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Leaf and tree age-related changes in leaf ecophysiological traits, nutrient, and adaptive strategies of *Alnus nepalensis* in the central Himalaya

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Leaf ecophysiological traits are known to change with leaf and tree age. In the present study, we measured the effect of leaf and tree age on leaf ecophysiological and morphological traits of nitrogen-fixing *Alnus nepalensis* (D. Don) which is a pioneer tree species in degraded lands. Three naturally occurring *A. nepalensis* forest stands, namely young (5–8 years old), mature (40–55 years old), and old (130–145 years old), were considered in this study. We also investigated the seasonal variations in leaf ecophysiological and morphological traits during leaf flushing, fully expanded, and leaf senescence phenological stages. The ecophysiological and morphological traits were compared between leaf and tree ages using a linear mixed-effect model (LMM) and Tukey's HSD test. Fully expanded leaves and young trees demonstrate ecophysiological traits consistent with acquisitive resource-use strategies. Our results revealed that net photosynthetic capacity (A_{area} and A_{mass}), leaf stomatal conductance (gsw_{area} and gsw_{mass}), transpiration rate (E_{area} and E_{mass}), specific leaf area (SLA), predawn and midday water potential (Ψ), leaf total chlorophyll concentration, photosynthetic N- and P-use efficiency (PNUE and PPUE) were higher in younger trees than mature and old trees. We found lower water-use efficiency (WUE) and intrinsic water-use efficiency (WUE_i) in young trees than in mature and old ones. Mass-based net photosynthetic capacity (A_{mass}) was positively correlated with PNUE, PPUE, transpiration rate, stomatal conductance, SLA and chlorophyll concentrations but negatively correlated with WUE and WUE_i. However, mass-based leaf nitrogen (N) and phosphorus (P) concentrations were the highest in fully expanded leaves and did not vary with tree age despite N concentration being negatively correlated with SLA. Overall, this study provides valuable insights into the age-related changes in leaf ecophysiological traits of *A. nepalensis*. The findings underscore the importance of considering tree age when studying plant ecophysiology and highlight the acquisitive resource-use strategies employed by young trees for rapid growth and establishment.

Keywords.   Age-related traits; gas exchange; resource-use efficiency; water relation; water-use efficiency

1. Introduction

Leaf morphological, chemical, and physiological traits and the ability to absorb nutrients may change as trees age. Increase in tree age can cause alterations in leaf morphological traits, vegetative/reproductive resource allocation, nutritional absorption, hormonal control, and environmental adaptability, all of which can lead to variations in the tree structure and functions (Thomas and Winner 2002; Thomas 2011a, b; Damián *et al.*

2018; Ji *et al.* 2021). All these traits may induce associated changes in the structure and functions of forests (Thomas 2010; Thomas 2011a, b; Martin and Thomas 2013; Damián *et al.* 2018).

Leaf ecophysiological traits vary significantly with plant life history, light, and nutrient availabilities (Reich *et al.* 1992; Ackerly *et al.* 2000; Han *et al.* 2020; Kumar *et al.* 2021; Maharjan *et al.* 2021; Sigdel *et al.* 2023). Many studies investigated the physiological traits (i.e., photosynthesis rate) that appear to

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decline in tall trees because of the limitation of hydraulic transport (Mencuccini and Grace 1996; Ryan and Yoder 1997). High values of leaf photosynthetic rate represent acquisitive strategies (high productivity) for plants, and low values represent conservative strategies (low productivity) (Wright et al. 2005; Gorne et al. 2022). Several studies have indicated that younger trees exhibit greater traits associated with resource acquisition strategies, while mature and older trees tend to display more traits indicative of conservative strategies (Damián et al. 2018; Dayrell et al. 2018). During forest stand development, plants shift from an acquisitive physiological strategy to a conservative physiological strategy (Han et al. 2020) to maintain the plant's overall productivity at its optimum according to metabolic need.

The leaf phenological cycle provides an interesting opportunity to explore the relationship between leaf gas exchange, water balance, and leaf functional traits in relation to leaf phenology (Fajardo and Siefert 2016). Leaf physiological traits significantly change with phenological phases (Escudero and Mediavilla 2003). Leaf phenological cycle (i.e., leaf initiation, expansion, and senescence) varied among species, among individual plants, and also among leaves on a plant (Chabot and Hicks 1982; Reich et al. 1991; Mediavilla et al. 2014; Bai et al. 2015; Joshi and Garkoti 2023c). Previous studies have revealed that with rise in leaf age and leaf mass per unit area (LMA), the leaf nitrogen (N) and phosphorus (P) decline (Niinemets and Lukjanova 2003; Niinemets et al. 2006; Athokpam and Garkoti 2015). Previous studies also revealed that leaf- and tree age-related decline in photosynthesis traits could be associated with the change in leaf morphology and nutrients over time (Reich et al. 1991). Leaf N is positively correlated with the activity of Rubisco (ribulose-1, 5-biphosphate carboxylase/oxygenase), and its concentration often decreases with age of tree and leaf (Kitajima et al. 2002, Wright et al. 2006; Fajardo and Siefert 2016; Chavana-Bryant et al. 2019). Leaf P is involved in various metabolic activities during photosynthesis and its concentration decreases with leaf and tree age (Wright et al. 2006; Mediavilla et al. 2011; Chavana-Bryant et al. 2017, 2019). Similarly, stomatal conductance and control (i.e., stomatal opening and closing) also decrease with leaf and tree age (Reich and Borchert 1988), which in turn affect photosynthesis.

Water, N, and P are essential resources for plant survival, growth, and photosynthesis. Leaf physiological traits such as net photosynthetic capacity, leaf diffusive conductance, and transpiration rate are indicators of CO_2 assimilation, resource-use strategies, and water exchange (Rawat et al. 2021; Singh et al. 2023). The water-use efficiency (WUE) and photosynthetic N- and P-use efficiency (PNUE and PPUE) are essential characteristics of plant species that determine leaf physiology, leaf economics, and strategies, and are expected to change with plant and leaf age (Robinson et al. 2001; Wright et al. 2004; Nabeshima and Hiura 2004; Bai et al. 2015). WUE, PNUE, and PPUE are important ecological indicators of species performance in different environmental conditions that are expected to change with plant and leaf age (Funk and Vitousek 2007). PNUE and WUE describe the N concentration per unit leaf area and the amount of water transpired, respectively, for a given rate of photosynthesis. At the leaf level, WUE is the ratio between net CO_2 assimilation and water loss via transpiration, and intrinsic water-use efficiency (WUEi) is the ratio between net CO_2 assimilation and stomatal conductance. The PNUE, PPUE, and WUE predict how photosynthetic assimilation is optimized per unit of N, P, and water in leaves (e.g., Poorter and Evans 1998; Castellanos et al. 2005; Sheng et al. 2011). Thus, the leaf phenological cycle and resource allocation strategies are physiologically related (Ackerly and Bazzaz 1995; Hikosaka 2005), making leaf age a crucial characteristic controlling plant carbon and nutrient economies, and eventually resulting in an adaptive modification in response to ecological heterogeneity.

Nitrogen-fixing Alnus nepalensis (D. Don) is one of the fast-growing early successional tree species which plays a crucial role in the ecosystem functioning and biogeochemical cycling in the central Himalaya (Joshi and Garkoti 2021b). In this region, A. nepalensis is an important economic and reforestation tree species (Joshi and Garkoti 2023a, b). Previous studies have demonstrated that A. nepalensis is important for soil conservation in degraded forests (Joshi and Garkoti 2021b, 2023a, b). Most studies on A. nepalensis have focused on ecosystem carbon dynamics (Joshi and Garkoti 2021b, 2023a), soil physicochemical properties, below-ground biomass, and litter dynamics (Joshi and Garkoti 2020, 2021a). Understanding the ecophysiology and nutrient dynamics of A. nepalensis may provide insights into its ecological importance and help develop sustainable forest management strategies.

Our study investigated variations in leaf ecophysiological traits, nutrient concentration, and adaptive strategies concerning leaf age and tree age. Leaf ecophysiological traits, such as photosynthetic rate, stomatal conductance, transpiration rate, and leaf nutrient concentrations of nitrogen and phosphorus were

measured across different leaf and tree age classes. In this study, we proposed the following questions: (1) How do the leaf ecophysiological and morphological traits change with tree age? We expected a shift from more acquisitive physiological strategies in the young age stand to conservative physiological strategies in the older age *A. nepalensis* stand. (2) How do the leaf ecophysiological and morphological traits change with leaf age? We believed that these traits varied with leaf phenophase.

2.   Materials and methods

2.1   *Study site*

The study was undertaken at 30°31′36.7″ N and 79°6′42.0″ E, 1612 meters above sea level in the proximity of Kedarnath Wildlife Sanctuary in the western part of the central Himalaya (table 1). The area exhibits a predominantly cool temperate climate and experiences noticeable changes in weather with season. The meteorological station near the study region assessed the annual rainfall, temperatures (maximum and minimum, mean), and precipitation during the study time (data recorded at the nearest meteorological station located at Ukhimath). The mean minimum temperature ranged from −1.1°C in January to 13.4°C in July, and the mean maximum temperature ranged from 11.6°C in January to 24.4°C in June. During the study, annual mean rainfall ranged from 7.3 mm in November to 637.1 mm in July. The cumulative yearly rainfall in the study area was 1983 mm, with over 70–80% of this usually occurring during the monsoon season (July–September) and moderate to heavy snowfall during December–February (Joshi and Garkoti 2020). The soil type in the study area was sandy loam, brown podzolic mixed with pebbles and gravel (Joshi and Garkoti 2021b). The dominant tree species representing forests are *A. nepalensis* D. Don, *Quercus leucotrichophora* A. Camus, *Myrica esculenta* Buch. Ham. ex D. Don, *Rhododendron arboreum* Smith, *Pyrus pashia* L., *Lyonia ovalifolia* (Wall.) Drude, *Litsea umbrosa* Nees, and *Symplocos paniculata* Miq (Joshi and Garkoti 2023b).

2.2   *Experimental design*

Field experiments were conducted in 0.1 ha permanent plots in three age groups of forest sites, i.e., age group I: young stage (5–8 years old), age group II: mature stage (40–55 years old), and group III: old stage (130–145 years old). All study sites were separated by at least 500–800 m. Detailed information for *A. nepalensis* individuals in the different tree age classes is given in table 1. Since we lacked precise information on the age of the forest, we used the basal area of *A. nepalensis* (which acts as an indicator of tree age). We corroborated it by interviewing elderly members of the local community who knew of the year when landslides occurred and subsequently when *A. nepalensis* was established. We further confirmed the data with the Forest Department.

The leaf life span of *A. nepalensis* was between 9 and 10 months. Leaf flushing starts during spring (March to April), and leaf production completes in 2 to 4 months. *Alnus nepalensis* retains 15–25% of leaves during winter. During the winter season, herbivory damages the leaves of *A. nepalensis* that are still retained and affect several leaf traits. Five healthy individuals each of young (5–8 years old), mature (40–55 years old), and old (130–145 years old) *A. nepalensis* were selected. To assess age-specific ecophysiological traits of both the tree and its leaves, we conducted gas exchange and water potential measurements for all 15 individuals during the three phenophases, each exposed to varying moisture and temperature conditions. The investigation dates for each sampling phenophase were 20–30 March 2021 (spring; leaf flushing), 20–30 June 2021 (early

Table 1.   Forest sites and geographical characteristics across the central Himalaya

Forest sites	Latitude (N)	Longitude (E)	Altitude (m)	Aspect	Slope (°)	Tree age (years)	Tree height (m)	Mean DBH (cm)
Young	30°31′51.61″	79°06′12.44″	1540	NE	25	5–8	3–5	8–15
Mature	30°31′36.39″	79°06′16.08″	1609	NE	28	40–55	8–12	35–40
Old	30°31′35.46″	79°06′19.71″′	1742	NE	24	130–145	12–16	120–155

NE, north-east.

monsoon summer; fully expanded leaf), and 20–30 October 2021 (fall; leaf senescing).

Area-based physiological traits, e.g., photosynthetic rate (A_{area}; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gsw_{area}; mol H_2O m^{-2} s^{-1}), transpiration rate (E_{area}; mol H_2O m^{-2} s^{-1}) were measured using an open-flow, portable measurement infrared gas analyzer (IRGA) (Li-6800, Li-Cor, Lincoln, NE, USA) (Evans and Santiago 2014) between 9:30 and 11:00 h local solar time (to minimize sources of diurnal heterogeneity and avoid mid-day depression) under ambient conditions and air temperature (T_{air},°C). The leaf temperature (T_{leaf},°C) and photosynthetic photon flux density (PPFD, µmol^{-2}s^{-1}) were recorded at each measurement by the IRGA using a 6 cm^2 chamber with red–blue light-emitting diodes on normal cloudless days. To avoid influence of fluctuating environmental conditions, photosynthetically active radiation (PAR) was set to 1200 µmol m^{-2}s^{-1}, while the concentration of CO_2, temperature, and humidity was set according to the ambient conditions of the study site. The cuvette's vapor pressure deficit (VPD) was maintained at 1 kPa. Specific leaf area (SLA; cm^{2}g^{-1}) represents the inverse of leaf mass area (LMA) and was calculated as the ratio of dry leaf area and leaf mass (Poorter *et al.* 2009). Leaf area was measured by leaf area meter (LI 3000C, LI-COR, Lincoln, Nebraska, USA). Mass-based assimilation rate (A_{mass}; µmol CO_2 kg^{-2}s^{-1}), mass-based stomatal conductance (gsw_{mass}; mol H_2O kg^{-1}s^{-1}), and mass-based transpiration rate (E_{mass}; mol H_2O kg^{-1} s^{-1}) were calculated as $A_{mass} = A_{area} \times SLA$; $gsw_{mass} = gsw_{area} \times SLA$, and $E_{mass} = E_{area} \times SLA$, respectively. Leaf functional traits measured included specific leaf area (SLA; cm^{2}g^{-1}), total nitrogen (leaf N; g kg^{-1}), total phosphorus (leaf P; g kg^{-1}) concentrations, and total chlorophyll (Chl; mg g^{-1}) concentrations. To estimate the mass-based leaf nitrogen (N_m) and phosphorus (P_m), eight to ten leaf discs of definite area (1.60 cm^2) were excised from the leaf (leaf without petiole), dried at 64°C to constant weight, and weighed. During the analysis, all samples were triplicated and averaged. Mass-based leaf nitrogen (N_m) and phosphorus (P_m) concentrations were calculated by $K_2Cr_2O_7$-H_2SO_4 oxidation, the Kjeldahl method, and the modified H_2O_2-H_2SO_4 method (Rapp *et al.* 1999), respectively. The concentration of P was determined at 725 nm using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). Mass-based leaf chlorophyll concentration (mg g^{-1}) was measured on fresh leaf discs, extracted using 5 mL of dimethylsulfoxide (DMSO), with three replicates for each tree and leaf age. After the sample test, the tube was preheated to 64°C in the water bath for 4 h and sample tissues were decolorized and cooled at room temperature; the absorbance of the supernatant was measured using a spectrophotometer (Shimadzu UV-1201, Kyoto, Japan). Chlorophyll a and b concentrations (mg g^{-1}) were calculated using 665 and 645 nm readings. Area-based leaf nitrogen (N_a) and phosphorus (P_a) concentrations were calculated as mass-based leaf nitrogen (N_m) and phosphorus (P_m) concentrations each divided by the specific leaf area (i.e., N_a and P_a = N_m/SLA and P_m/SLA, respectively). The photosynthetic N- and P-use efficiency were measured by calculating nitrogen- and phosphorus-use efficiency (PNUE or PPUE = A_{area}/N_{area} or A_{area}/P_{area} µmol CO_2 N and P s^{-1} g^{-1}). Intrinsic water-use efficiency (WUE$_i$; µmol CO_2 µmol^{-1} H_2O) was measured as the ratio of A_{area}/gsw_{area}, and water-use efficiency (WUE; µmol CO_2 µmol^{-1} H_2O) was derived as the ratio of A_{area} /E_{area} (Farquhar and Sharkey 1982). We measured mid-day water potential on the same branch on which leaf gas exchange experiments were conducted using a pressure chamber (Model 1000, PMS Instrument, Corvallis, OR). We also measured pre-dawn water potential on each tree before the leaf physiological traits measurement.

2.3   *Statistical analysis*

All statistical analysis in this study was performed using the R programming language, version 4.0 (R Core Team 2021). Data sets of physiological, leaf morphological, and chemical traits were tested for normality and homoscedasticity by the Shapiro–Wilk and Levene tests, respectively. For leaf ecophysiological, morphological, and chemical traits, tree age (young, mature, and old) and leaf age (flushing, fully expanded, and senescing) were considered fixed effects for the mixed-model ANOVA, where interactions among fixed effects were also assessed. In order to analyze results of the mixed models, we employed the R package 'lme4'. Additionally, we utilized the 'car' package to determine the significance of individual fixed factors as well as their interactions. Moreover, after conducting an ANOVA to detect significant variations, a *post hoc* test (Tukey's HSD) was employed utilizing the 'emmeans' package. This test was carried out for pairwise comparisons of means to determine significant differences. Linear regression was performed to observe the correlations between physiological, leaf morphological, and chemical traits with regression equations. All analyzed correlations were considered significantly different when $p<0.05$.

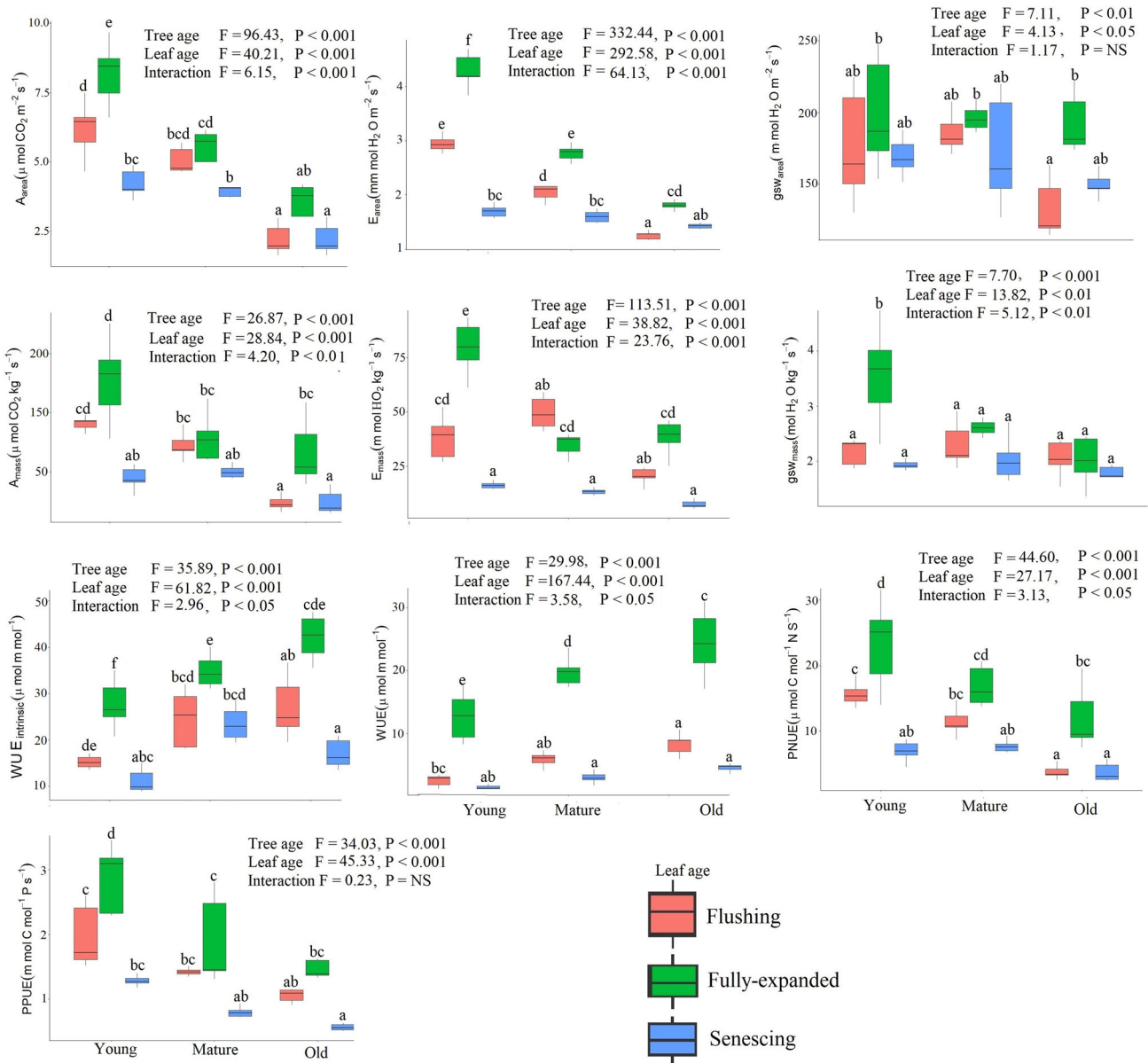**Figure 1.** Seasonal variation in different leaf physiological traits in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya. Lower and upper box boundaries represent the 25% and 75% quantiles, respectively; the solid lines across each box are the median. Different lowercase letters indicate significant differences at *p*<0.05.

3. Results

3.1 *Variation in leaf ecophysiological traits with leaf and tree age*

Although influence of tree age and leaf age manifested significant physiological trait differences in *A. nepalensis*, fully expended leaves had the highest values of all the physiological traits at the flushing and senescing stages regardless of tree age (figure 1). Most of the leaf physiological trait values were higher in young trees compared with mature and old trees (figure 1). Most of the leaf trait values followed a descending order from summer (fully expanded) to spring (flushing) to autumn (senescing). Fully expanded leaves showed greater capacity for photosynthesis compared with leaves either at the flushing or senescing stage. Like the physiological traits, SLA, total chlorophyll concentrations, and N and P concentration per unit leaf mass followed a similar leaf age-related pattern (higher for the fully expanded stage than flushing and senescing) (figure 2).

A_{area} and A_{mass}, E_{area} and E_{mass}, gsw_{area} and gsw_{mass}, PNUE, and PPUE tend to decrease with tree age. WUE_i and WUE tend to increase with tree age. Despite high
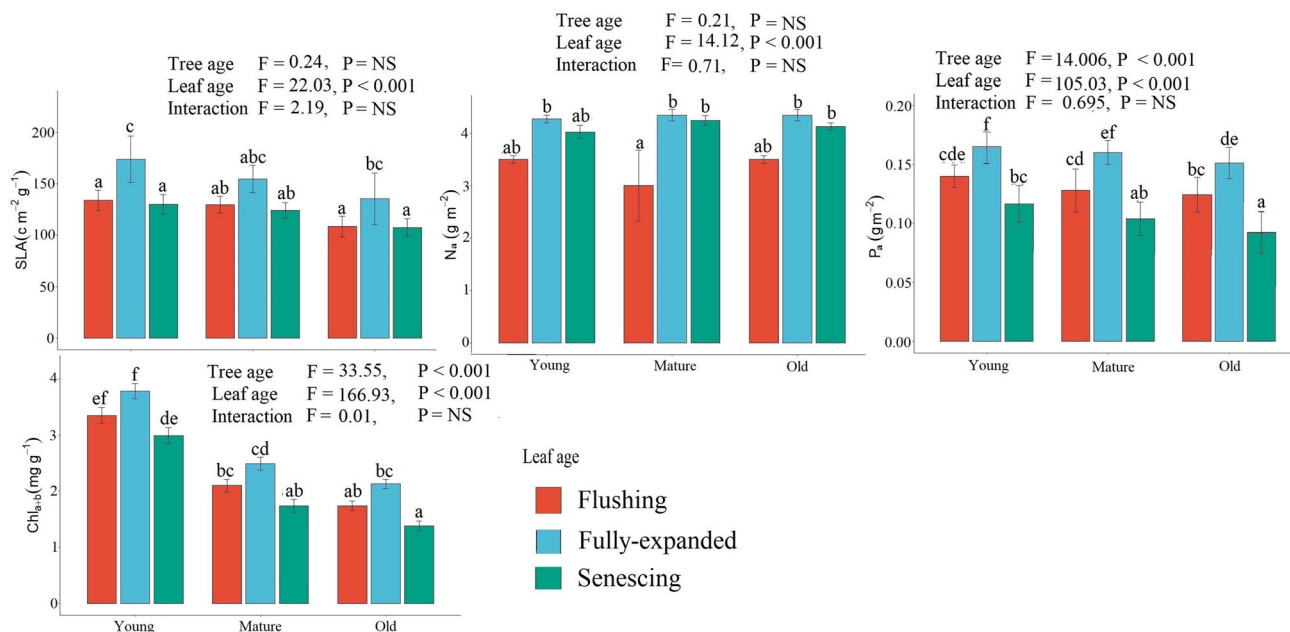
Figure 2. Seasonal variation in different leaf morphological and chemical traits in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya. Different lowercase letters indicate significant differences at $p<0.05$.
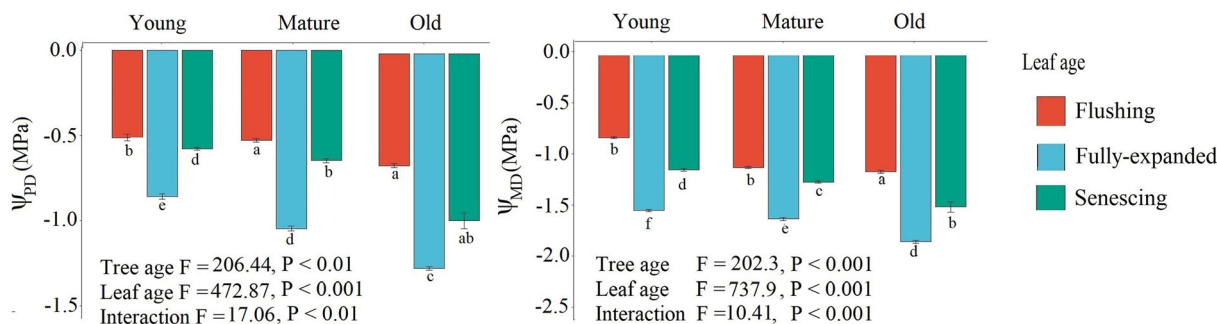

Figure 3. Seasonal variation in leaf water in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya. (Ψ_{PD}) and (Ψ_{MD}) refer to pre-dawn and mid-day water potential. Different lowercase letters indicate significant differences at $p<0.05$.

physiological traits in the fully expanded leaves, pre-dawn (Ψ_{pd}) and mid-day (Ψ_{md}) water potential were more negative during the fully expanded stage and leaf senescing stage in old trees (figure 3). However, SLA, N, and P per unit area did not change significantly with tree age. Total chlorophyll concentrations peaked when leaves were fully expanded, which decreased with tree age.

3.2 *Relationship between leaf traits*

The present study found a positive correlation between E_{mass}, gsw_{mass}, PNUE, and PPUE with A_{mass}; E_{area}, gsw_{area} with A_{area}; E_{area} with gsw_{area}, and a negative correlation between WUE_i and WUE with A_{area}; gws_{area} with WUE_i, and E_{area} with WUE across the tree age classes (figure 4). Intrinsic water-use efficiency and water-

use efficiency were negatively correlated with PNUE in all three tree age stages. Midday water potential (Ψ_{md}) was positively correlated with A_{area}, E_{area}, and gsw_{area} (figure 5). Across tree age stages, SLA was positively correlated with A_{mass}, E_{mass}, PNUE, and PPUE, but negatively related to N concentration per unit leaf mass. Tree age exhibited a significant negative relationship between N_{area} and PNUE, P_{area}, and PPUE (figure 6).

4. Discussion

4.1 *Variation in leaf ecophysiological traits with tree age*

Our first objective was to understand how key eco-physiological tree traits change from resource
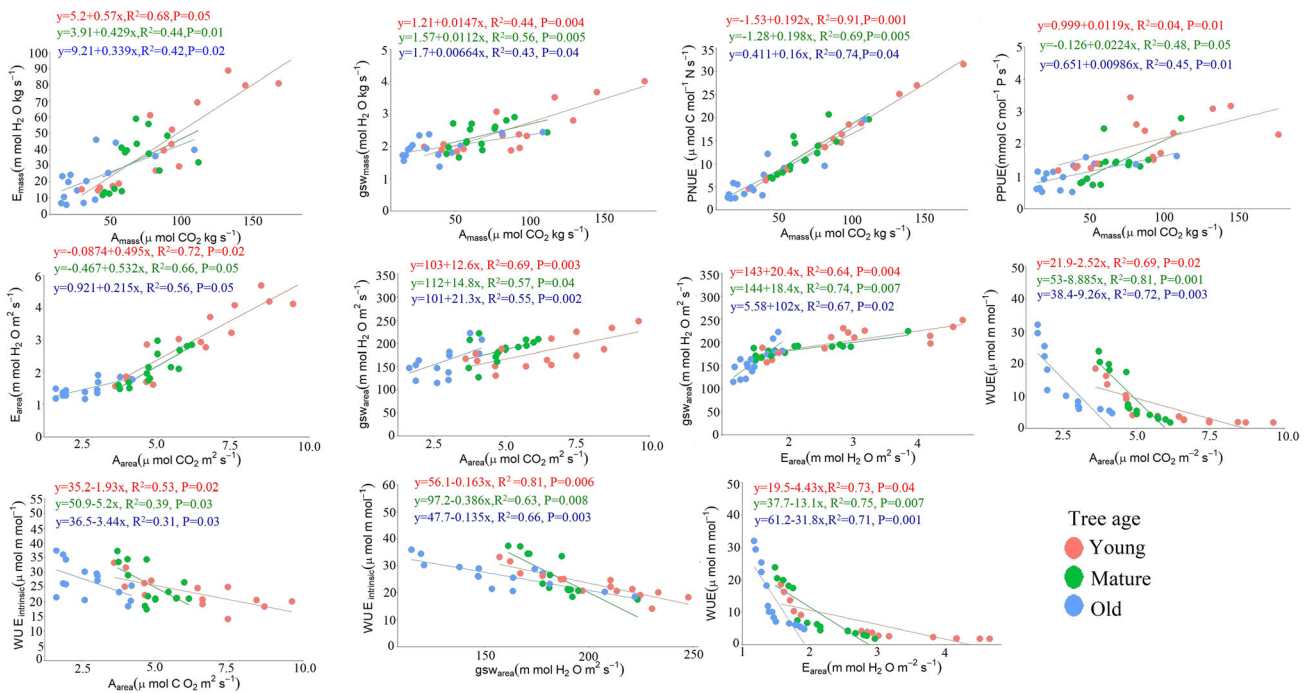
Figure 4. Variation in the relationship among the leaf physiological traits in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya.

acquisition to resource conservation with plant age and leaf age. As expected, we noticed a shift toward resource-conservative features with tree age. Thus, our results reinforce the finding that older trees have more conservative traits (Martin and Thomas 2013; Damián *et al.* 2018; Dayrell *et al.* 2018; Funk *et al.* 2021). The older trees had lower SLA, lower leaf N_{mass}, and reduced photosynthetic traits than young trees. Lower SLA values indicate a more conservative strategy, while higher SLA values indicate a more resource-acquisitive strategy, where plants invest more in leaf area to capture resources (Khan *et al.* 2022).

Modifications in photosynthetic rate were strongly dependent on E, gsw, PNUE, PPUE, WUE, and SLA (figure 4). Our results demonstrated that leaf age and tree age had a significant effect on gsw, which may be due to the leaf age- and tree age-related changes in hydraulic conductance. The lower gsw of the old trees than young and mature trees would be consistent with the hydraulic limitation hypothesis and the influence of the gravitational hydrostatic gradient (Ryan and Yoder 1997). The hydraulic limitation hypothesis proposes that as plants grow taller, the hydraulic resistance to water flow in the vascular system increases, making it increasingly difficult to transport water from roots to leaves (Ryan *et al.* 2006). This can lead to a situation where the leaves at the top of the plant experience water stress and cannot perform photosynthesis effectively,

hence limiting a plant's overall growth and productivity (Ryan and Yoder 1997). Stomatal closure is well recognized as linked to decreased soil-to-leaf hydraulic conductance and variations in Ψ_{leaf} (Hubbard *et al.* 1999; Kolb and Stone 2000). In the present study, young trees generally have less negative water potential, indicating that water is more readily available to them compared to old trees. This can be attributed to their higher water absorption capacity, efficient water transport systems, and generally healthier root systems. Additionally, their higher transpiration rates can help maintain favorable water potential by effectively drawing up water from roots (McDowell 2011). As a result, plant strategies gradually shift from resource acquisition to resource conservation (Guariguata and Ostertag 2001). Similarly, shifts in plant strategies were also reflected through higher leaf chlorophyll concentrations and greater PNUE and PPUE in young trees which declined as trees grow old. Higher leaf chlorophyll concentration and greater PNUE and PPUE in young trees reflect leaf traits with faster growth strategies involving greater photosynthesis and light capture.

Differences in chemical and morphological traits, driven by leaf and tree age, significantly shape physiological traits observed at the leaf scale. In the present study, the total chlorophyll, nitrogen mass (N_{mass}), and phosphorus mass (P_{mass}) values were higher in younger trees compared with older ones, suggesting a higher
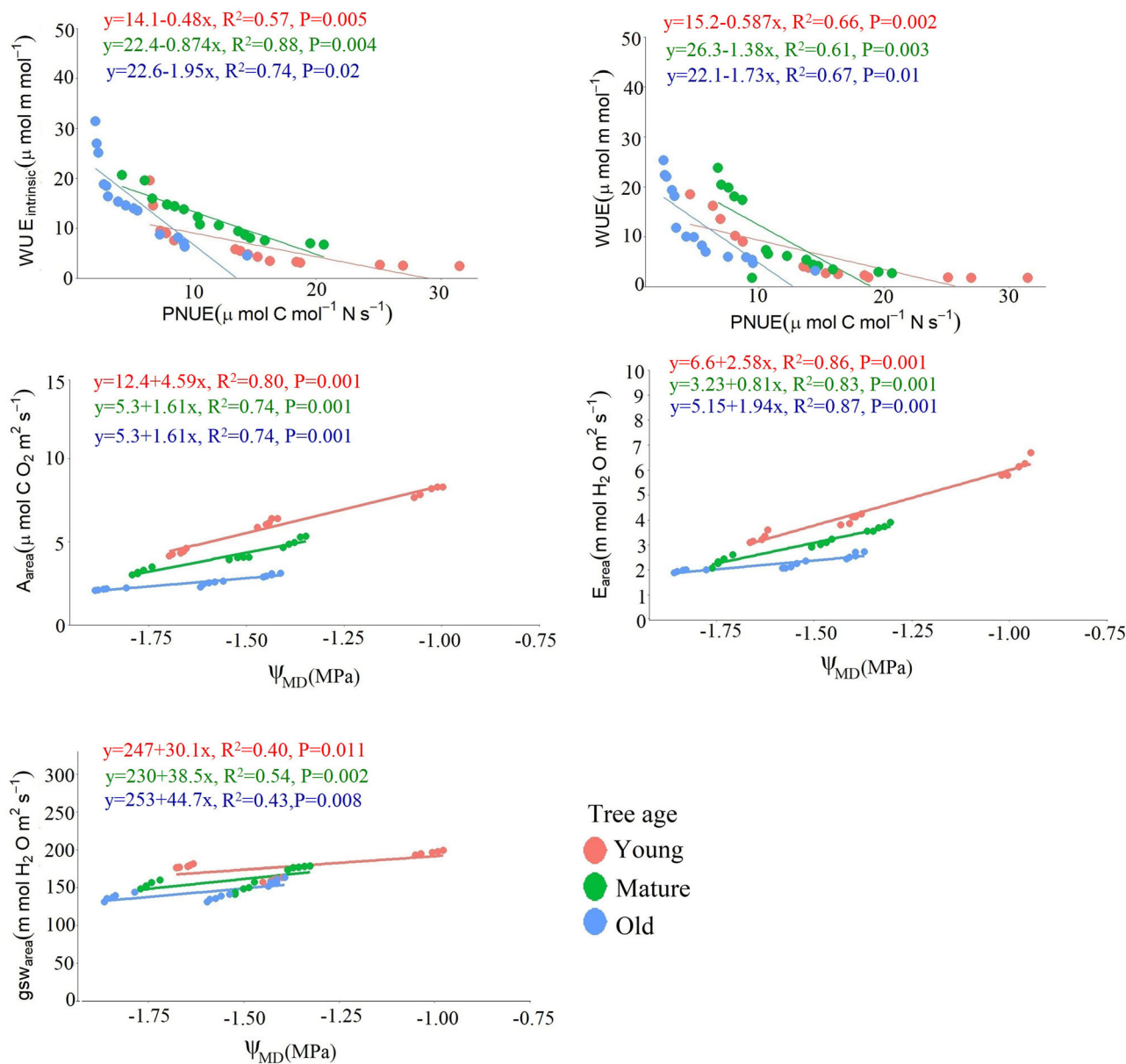
Figure 5. Variation in the relationship among the physiological, photosynthetic nitrogen use efficiency and photosynthetic phosphorus use efficiency and midday water potential in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya.

growth rate and productivity in young trees. These traits may have positively influenced the cycling of nutrients and water within the young trees.

4.2   *Variation in leaf ecophysiological traits with leaf age*

The results supported optimum productivity during summer when the leaf was fully expanded (summer) then leaf flushing (spring) and leaf senescing stages (autumn). Previous studies also suggested similar leaf

age trends of physiological traits in that they showed peak values during the fully expanded leaves (Wilson *et al.* 2000, 2001; Bauerle *et al.* 2004; Grassi and Magnani 2005). In the present study, physiological traits showed peak values during the fully expanded leaf stage. During summer, various factors such as longer daylight hours, increased photosynthetically active radiation, ideal air temperature, and higher leaf nitrogen and phosphorus content contribute to the optimal physiological traits of plants (Grassi and Magnano 2005; Wright *et al.* 2006; Hikosaka *et al.* 2007). However, as leaves enter the senescence stage,
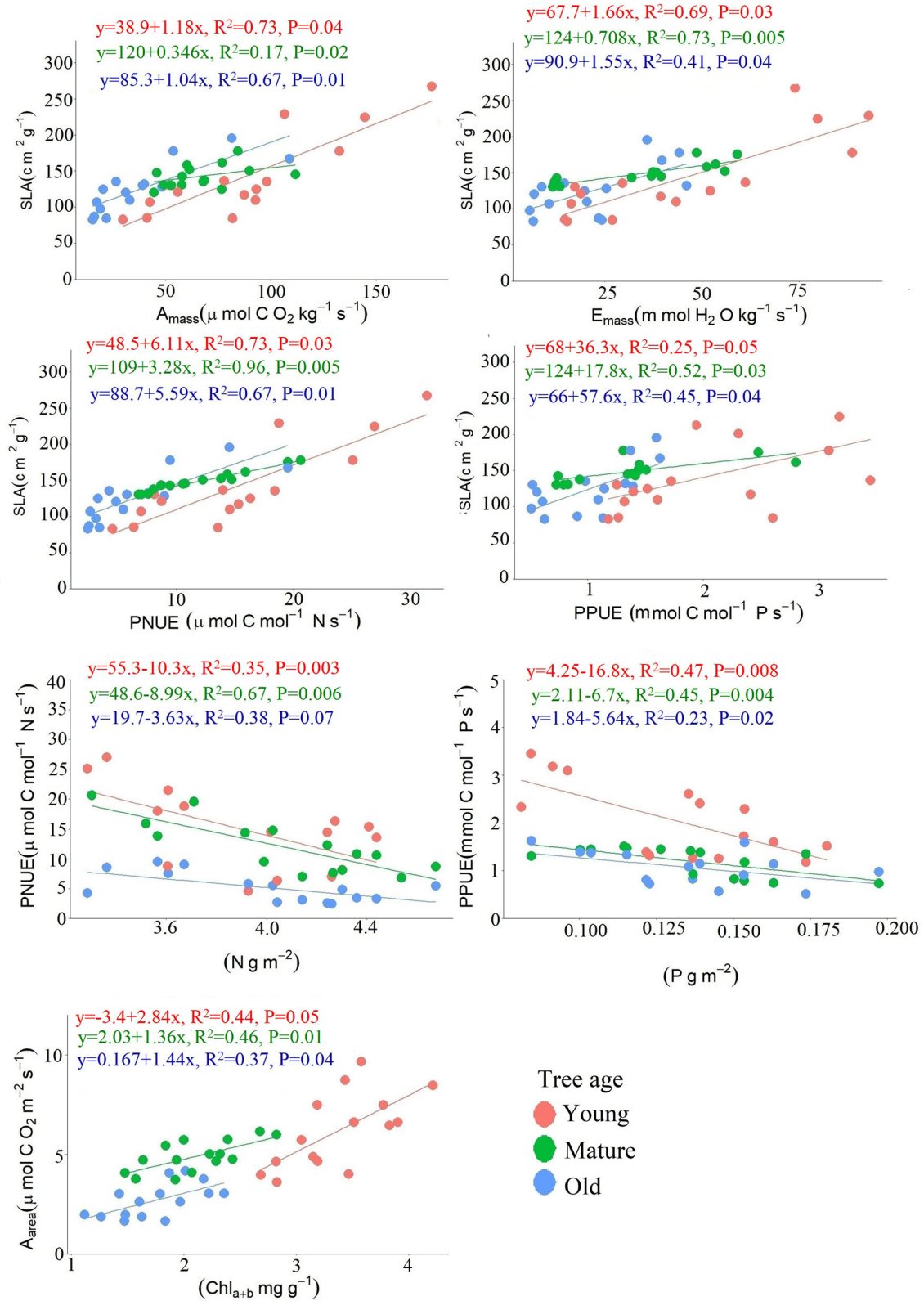
Figure 6. Variation in the relationship among selected leaf morphological, chemical, and physiological traits in the young, mature, and old stages of *A. nepalensis* trees in the central Himalaya.

the levels of nitrogen and chlorophyll in the leaf reach their lowest point due to a nutrient remobilization process (Maillard *et al.* 2015). This leads to reduced physiological traits following leaf senescence. Such leaf age- and tree age-dependent decline in leaf morphological and chemical traits are significantly associated with changes in leaf physiological traits (i.e., photosynthetic rate, stomatal conductance, and transpiration rate) (Reich *et al.* 1991). In addition, the nutrient resorption process during leaf senescence negatively affected the overall productivity during the leaf senescing stage (Crous *et al.* 2019). The photosynthetic rate in a fully expanded leaf was significantly associated with other physiological traits, including transpiration rate, stomatal conductance, water potential, and photosynthetic nutrient-use efficiency (Reich *et al.* 1998; Wright *et al.* 2005). The tight connection between levels of photosynthesis, chlorophyll, and N_{mass} reflected the contribution of nitrogen to the Calvin–Benson cycle enzyme (in particular, ribulose-1,5-biphosphate carboxylase/oxygenase, Rubisco) and chlorophyll for better light harvesting (Whitmarsh 1999). The high chlorophyll concentrations in the fully expanded leaves supported high light-harvesting and higher carbon assimilation per unit leaf area (Li *et al.* 2018).

4.3   *Acquisitive vs. conservative resource-use strategy*

The present study found a significant PNUE–WUE trade-off in young, mature, and old trees. Young trees achieved higher PNUE and lower WUE, whereas mature and old trees followed the reverse trend. The trade-off between WUE and PNUE may explain the greater rates of physiological traits in the young trees. As trees mature and grow old, they may become more resource-limited and face environmental stressors such as competition for resources, drought, or nutrient limitations. In response, mature and old trees adapt by reducing their water requirements and increasing water-use efficiency, potentially sacrificing some of their photosynthetic efficiency (Niinemets *et al.* 2006; Niinemets 2010). PNUE was 12% higher for young trees than for mature trees and 18% higher than for old trees. Young trees exhibited higher water potential, suggesting a trade-off between leaves with greater photosynthetic rates in young trees and leaves that are water-stressed (low water potential) in old trees. Young trees may have a higher capacity for water uptake and transport, allowing them to maintain higher water potentials despite their higher

rates of water loss through transpiration. This may be due to various differences including root architecture, root morphology, physiology and anatomy of young and old tree leaves, and environmental stress (Freschet *et al.* 2021). PNUE and PPUE were higher in the young trees, favoring low investment and quick resource return strategy than in mature and old trees, which favored a slow resource return strategy (Wright *et al.* 2004; Reich and Flores-Moreno 2017). In the present study, the young trees showed greater N assimilation efficiency and more allocation of N to photosynthetic chlorophyll tissue than other non-photosynthetic tissues to ensure optimum growth. This was supported by the comparatively higher SLA values in young trees than mature and older trees (Abdul-Hamid and Mencuccini 2009), to reduce resource investment in non-photosynthetic tissues of young trees. Previous studies have also reported the trade-off in N partitioning between photosynthetic and non-photosynthetic tissues in varying tree ages (Hikosaka and Hirose 2000; Hikosaka 2004).

In the present study, the majority of mass based-physiological traits (i.e., A_{mass}, E_{mass}, and gsw_{mass}) and photosynthetic nutrient-use efficiency showed positive correlation with SLA, which is similar to several previous reports (Wright *et al.* 2005; Crous *et al.* 2017). Onoda *et al.* (2017) also revealed that leaves with greater SLA tend to enhance A_{mass}, $E_{mass,}$ and PNUE to support fast growth. Old trees had higher WUE than young and mature trees, leading to significantly lower physiological traits (Forrester 2015). In general, high WUE indicates a more conservative resource-use pattern (Lambers *et al.* 2008). Mid-day water potential (Ψ_{md}) was more negative during the summer in old trees, which may be due to a combination of factors, including differences in leaf morphology, increasing temperature, vapor pressure deficit (VPD), biochemical characteristics, larger overall biomass, root distribution, and physiological regulation of stomatal conductance (Rodriguez-Calcerrada *et al.* 2008; Abdul-Hamid and Mencuccini 2009; Schönbeck *et al.* 2022). Consequently, to adapt to the pressure and balance of demand and supply, old trees acquire a more conservative approach to shift toward higher WUE, which indicates that at a given photosynthetic rate, the transpiration rate, stomatal conductance, and photosynthetic nutrient-use efficiency were higher in young rather than mature and old trees (Abdul-Hamid and Mencuccini 2009).

4.4   *Ecosystem implication*

Leaf and tree age lead to adaptive changes in the ecophysiological processes, such as variation in water

relation, gas exchange, and growth rate among varying tree age stages (Sala *et al.* 2010). Physiological responses, such as evolution and adaptation to changing environments, are influenced by phenotypic plasticity, which is considered the primary underlying process with implications for ecological processes (Hovenden and Vander Schoor 2003; Thomas 2011a, b). Better comprehension of physiological traits of major native trees is required for introducing resilient forest management strategies to minimize expected effects of climatic change on plant development and water stress. Age-specific physiological traits of *A. nepalensis* appear important for ecosystem processes. WUE, PNUE, and PPUE play an essential regulatory role in functioning of *A. nepalensis*-dominated ecosystems. PNUE and PPUE were significantly greater in the young stage, providing additional evidence that *A. nepalensis* has developed a mechanism for the efficient use of N and P nutrients and modifications in physiological traits. Ishida *et al.* (2005) have also explained similar ontogenetic morphological, anatomical, and chemical modifications leading to evolution and adaptation in leaf physiology along the age gradient for the pioneer tree species *Macaranga gigantea*. Furthermore, different-aged stands of *A. nepalensis* may exhibit different adaptive strategies in response to soil resource availability (soil water and nutrient availability), which may potentially affect growth, survival, and competitive ability. Further studies are needed to test these hypotheses and to better understand the underlying mechanisms and ecological implications of these relationships.

5. Conclusion

In conclusion, our findings indicate that most of the physiological traits of *A. nepalensis* decreased with tree age, indicating that the ecological strategy of *A. nepalensis* changed from a resource-acquisitive approach to a resource-conservative approach along the tree age gradient. The relationship between leaf traits and structural and chemical traits changed through tree age, indicating different trade-off strategies across the age gradient. High photosynthetic nitrogen- and phosphorus-use efficiency in young trees could support rapid growth of *A. nepalensis*. Specific leaf area and total chlorophyll concentration strongly influence many physiological traits and serve as vital regulators. Our results contributed to a more dynamic understanding of the relationship between leaf physiological traits and their interaction with leaf morphological and chemical

traits. Additional studies are needed to understand the interaction with soil physicochemical properties and soil moisture concentration along the age gradient in *A. nepalensis* forest stands in the central Himalaya.

Author contributions

SCG and RKJ conceived the idea; RKJ, AM, and RG, designed and conducted the field experiment and analyzed the data; RKJ, RG, and AM contributed reagents/materials/analysis tools; RKJ wrote the manuscript, and SCG, AM, and RG edited the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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