



Functional leaf traits indicate phylogenetic signals in forests across an elevational gradient in the central Himalaya

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Abstract

Traits are the primary attributes that distinguish a species niche. Species and higher taxa are part of a structured phylogeny, and variation in plant traits depends on lineage as well as on environmental conditions. Therefore, it is crucial to investigate linkages between taxonomic identity, shared ancestry, and environment for understanding the variation in leaf traits. We investigated the evolutionary relationships, based on multiple gene sequences among 26 plant species sampled along an elevational gradient from 650 to 3600 m a.s.l. in the central Himalaya. We tested for the phylogenetic signal based on three different measures in 10 leaf traits having a significant association with the resource acquisition-conservation trade-offs axis and influencing plant growth, development, and ecological performance. We further assessed the role of elevation and growth forms as the potential drivers of leaf traits variation while controlling for phylogeny. 5 out of 10 leaf traits showed significant phylogenetic signal. Plant species clustered more often by growth forms at the tips of the phylogeny indicating multiple instances of independent evolution. Evergreen taxa showed niche separation with deciduous and incorporated larger trait variation. Trait variations were guided by both growth forms and elevation when accounted for phylogeny. Growth form has a higher contribution to trait variation compared to elevation. Trade-offs were detected between resource conservation and resource acquisition machinery traits (that would maximise carbon gain), differing between growth forms and along elevation.

Keywords Adaptive traits · Carbon gain · Elevation · Growth forms · Phylogeny · Resource acquisition · Resource conservation

Introduction

The occurrence of a species in an ecosystem is the outcome of various adaptive strategies by the species to manage and overcome abiotic and biotic constraints (Baraloto et al. 2012; Chesson 2000; Silvertown 2004; Valladares et al. 2015). The concept of functional traits has been widely applied to understand the key ecological strategies by which plants modulate and adapt to their environment to fulfil their

ecological requirements (Li et al. 2015). Traits are the primary attributes that distinguish a species niche and are liable to change, both at an evolutionary scale and at various stages of species development (Burns and Strauss 2012; Donovan et al. 2011). Trait changes could occur directly due to genetic control or indirectly due to biotic interactions (Burns and Strauss 2012).

Plant functional traits involved in the exchange of gas and water by leaves have received considerable attention due to their crucial role in ecosystem productivity and ecological function (Ackerly 2004; Li et al. 2015; Reich 2014; Westoby et al. 2002). Consequently, the worldwide leaf economic spectrum (WLES), a set of correlated leaf traits covering multiple niche dimensions, was developed (Donovan et al. 2011; Reich 2014; Reich et al. 1999; Westoby et al. 2002). WLES effectively summarizes trade-offs between investment and benefit in leaf construction and carbon fixation, respectively (Kikuzawa 1991, 1995; Reich 2014; Reich et al. 1999). At one end, the spectrum represents species with faster investment return with high specific leaf area,

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high leaf nitrogen content and shorter leaf lifespan, whereas, at other end species with slow investment return with low specific leaf area, low nitrogen content, and longer leaf lifespan are present (Onoda et al. 2011; Reich 2014; Wright et al. 2004).

Species and higher taxa, however, are not phylogenetically independent but a part of hierarchically structured phylogeny (Felsenstein 1985). Therefore, it is important to focus on the links between taxonomic identity, shared ancestry, and environmental conditions to understand the variation in leaf traits (Ackerly 2003, 2009; Losos 2008). Broadly, two processes governed by the same forces, albeit in opposite directions, are attributed to trait evolution (Ackerly 2009). First, adaptive radiation is rapid diversification of a lineage and its traits to take advantage of novel ecological niches, provided by events such as colonization and transition to native adaptive zones, or in response to various biotic interactions (especially competition). In contrast, niche conservatism, the second process, is the degree to which organisms retain their niches and ecological traits in space and time (Ackerly 2009). Phylogenetic comparative methods (PCMs) that evaluate trait variation by taking phylogenetic relatedness among the species are most suitable for assessing phylogenetic nonindependence (Cooper et al. 2010).

Mountains with steep elevational gradients provide natural climatic variation to which plants adapt and adjust their performance through variation in its traits. As a result, these mountains serve as excellent eco-evolutionary landscapes for understanding trait variations in response to environmental changes (Chun and Lee 2017; Gerhold et al. 2015; Read et al. 2014; Xu et al. 2017). Generally, low elevation with the longer favourable season, high temperature, and high resource availability tends to favour acquisitive resource strategies to help individuals to face higher competition for the resources (Read et al. 2014). Conversely, high elevation characterised by short favourable season, low temperature and lower competition among plant species push them to invest more towards resource conservation machinery (Read et al. 2014; Reich et al. 1997). Apart from the elevation gradient, traits also vary according to the growth forms (Chabot and Hicks 1982; Givnish 1988; Kikuzawa 1991). In general, deciduous species exhibit a more resource acquisitive leaf strategy with higher leaf nitrogen, lower leaf mass per unit area. In contrast, evergreens exhibit conservative resource strategies such as longer leaf span, a lower percentage of leaf nitrogen, high leaf mass per unit area (Reich et al. 1999). In the present study, we assessed variation in the functional leaf traits of the selected tree species with contrasting growth forms (evergreens versus deciduous) taking into account the shared evolutionary relationships across an elevation gradient in the central Himalaya. Himalaya in the past has served as a biological corridor for the immigrant taxa which have diversified due to major geophysical upheavals associated

with Himalayan orogeny (Manish and Pandit 2018a; Singh and Singh 1987). Our primary focus was to test the hypothesis that variation in the functional leaf traits in the sampled tree species, attuned to maximise photosynthetic gains, is guided by both growth forms and elevation when controlled for phylogeny.

Our main objectives were to: (1) investigate variations in leaf traits of the sampled tree species distributed across an elevation gradient in the central Himalaya, (2) investigate the evolutionary relationships among the sampled tree species and determine the magnitude of phylogenetic signals in the leaf functional traits, (3) understand the relative role of elevation and growth form in driving variation in the leaf traits with phylogeny taken into account.

Materials and methods

Study area and sampling

This study was carried out in the Indian central Himalaya across an elevational range from 650 to 3200 m a.s.l. with a steep bioclimatic gradient. The elevational gradient traverses climatic zones from subtropical at low elevations to temperate and subalpine at intermediate and high elevations. Clouds generally form above 1400 m a.s.l. but more frequently above 2200 m a.s.l. Mid-elevation slopes, i.e. 1100–2000 m a.s.l., generally receive the highest rainfall (Bhattarai and Vetaas 2003; Carpenter 2005; Singh 2014). The year is divisible into three seasons: a relatively dry and cold winter (mid-December through February), warm and dry summer (from mid-April to mid-June), warm and wet rainy season (mid-June to mid-September). The most notable feature of the climate is the distinct monsoonal pattern of rainfall with about 3/4th of the annual rainfall (1500–2500 mm) concentrated in the rainy season (Singh 2014).

The study area was divided into three elevation bands : low (≤ 1000 m a.s.l.) ($30^{\circ} 12' 6.03''$ N– $30^{\circ} 13' 14.8''$ N and $78^{\circ} 47' 30''$ E– $78^{\circ} 40' 36''$ E), intermediate (1100–2000 m a.s.l.) ($30^{\circ} 31' 36.7''$ N and $30^{\circ} 32' 34.5''$ N and $79^{\circ} 64.200'$ E– $79^{\circ} 7' 23.9''$ E), and high (≥ 2100 m a.s.l.) ($30^{\circ} 29' 45''$ N– $30^{\circ} 29' 54''$ N and $79^{\circ} 12' 45''$ E– $79^{\circ} 13' 24''$ E). These regions are highly vulnerable to landslips and landslides, with large accumulations of debris from fallen and fractured rocks. Sampling sites were in natural and relatively undisturbed forest with minimal anthropogenic activities.

We established five relatively undisturbed study sites of 0.1 hectare at each elevation. A total of 26 tree species representing 15 families, with a sufficient number of individuals (minimum 10) in the same age group, were chosen for the study. Age group was taken into account to minimize the effect of age differences on intraspecific variations in the trait measurements (Liu et al. 2019).

Functional leaf traits

We selected a total of 10 leaf functional traits based on their significant association with the resource acquisition-conservation trade-offs axis and has important influence on plant growth, development and ecological performance. The selected leaf traits were: LA = Leaf area (cm^2), LL = Leaf life-span (days), LDM = Leaf dry mass (g), LMA = Leaf mass per unit area (g cm^{-2}), LEP = Leaf expansion period (days), DML = Dry mass loss (%), LnM = Leaf nitrogen in mature leaves (mg g^{-1}), LnS = Leaf nitrogen in senesced leaves (mg g^{-1}), NRE = Nitrogen resorption efficiency (%), LmRS = Leaf mass accumulated before rainy season as a fraction of leaf mass of matured leaves (%). Thirty sunlit leaves were marked in the selected individuals (10–15 in number) of each species at the time of budburst for measuring leaf expansion and leaf longevity. All marked leaves were in the middle of crowns on south-facing slopes. Leaf area (LA) was measured using an LI-3000 A leaf area meter (LI-COR, Nebraska, USA), and were assigned to a particular size classes following Raunkiaer's classification (Gillison 2006; Raunkiaer 1934). LA is considered an important functional trait as it approximates water-energy balance of the plants. Leaf expansion period (LEP), defined as the interval (in days) between the day of budburst and the day when no further expansion in leaf area is observed, was measured weekly. Leaf life span (LL, days) was calculated based on the average number of days the leaf remained in the canopy since budburst (Athokpam et al. 2014). Other leaf traits were measured on approximately 1000 more leaves tagged separately on another 5–10 individuals (approx. 25 leaves per individuals) with similar canopy positions, following (Cornelissen et al. 2003).

Traits such as leaf dry-mass (LDM) and leaf mass per unit area (LMA), were measured on fully expanded leaves from 10:00 to 12:00 to minimize diurnal variations, following Cornelissen et al. (2003). LMA generally serves as a proxy for the cost of construction of leaves and light interception. Loss of dry mass (DML) was calculated as the fractional loss of the dry mass of mature leaves from the dry mass of senesced leaves (Negi 2006; Ralhan and Singh 1987). A higher DML prior to leaf senescence alludes to lesser dependence on soil resources. Further, leaves were oven-dried, powdered, and digested using the wet-ash nitric acid/perchloric acid procedure. Nitrogen (N) content was estimated in mature leaves (LnM) and senesced leaves (LnS) using the micro-Kjeldahl technique. N-resorption efficiency (NRE), defined as the percentage withdrawal of N mass from senesced leaves, was calculated using a standard formula (Killingbeck 1996; Yuan et al. 2005). A higher NRE refers to internal re-translocation of nitrogen and thus reduce the dependency on soil nitrogen. Leaf mass before the rainy season (LmRS) is the fraction of leaf mass of the mature leaves

gained prior to the arrival of monsoon season. LmRS is an important leaf trait as it underlies the synchronisation of leaf growth with monsoon rains which accounts for more than 3/4th of total rainfall in the Himalaya.

The data for the leaf traits were assessed for normality using Shapiro-Wilk's test, which is based on the correlation between the data and the corresponding normal scores. Variables that differed significantly from normality were either natural log-transformed (LL, LMA, LEP, DML, and LmRS) or square-root transformed (LA and LDM) to avoid negative values.

Plant phylogeny

We downloaded sequences from NCBI for the matK, rbcL, and 5.8 S genes, when available, for inferring the phylogenetic relationships among the 26 tree species. Sequences for each gene were aligned using ClustalW and were then concatenated. The concatenated alignment was trimmed using trimAl (Capella-Gutierrez et al. 2009) with the option 'automated1' using the Phylemon 2.0 web server (Sánchez et al. 2011; <http://phylemon.bioinfo.cipf.es>). The program jmodeltest2 was used to identify the evolutionary model and other parameters that best accounted for the alignment data. The TVM+I+G model with a proportion of invariant sites (0.176 invariant sites) and rates at other sites varying in a gamma distribution (gamma shape = 0.720, number of discrete gamma categories = 4) best described the sequence data based on the Akaike information criterion corrected for small sample sizes (AICc). The phylogenetic relationships among the tree species was reconstructed using PhyML (Guindon et al. 2010) with 1000 bootstraps to assess the confidence of the nodes (Fig. 1).

Phylogenetic signals of the leaf traits

The phylogenetic tree was rooted with the fern species *Psilotum nudum*, but this species was not included in the data set for trait analysis. Phylogenetic signals represent trait variations due to phylogeny. Our null hypothesis is that traits evolved independent of phylogeny. To test the null hypothesis, phylogenetic signals were identified using three statistical indices to quantify the influence of phylogeny. These three measures were Pagel's λ (Pagel 1999), Blomberg's K (Blomberg et al. 2003), and phylogenetic autocorrelation measured using Abouheif's C_{mean} (Abouheif 1999) and were tested using the R package phylosignal (Keck et al. 2016). Both Pagel's λ and Blomberg's K assume a random-drift model of trait evolution, and C_{mean} is an autocorrelation index not based on an evolutionary model.

Pagel's λ represents a quantitative estimate of the phylogenetic signal for a trait and varies between zero and

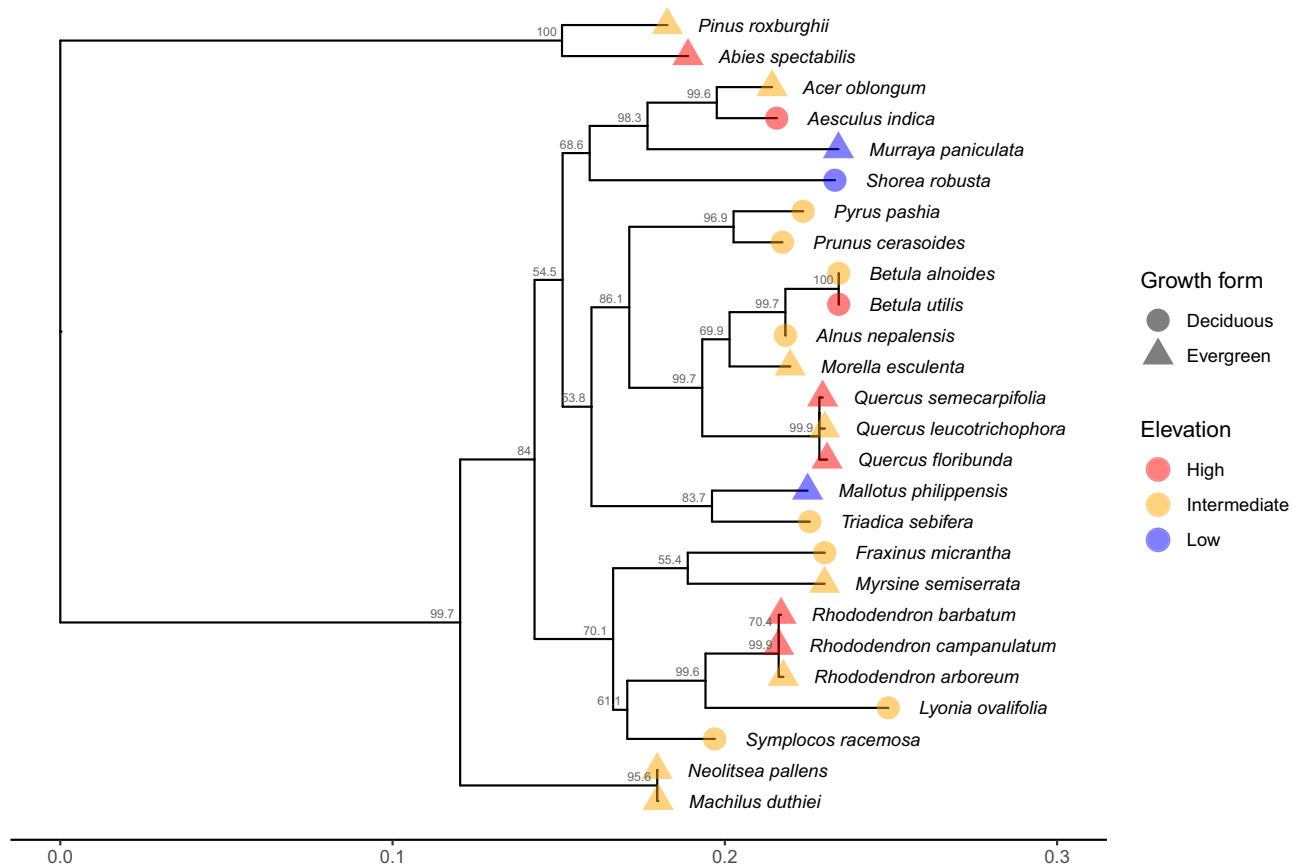


Fig. 1 The Phylogenetic tree with growth characteristics and elevation category. Node values are bootstrap support from 1000 bootstraps. Phylogenetic tree was constructed based on 3 gene sequences (matK, rbcL, 5.8 S)

one. A value of $\lambda = 0$ indicates that the trait has evolved independently of phylogeny, i.e. average trait values are not more similar between close relatives than between distant relatives. A value of $\lambda = 1$ represents a strong phylogenetic signal, i.e. trait evolution by the Brownian-motion model, indicating random genetic drift. Intermediate values of λ suggest that traits may have evolved by a process other than random drift, despite phylogenetic influence (Kamilar and Cooper 2013; Pagel 1999). Blomberg's K is also based on the assumption of random drift in trait evolution. K, however, varies from zero to infinity, with $K = 0$ suggesting that trait evolution is independent of phylogeny and $K = 1$ indicating that trait evolution conforms to the phylogeny under the Brownian-motion model. Values of $K > 1$ indicate stronger similarity between close relatives than expected under random drift (Blomberg et al. 2003; Kamilar and Cooper 2013). C_{mean} is an autocorrelation measure of the covariation of trait values across species relative to the phylogenetic distances between the species. $C_{\text{mean}} = 1$ indicates strong similarity across close relatives, and $C_{\text{mean}} = -1$ indicates strong negative associations between species and trait similarity (Abouheif 1999).

Statistical analysis

We assessed the phylogenetically independent correlations among leaf by constructing a phylogenetic Pearson correlation matrix using the *phyr* R package and the *cor_phylo* function (Li et al. 2020). The significance of correlations for all unique pairs of traits was tested using the *psych* R package and the *corr.p* function (Revell 2020). A phylogenetic PCA was conducted using the *phyl.pca* function in the *phyltools* package (Revell 2009, 2012) after centering and scaling the data for functional leaf trait. Phylogenetic principal components (PCs) provide estimates of the eigen structure that will have lower variance relative to nonphylogenetic procedures, reducing type 1 errors (false positives) relative to a regular PCA when scores are subsequently analyzed using phylogenetic methods (Revell 2009).

A composite variable in the form of a phylogenetic primary-axis species score (PPASS) was created by extracting the first component from the respective PCAs to account for maximum trait variations and the influence of phylogeny. Traits variation are not phylogenetically independent, so PGLS models were constructed using the *caper* R package

(Orme et al. 2018). PPASS was set as the dependent variable, and elevation and growth form were set as predictor variables. λ was estimated using maximum likelihood. Figures were made using the ggplot2 (Wickham 2016) and ggtree (Yu et al. 2017) and corplot (Wei and Simko 2017) R packages. An analysis of variance (ANOVA) of the PGLS model was conducted to examine the overall effect of the categorical variables.

Results

Ecology of the himalayan tree species and the evolutionary relationships

In the present study, 26 tree species sampled represented 15 families and were distributed unevenly in an inverted J-shaped pattern. Intermediate elevation had the maximum tree species followed by high and low elevation. At each elevation, the evergreens' frequency was higher than the deciduous tree species (Table S1). Out of 26 tree species sampled among the growth forms, 15 tree species were evergreen while 11 were deciduous. Further, tree species belonging to families Fagaceae, Ericaceae, Betulaceae assorted across intermediate to high elevation (Table S1). The phylogeny based on the multiple gene sequences similarity shows that species shared the evolutionary history (matK, rbcL, and 5.8 S) and revealed the clustering of taxa, more commonly at the tips of the tree than at deeper nodes (Fig. 1). The coniferous species *Pinus roxburghii* and *Abies spectabilis* formed a monophyletic clade distinct from all other species. Taxa from the same family clustered more often by growth form than exclusively by elevation. For example, taxa representing family Pinaceae, *P. roxburghii* and *A. spectabilis*; three *Quercus* species of family Fagaceae; *Betula alnoides*, *Betula utilis*, and *Alnus nepalensis* of family Betulaceae; and three *Rhododendron* species of family Ericaceae clustered by growth form and assorted along intermediate-to-high elevations. Both growth form and elevation, however, governed the clustering for some cases, e.g. the clustering of *Pyrus pashia* with *Prunus cerasoides* (Family: Rosaceae) and *Neolitsea pallens* with *Machilus duthiei* (Family: Lauraceae).

Family Fagaceae, which included three *Quercus* species, was paraphyletic with the family Betulaceae represented by *Alnus nepalensis*, *Betula alnoides*, *Betula utilis* and *Myrica esculenta* and distributed across intermediate to high elevation. *P. pashia* and *P. cerasoides* belonging to family Rosaceae formed a monophyletic clade. Family Ericaceae, though, diverged into evergreen *Rhododendron* species and the deciduous *Lyonia ovalifolia* was distributed among intermediate-to-high elevations. *Symplocos racemosa*, an intermediate-elevation deciduous species, was paraphyletic

with the deciduous *L. ovalifolia* and the evergreen *Rhododendron arboreum*, both at intermediate elevations.

Assessment of phylogenetic signal in leaf traits

Out of 10 leaf functional traits taken for the study, 5 leaf traits had significant phylogenetic signal based on three different measures for detecting signals. Leaf area (LA), which approximates the water-energy balance, and leaf mass per unit area (LMA), a surrogate for the cost of constructing leaves and intercepting light, had strong phylogenetic signals (Pagel's λ , Table 1), with $\lambda = 0.727$ for LA and $\lambda = 0.914$ for LMA. LA and LMA also had a significant C_{mean} , indicating a significant positive autocorrelation between close relatives (Table 1). Leaf expansion phase (LEP), which underlies maximizing resource capture during favourable conditions, and leaf nitrogen in a senesced leaf (LnS), had significant phylogenetic signal based on C_{mean} . Leaf dry mass (LDM), had a significant phylogenetic signal (Blomberg's K, Table 1).

Phylogenetic PCA

Any trait used in a comparative analysis should be phylogenetically corrected to remove pseudo-replication from current or downstream analyses (Revell 2009). The PPCA was fitted with a maximum-likelihood value of $\lambda = 0.547$, and PPC1 and PPC2 explained 29.5 and 26.2% of the total variance of the leaf traits, respectively. Evergreen and deciduous species distinctly grouped along PC1 (Fig. 2). Evergreen species covered the full range of PC2, but deciduous species were more localized. The loading plot shows the groupings and relationships between the various foliar traits according to species elevation category and growth form (Fig. 3).

The grouping of LnM, LmRS, and LnS and their close relationships are visible in the third quadrant of the loading plot, along with lesser associations between other traits in the first, second, and fourth quadrants. The grouping of LnM, LmRS, and LnS in the third quadrant is important, because these traits had nonsignificant phylogenetic signals (Table 1). The first PC that captures the major variation from the leaf trait variables is dominated by the difference in growth forms, with deciduous species grouping separately from evergreen and deciduous correlating negatively with PC1. PC1 also shows grouping by elevation, with intermediate elevation correlating negatively. Based on their loadings, NRE and LnM and LmRS are increased in deciduous and intermediate elevation species. LL is higher in evergreen species. The second PC is dominated by growth form, with deciduous being positively correlated and evergreen negatively correlated. Based on their loadings, NRE, DML, LEP and LDM are higher in deciduous compared to evergreen

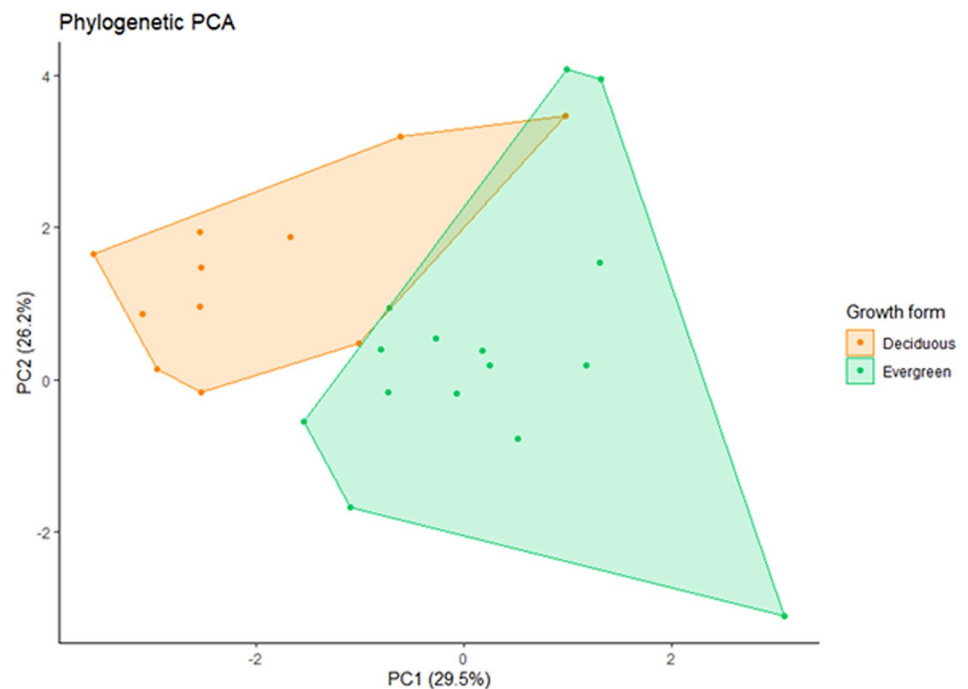
Table 1 Phylogenetic signal of plant functional traits for trees at the site. Significant values ($P < 0.05$) are in bold

Trait	λ	P-value	C_{mean}	P-value	K	P-value
LA	0.727	0.023	0.176	0.054	0.004	0.293
LL	0.555	0.072	0.036	0.268	0.006	0.216
LDM	0.901	0.068	0.140	0.096	0.050	0.006
LMA	0.914	0.000	0.512	0.001	0.004	0.338
LEP	0.000	1.000	0.263	0.014	0.000	0.800
DML	0.933	0.326	0.053	0.250	0.004	0.256
LnM	0.276	0.552	0.089	0.170	0.000	0.953
LnS	0.407	0.492	0.259	0.014	0.000	0.728
NRE	0.000	1.000	-0.181	0.842	0.001	0.424
LmRS	0.000	1.000	0.011	0.339	0.001	0.575

λ = Pagel's Lambda; $\lambda = 0$ indicates traits evolve independent of phylogeny; $\lambda = 1$ indicates traits evolve dependent on phylogeny by random drift. C_{mean} = Abouheif's C_{mean} ; an index of autocorrelation where deviations from zero indicate stronger relationships between trait values and the phylogeny. K = Bloomberg's K; $K > 1$ indicates species are more similar than expected under random drift; $K < 1$ indicates species are more divergent than expected under random drift. P-value is determined by 5000 randomizations. Traits with significant P-values are in bold. Significant values ($P < 0.05$) are in bold

LA leaf area, LL leaf life span, LDM leaf dry mass, LMA leaf mass per unit area (g m^{-2}), LEP leaf expansion period (weeks), DML dry mass loss percentage, LnM leaf nitrogen (mature), LnS leaf nitrogen (senesced), NRE nitrogen resorption percentage, LmRS leaf mass before rainy season

Fig. 2 Phylogenetic Principal Components Analysis of plant functional traits by growth characteristics. Trait variable data was square-root or log-transformed, then centred and scaled prior to PCA analyses



growth forms. In contrast, evergreen forms have higher LL and LnS.

Phylogenetic correlations between leaf traits

A correlation analysis controlling for phylogenetic dependence found that most pairwise correlations between traits

were not significant (Fig. 4). Of the 8 that were significant, 5 were negatively correlated and 3 were positively correlated. Leaf lifespan (LL) was negatively correlated with dry mass loss (DML) and nitrogen resorption efficiency (NRE). Leaf mass per unit area (LMA) showed a highly significant positive correlation with nitrogen resorption efficiency (NRE).

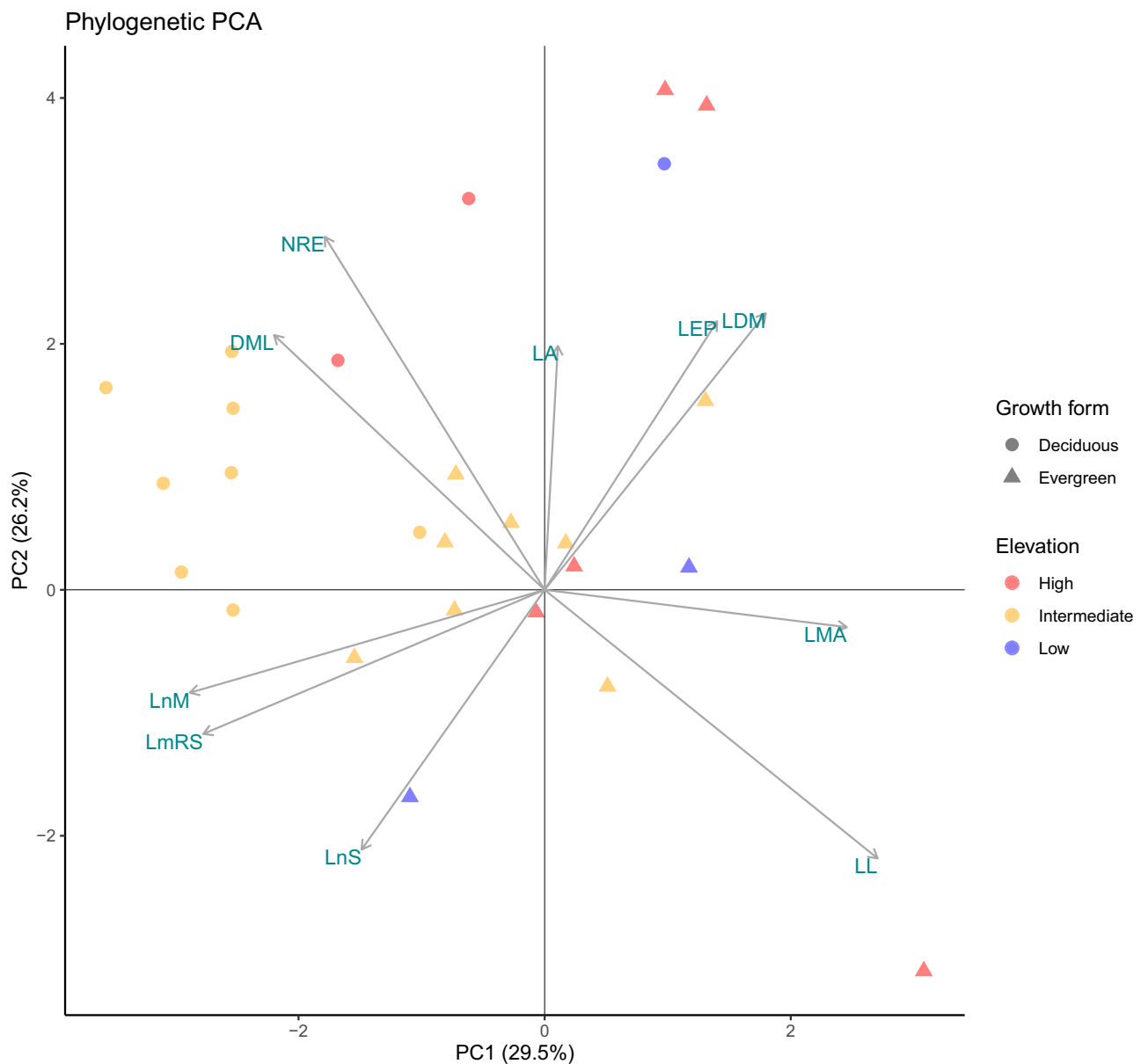


Fig. 3 Phylogenetic PCA biplot of loadings and scores of plant functional traits by growth form and elevation. Trait variable data was square-root or log-transformed, then centred and scaled prior to PCA analyses

Factors affecting leaf trait variations

As a composite variable accounting for maximum trait variation, PPASS was used to assess the variation in foliar traits as a response to elevation and growth forms across species. Both elevation and growth form were significant factors in the PGLS models, driving the trait variation represented by PPASS when controlled for phylogeny (Tables 2, 3). Intermediate elevation had a significant negative effect on the composite traits compared to high elevation (Table 2). Evergreen species had a significant positive effect on composite traits compared to deciduous species (Table 2). Of the two

predictor variables with significant effects, growth form had a larger coefficient and thus contributed more to the model (explained more variance in PPASS) than did elevation (Table 3).

Discussion

This study investigated the evolutionary relationships among tree taxa distributed across an elevational gradient in the central Himalaya. We sought to understand the variations in foliar traits of the sampled tree species taking phylogenetic

Fig. 4 Phylogenetic Pearson correlation plot of leaf traits. Coloured circles indicate significant correlations ($P < 0.05$)

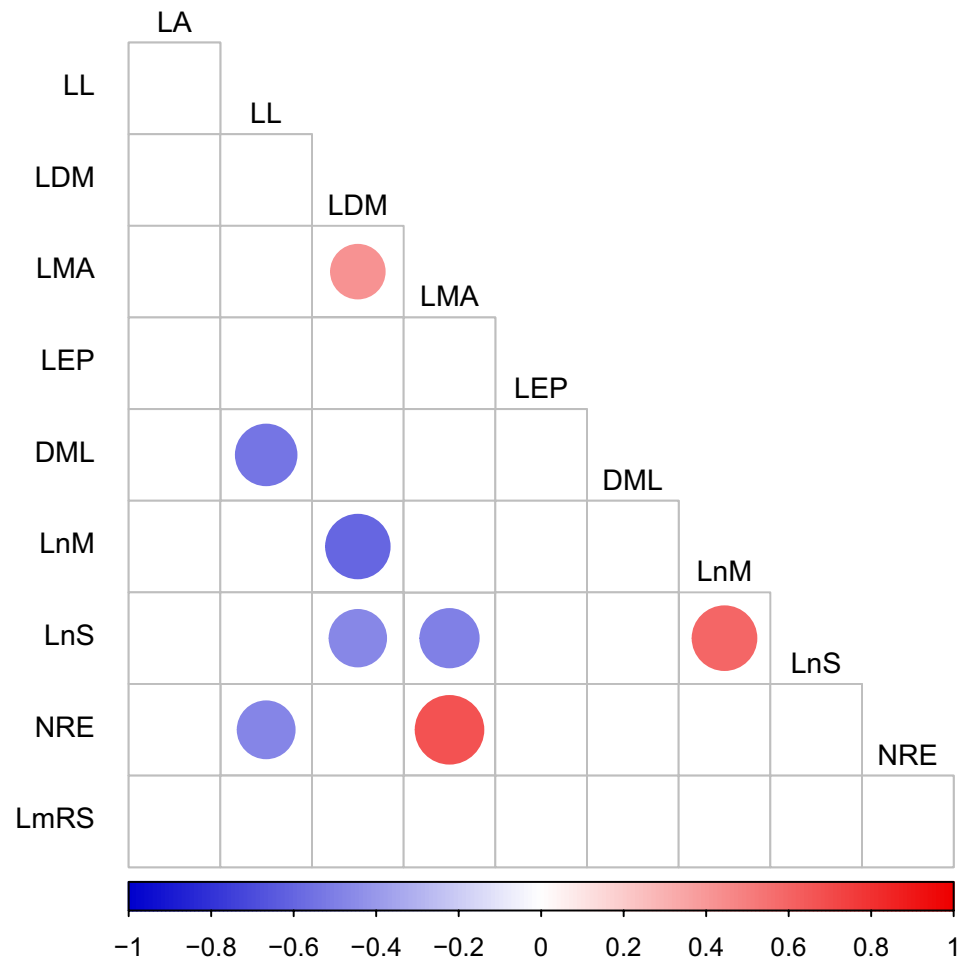


Table 2 Results from PGLS model predicting functional foliar trait variation (Phylogenetic Primary Axis Species Score, PPASS)

Phylogenetic PC1 (29.5%) of functional foliar traits				
Variable	Estimate	Std. Error	t-value	P-value
(Intercept)	-0.601	0.723	-0.831	0.415
Elevation (Intermediate)	-1.407	0.447	-3.149	0.005
Elevation (Low)	0.189	0.733	0.258	0.799
Growth form (Evergreen)	1.895	0.422	4.486	0.000

PGLS model = PPASS ~ Elevation + Growth; lambda (ML) = 0.44, kappa = 1.00, delta = 1.00, $R^2_{adj} = 0.599$, $F(3,22) = 13.47$, P-value = 0.000033. Significant values ($P < 0.05$) are in bold

relatedness in the account. Sister species of taxa at the tips of the phylogenetic tree and taxonomically distant species (Fig. 1) shared the same growth forms, suggesting multiple independent evolution of similar traits (i.e. convergent evolution). Himalayan flora is a combination of endemic taxa and taxa from various regions of the world that have diversified in the past due to major geophysical upheavals during the formation of the Himalaya (Manish and Pandit 2018a; Singh and Singh 1987). One of the geophysical upheavals event

Table 3 Analysis of Variance Table for PGLS model of factors affecting PPASS

Predictor	Df	F value	Pr(>F)
Elevation	2	10.141	0.0008
Growth form	1	20.121	0.0002

Results from ANOVA (sequential SS for pglS) showing associations between the PPASS of 26 Himalayan tree species and two predictor variables (mean elevation of species site and deciduous vs. evergreen growth). PGLS model: lambda = 0.44, delta = 1.00, kappa = 1.00 Significant values ($P < 0.05$) are in bold.

related to the Himalayan orogeny is the geological upliftment and consequent establishment of the Indian monsoon system (Pandit et al. 2014; Singh and Singh 1987). This led to the creation of a climatic gradient from tropical to temperate and subalpine along the elevational gradient and thus served as a refuge to the immigrant taxa from colder regions. The monophyletic groupings of (A) *nepalensis*, (B) *alnoides*, and *B. utilis* in conjunction with the three *Quercus* species represent the immigration of taxa from temperate regions (Manish and Pandit 2018b; Pandit et al. 2014).

Climatic cyclicality during the Quaternary period demonstrating periodic glacial advance and retreat is another geophysical event associated with the Himalayas. Such events led to the creation of multiple refugia and alternating shrinkage and enlargement of environmental niches. Domination of *Quercus semercarpifolia* and *A. nepalensis* at higher elevations during the Pleistocene epoch are corroborated from pollen records (Mehrotra et al. 2005; Phadtare 2000). In our study too, taxa belonging to the families Fagaceae (3 species of *Quercus*), Ericaceae (*Lyonia ovalifolia*, and three species of *Rhododendron*), and Betulaceae (*Alnus nepalensis* and two species of *Betula*) were distributed across intermediate to high elevation. Alternative shrinkage and enlargement may be why the plant species from the same family were clustered more often by growth forms than by elevation. Conifers in our study (*P. roxburghii* and *A. spectabilis*) formed a distinct monophyletic clade from the other species. Previous palynological reports suggest that these conifers migrated in response to climate warming leading to the subsequent replacement of *Quercus* spp. and *A. nepalensis* (Manish and Pandit 2018a; Singh and Singh 1987; Singh et al. 2018).

Alternating glacial advances and retreats ensured multiple recolonizations, frequent mixing of floral taxa, and periodic hybridization among plant species. Hybridization likely led to the successful colonization of *Rhododendron* species in the Himalaya (Zha et al. 2008).

Shared evolutionary history among the plant species was imprinted in the form of significant phylogenetic signals in various functional leaf traits (Table 1). Our study concurs with the previous study conducted across Chinese grassland biomes that reported phylogenetic differences among species as one of the drivers of the total variation in leaf traits (accounted for an average 27%) (He et al. 2010). The significant phylogenetic signal in LMA agreed with previous reports based on the global analysis of phylogenetic signals in species of vascular plants (Flores et al. 2014). Furthermore, our result was consistent with previous reports from a tropical-forest community in Laos, where LA had a significant phylogenetic signal (Satdichanh et al. 2015). The distinct grouping of evergreens and deciduous trees along PC1 suggests that niche separation has occurred between these growth forms for most of the leaf traits. The larger variation in leaf traits in evergreen species alludes to larger niche occupancy compared to deciduous form. This may provide an adaptive advantage to evergreens in the Himalaya where there is a high environmental heterogeneity. This may be a reason that evergreen taxa dominate over deciduous in the central Himalaya (Singh et al. 1994).

Controlling for phylogeny, both elevation and growth form were significant predictors of variation in a composite leaf trait in our study. The importance of elevation in influencing phylogeny and trait variation agreed with a previous

report from Taibai Mountain in central China (Xu et al. 2017). Evergreen growth form had a significant positive effect on the composite traits compared to deciduous form. In addition, intermediate elevation had a significant negative effect on the composite traits compared to high elevations. The negative coefficient for the intermediate elevations indicated that these elevations were most strongly associated with LMA, LnM, and LmRS. Interestingly, these traits had nonsignificant phylogenetic signals. Also, these traits i.e. LMA, LnM, LmRS are related to the resource acquisitive strategies. As intermediate elevation caters maximum species diversity, its association with these traits may help individuals to face higher competition for the available resources. Additionally, intermediate elevations of the Himalaya are in the main region of central thrust and are prone to tectonic movement. Environmental heterogeneity is consequently more common at intermediate elevations and may induce higher trait plasticity in the plant species. This may explain the association of the intermediate elevation with leaf traits with the weakened or non-phylogenetic signal.

Further, in our study DML, NRE, LEP, LDM, LnM and LmRS are higher in deciduous compared to evergreen growth form. In contrast, evergreen forms have higher LL and LnS. The higher proportion of dry mass retranslocation (DML), nitrogen resorption (NRE) may provide concession to deciduous taxa to remobilise assimilates, nutrients to support growth in next growing season (Chabot and Hicks 1982; Kikuzawa 1995; Reich et al. 1999). Our findings are consistent with the previous reports documenting higher leaf dry mass in deciduous species than in evergreen species (Athokpam et al. 2014; Kikuzawa and Ackerly 1999; Ralhan and Singh 1987). Also, internal remobilisation of the assimilates may allow earlier leafing in the deciduous species. However, as LEP (Leaf expansion phase) chases the length of favourable growing period initiating from leaf bud burst, it is expectedly higher for deciduous species in our study due to earlier leaf budburst. Earlier leafing also allows deciduous taxa to accumulate a larger fraction of leaf dry mass before the arrival of the rainy season (LmRS). The last adds another perspective that deciduous species may be more sensitive to early summer drought as photosynthetic carbon fixation may decrease due to stomatal limitation in response to drought (Negi 2006).

The higher LDM in deciduous growth form is consistent with previous central Himalaya findings (Negi and Singh 1992). A higher percentage of leaf nitrogen in mature leaves (LnM) maximises carbon gains within a shorter leaf lifespan by constructing highly efficient photosynthetic leaves. On the contrary, longer leaf lifespan in evergreen taxa means a longer time before the leaf payback its construction cost (Saeki and Nomoto 1958). Species with longer leaf lifespan (LL) require investment in machinery that allows them to

resist abiotic stresses and herbivory, which requires significant investment in the leaf's structural component (Reich et al. 1997; Wright et al. 2004). Therefore, we argue that the higher nitrogen percentage in senesced leaves (LnS) in evergreens in our study is due to its investment in the structural part, possibly in the leaf's cell wall. As a result, nitrogen resorption in evergreen was lower than deciduous growth forms (Athokpam et al. 2014).

Although elevation has a lesser contribution relative to growth form in explaining leaf trait variation, our study indirectly shows that high elevation is associated with resource conservation traits. The last may be due to the shorter growing season, which is more pronounced as we move from intermediate to high elevation.

Overall, based on our results, it can be inferred that both growth forms and elevation, in conjunction, guide the variation in leaf traits of the Himalayan taxa in the central Himalaya. Growth form has a higher contribution in explaining leaf traits variation compared to elevation. Elevation due to geophysical upheavals associated with Himalaya formation may likely act as an ecological factor to distribute plant taxa along a bioclimatic gradient.

Future perspectives

Recent reports suggest that forests' global productivity would be determined by trait responses to climate change (Madani et al. 2018). Therefore, we suggest incorporating a diverse set of functional traits such as hydraulic and below-ground traits along an elevation gradient with a larger dataset while controlling for phylogeny to understand and predict the response of the eco-evolutionary landscape of the Himalaya.

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Author contributions MK, SCG conceptualised the original study. MK collected field data, performed leaf trait measurements. MK & JW led the analysis of the data. MK wrote the manuscript with the guidance provided by JW, SCG and JP. All authors contributed substantially to the drafts and gave final approval for the publication.

Data accessibility Once the article be accepted for publication, all the data supporting the results will be made available on request.

Declaration

Conflict of interest The Authors declare no conflict of interests.

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