higher: 2500-3000 m asl) and changing season (red colour indicates summer, green indicates monsoon and blue indicates winter season).



Fig. 3. Correlation among environmental factors among study sites. MC refers to Soil moisture content (water availability), Soil type represents soil texture (percentage of sand:silt:clay), RI refers to relative radiation intensity (light intensity) and temperature is the monthly average soil temperature.

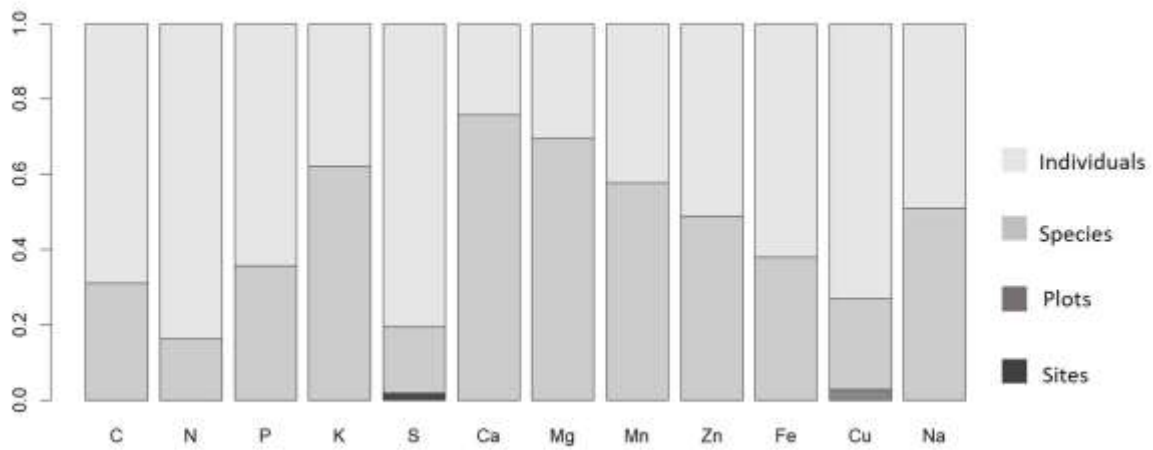


Fig. 4. Variance components of plant traits (leaf and stem traits) based on nested ANOVA across ecological scales including sites (variation among different sites), plots (variation among plots), species (variation among species) and individual level (variation among organs).

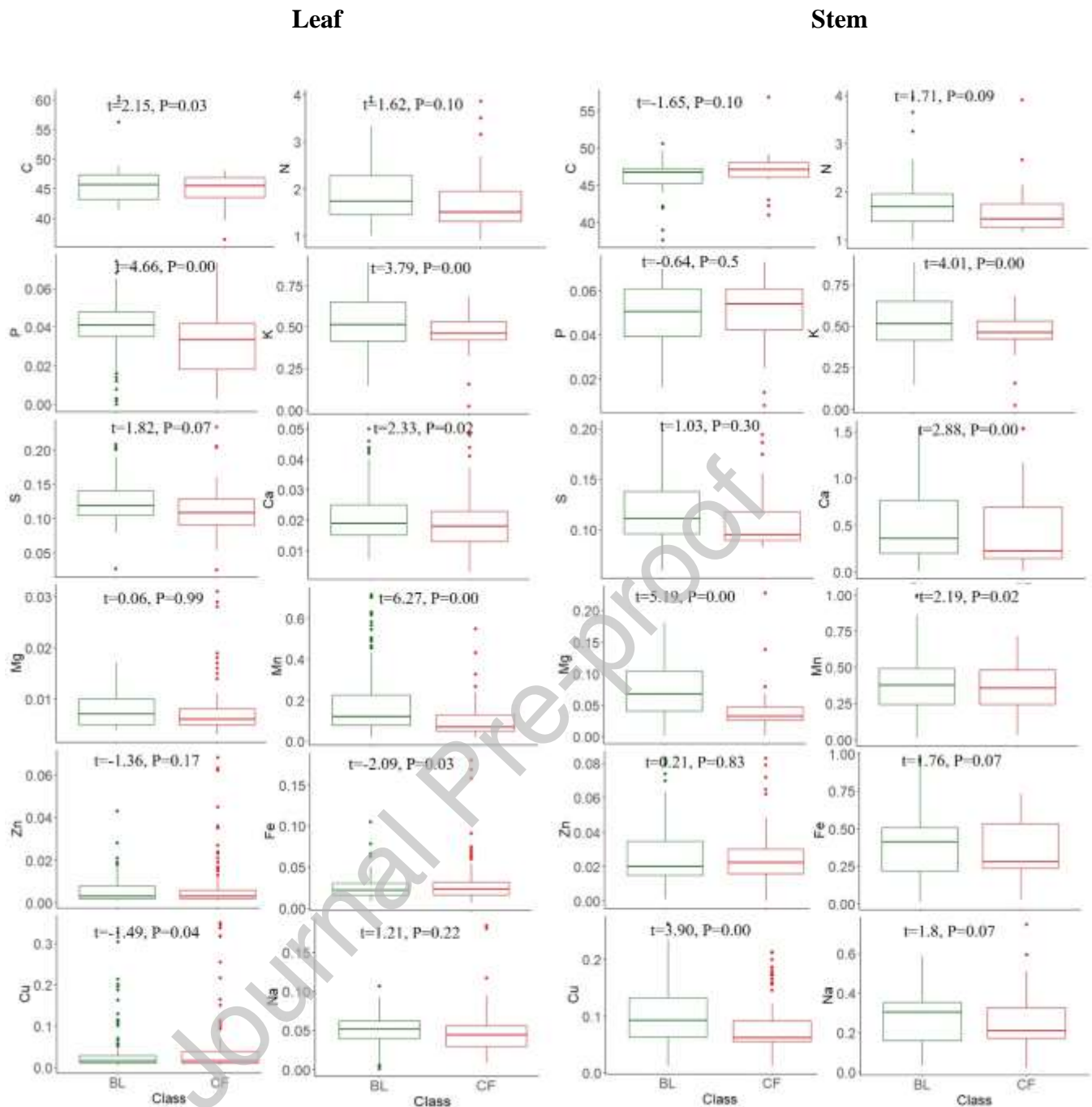




Fig. 5. Boxplots indicating differences between broad-leaved (BL, green) and coniferous (CF, red) tree species for leaf (left) and stem (right) chemical traits.

Class  
 BL  
 CF

The unit for each chemical trait is mentioned in Fig. 2. The trait values shown indicates overall variation in both broad-leaved and coniferous species. The limits of boxes indicate the first and third quartiles, and the horizontal line within boxes corresponds to the median. The upper whisker extends from the third quartile to the highest value within

$1.5 \times \text{IQR}$  (interquartile range) of the third quartile. The lower whisker extends from the first quartile to the lowest value within  $1.5 \times \text{IQR}$  of the first quartile. Box colours indicate the different classes (broad-leaved versus coniferous) and the circles are outliers. Test statistics indicate significant differences between BL and CF species based on t-test and P-values.

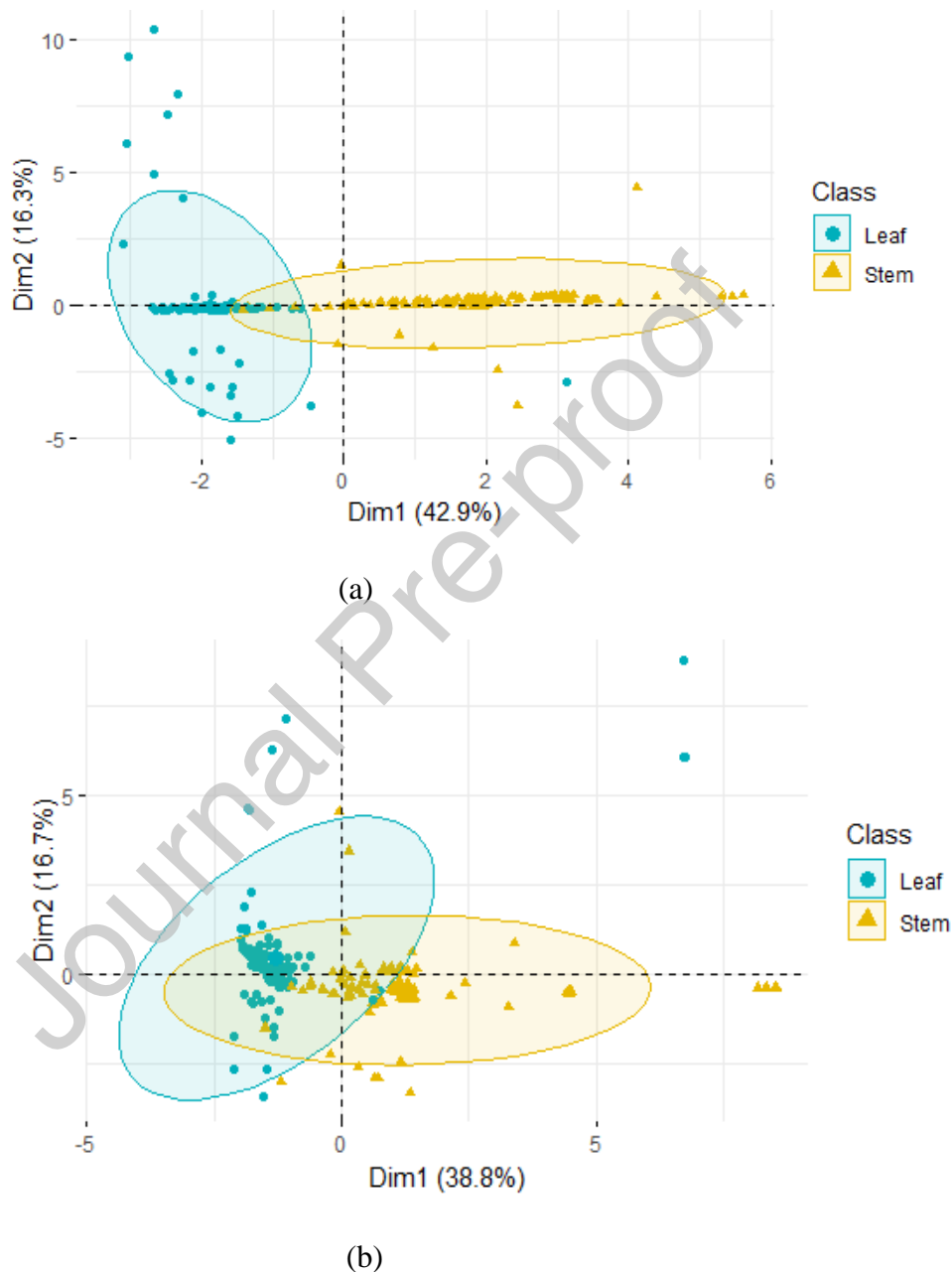


Fig. 6. Principal Component Analysis (PCA) of plant organs: Leaf (blue) and stem (yellow) for (a) broad-leaved (BL, above) and (b) coniferous (CF, below) species representing plant chemical trait coordination.

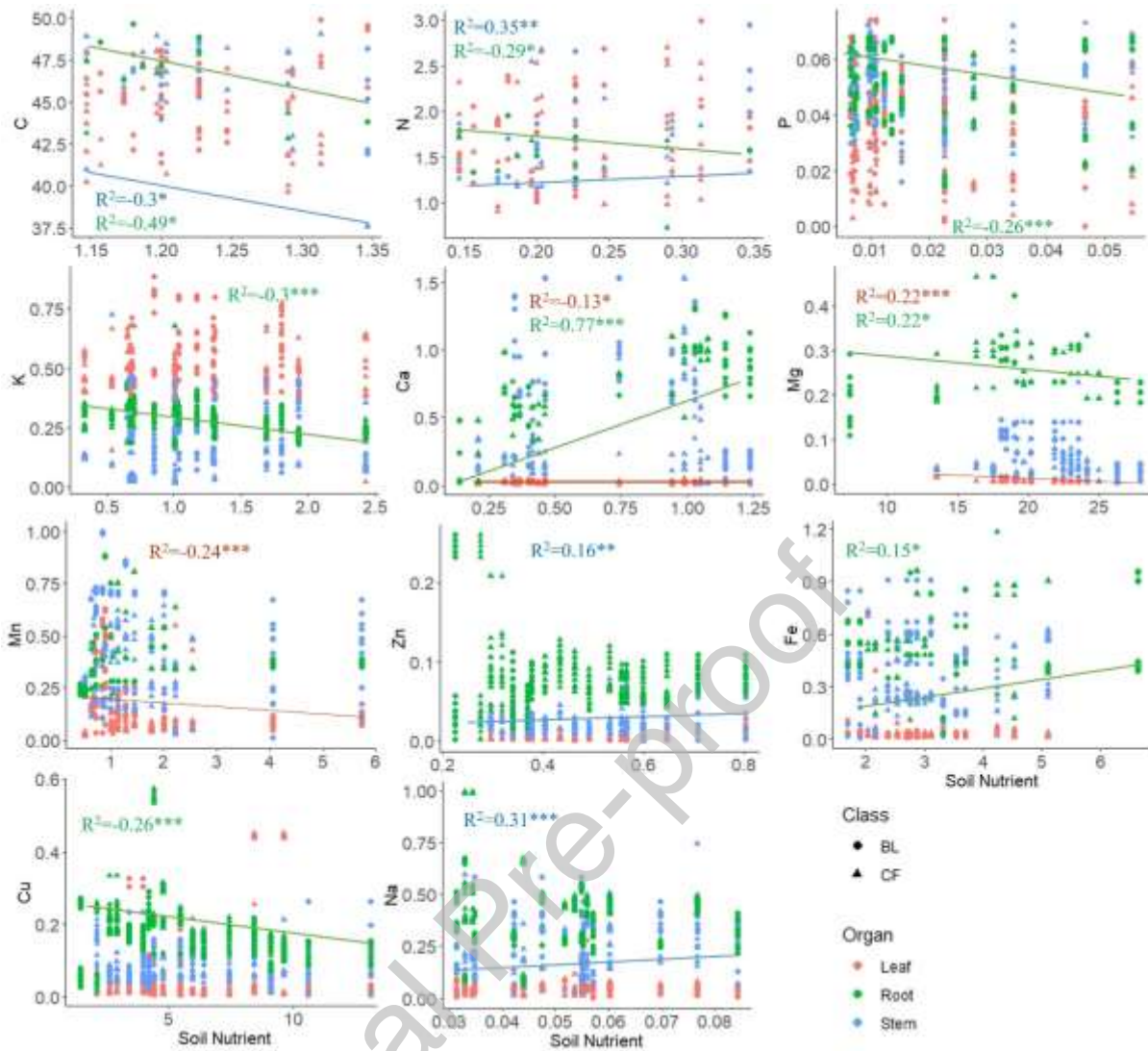


Fig. 7. Relationship between plant chemical trait concentrations of different organs and their respective soil nutrient concentrations for broad-leaved (BL, denoted with solid circles) and coniferous (CF, denoted with solid triangles) species: Leaf (red), stem (blue) and Root (green) for broad-leaved and coniferous species. Carbon (C), Nitrogen (N), Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Manganese (Mn), Zinc (Zn), Iron (Fe), Copper (Cu), Sodium (Na). The trait values are shown in percentage. Each point in the figure represents averaged trait values for leaf, stem, and root for three seasons. The regression lines were plotted for significant relationships with  $p < 0.05$ . Significance codes: '\*\*\*' 0.001, '\*\*' 0.01, '\*' 0.05.

Table 1. Results of multiple linear regression showing the effects of environmental factors.

Traits	Intercept		Light availability		Water availability		Soil type		Temperature	
	t-value	P-value	t-value	P-value	t-value	P-value	t-value	P-value	t-value	P-value
<b>(Leaf)</b>										
C	3.09	<b>0.00</b>	1.49	0.14	0.52	0.60	1.29	0.19	-1.38	0.17
N	0.43	0.66	0.92	0.36	-0.66	0.51	-0.12	0.90	-1.19	0.23
P	1.15	0.24	0.69	0.48	1.27	0.20	-0.04	0.96	-0.45	0.64
K	1.57	0.11	-0.67	0.49	2.73	<b>0.00</b>	0.90	0.36	0.91	0.35
S	1.61	0.11	1.00	0.32	-1.23	0.22	0.70	0.48	-2.27	<b>0.02</b>
Ca	-1.17	0.24	-0.47	0.63	0.21	0.82	-1.22	0.22	0.59	0.55
Mg	0.72	0.47	-1.07	0.28	-2.05	<b>0.04</b>	0.07	0.93	-1.71	0.08
Mn	1.78	0.07	-3.50	<b>0.00</b>	-1.21	0.22	1.62	0.10	0.27	0.78
Zn	0.07	0.93	-0.22	0.82	-1.74	0.08	-0.91	0.36	-3.06	<b>0.00</b>
Fe	-0.04	0.96	0.99	0.32	-1.18	0.23	-0.79	0.42	-2.32	<b>0.02</b>
Cu	0.91	0.35	-0.49	0.62	-2.73	<b>0.00</b>	0.23	0.81	-2.47	<b>0.01</b>
Na	0.97	0.33	-0.78	0.43	-2.84	<b>0.00</b>	0.20	0.83	-2.56	<b>0.01</b>
<b>(Stem)</b>										
C	1.39	0.17	-1.62	0.11	2.18	<b>0.04</b>	-0.13	0.89	1.20	0.24
N	-1.48	0.15	-1.94	0.06	1.18	0.24	-1.60	0.12	0.94	0.35
P	0.28	0.77	-5.05	<b>0.00</b>	1.58	0.11	-0.55	0.57	5.80	<b>0.00</b>
K	-1.22	0.22	-3.5	<b>0.00</b>	-1.40	0.16	-2.32	<b>0.02</b>	-2.21	<b>0.02</b>
S	-2.07	<b>0.05</b>	-2.41	<b>0.02</b>	-0.46	0.64	-2.51	<b>0.02</b>	0.36	0.72
Ca	-1.8	0.06	-7.43	<b>0.00</b>	-1.76	0.07	-2.18	<b>0.02</b>	1.21	0.22
Mg	1.49	0.13	-7.00	<b>0.00</b>	-2.45	<b>0.01</b>	1.27	0.20	1.47	0.14
Mn	2.24	0.002	-6.48	<b>0.00</b>	-2.15	<b>0.03</b>	1.96	<b>0.05</b>	0.35	0.72
Zn	-1.42	0.15	3.33	<b>0.00</b>	2.82	<b>0.00</b>	-1.52	0.12	1.36	0.17
Fe	1.22	0.22	0.85	0.39	-2.73	<b>0.00</b>	0.58	0.55	-1.57	0.11
Cu	-1.03	0.30	2.46	<b>0.01</b>	-1.36	0.17	-1.63	0.10	0.54	0.58
Na	0.07	0.94	0.12	0.90	-1.85	0.06	-1.49	0.13	-3.61	<b>0.00</b>
<b>(Root)</b>										
C	2.80	0.06	-2.2	0.11	0.11	0.91	0.92	0.42	1.50	0.23
N	-0.23	0.83	-1.26	0.29	-0.68	0.54	-0.30	0.78	0.81	0.47
P	2.95	<b>0.00</b>	-4.87	<b>0.00</b>	0.62	0.53	2.40	<b>0.01</b>	4.53	<b>0.00</b>
K	-1.38	0.16	-0.64	0.52	4.57	<b>0.00</b>	-1.24	0.21	5.39	<b>0.00</b>
S	0.09	0.93	-0.70	0.53	-2.08	0.12	-0.32	0.76	0.19	0.85
Ca	2.20	<b>0.02</b>	-7.48	<b>0.00</b>	3.00	<b>0.00</b>	1.50	0.13	-0.59	0.55
Mg	4.63	<b>0.00</b>	-9.61	<b>0.00</b>	-0.50	0.61	3.44	<b>0.00</b>	-2.04	<b>0.04</b>
Mn	9.43	<b>0.00</b>	-7.28	<b>0.00</b>	-4.40	<b>0.00</b>	7.99	<b>0.00</b>	-5.29	<b>0.00</b>
Zn	0.03	0.97	1.97	<b>0.04</b>	-0.77	0.43	-0.42	0.67	0.34	0.73
Fe	3.55	<b>0.00</b>	-3.26	<b>0.00</b>	-3.69	<b>0.00</b>	2.10	<b>0.03</b>	-4.33	<b>0.00</b>
Cu	-0.78	0.43	-6.27	<b>0.00</b>	0.49	0.61	-0.86	0.38	3.05	<b>0.00</b>
Na	0.211	0.83	-1.16	0.24	0.31	0.75	-0.86	0.38	-2.11	<b>0.03</b>

Test statistics represent t-test values (coefficients divided by standard errors) and P-value

<0.05 (highlighted in bold). Light availability is denoted by Relative radiation intensity,

Water availability is denoted by soil moisture content, Soil type (texture) and Temperature is

denoted as monthly average soil temperature.











**Credit Author Statement**

**Shipra Singh:** Conceptualization, Formal analysis, Writing - Original Draft. **Abhishek K.**

**Verma:** Data curation, Formal analysis. *Satish Chandra Garkoti:* Writing - Review & Editing Supervision.

**Declaration of interest**

There is no potential conflict of interest among the authors.

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