

Effect of leaf phenology, and tree age on leaf ecophysiology traits and adaptive strategies of *Alnus nepalensis* in central Himalaya

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Abstract

The leaf ecophysiological traits are expected to change with the leaf age, and tree age. Leaf phenology and tree age (seedling, sapling, and tree stages) was a stronger driver of changes in ecophysiological traits. In the present study, we measured effect of leaf phenophases (initiation stage, expansion stage, and senescence stages) and tree age (seedling, sapling, and tree stages) on the leaf physiological and morphological traits of nitrogen-fixing *Alnus nepalensis* (D. Don), a pioneer tree species in the central Himalaya. In fully expanded leaf and seedling stage demonstrate ecophysiological traits consistent with an acquisitive resource-use strategy. Results revealed that net photosynthetic capacity (A_{area} and A_{mass}), leaf stomata conductance ($g_{sw_{area}}$ and $g_{sw_{mass}}$), transpiration rate (E_{area} and E_{mass}), specific leaf area (SLA), pre-dawn and mid-day water potential (Ψ), leaf total chlorophyll concentration, photosynthetic N-, and P-use efficiency (PNUE and PPUE) were highest in seedling stage and sapling than trees. Seedling stage and sapling had significantly higher transpiration rates (E_{area} and E_{mass}) and stomatal conductance ($g_{sw_{area}}$ and $g_{sw_{mass}}$), therefore showing significantly lower water use efficiency (WUE) and intrinsic water use efficiency (WUE_i). Mass-based net photosynthetic capacity (A_{mass}) were positively correlated with PNUE, PPUE, transpiration rate, stomatal conductance, SLA, and chlorophyll concentrations while negatively correlated with WUE and WUE_i . However mass-based leaf nitrogen (N), and phosphorus (P) concentrations were higher in fully expended leaf; they did not vary significantly, despite N concentration negatively correlated with SLA. Collectively, our results indicated that seedling *A. nepalensis* displayed characteristic values associated with a more acquisitive resource-use strategy. Consequently, this may explain their survival and replacement strategies during secondary succession and should be considered for the vegetation restoration model of degraded forest in the central Himalaya.

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Introduction

Tree age may result in variation in leaf morphological, chemical, physiological traits and the ability to absorb nutrients. Increasing tree age can cause alterations in leaf morphological traits, vegetative/reproductive allocation, nutritional absorption, hormonal control, and environmental adaptability, all of which can lead to variation in tree structure and function (Thomas et al., 2002; Thomas 2011; Damián et al. 2018). All of these traits may induce the associated differences in the structure and function of the forest (Thomas 2010; Thomas 2011; Martin and Thomas 2013; Damián et al. 2018).

The leaf ecophysiological traits vary significantly with plant life history, light and nutrients availabilities (Reich et al., 1992; Ackerly et al. 2000; Han et al. 2020). Many studies investigated the physiological traits (i.e. photosynthesis rate) appear to decline in tall trees, because of the limitation of hydraulic transport (Mencuccini and Grace 1996a). It has been observed that hydraulic transport may reduced with tree age as a results of the longer path length from soil to stomata, which derive the reductions in stomatal conductance and photosynthetic rate that directly influence tree productivity (Ryan and Yoder 1997). The high values of leaf

physiological traits represent acquisitive strategies (high productivity) for plants, and low values represent conservative strategies (low productivity) (Wright et al. 2005a; Gorne et al. 2020). Several investigations evaluated that younger tree have greater trait values related to resource acquisition, but mature and older have more conservative traits (Damián et al. 2018; Dayrell et al. 2018). During the forest chronosequence, plants shift from acquisitive physiological strategy to conservative physiological strategy (Han et al. 2020) to maintain the plant overall productivity at its optimum as per the metabolic need.

The leaf phenological cycle offers an intriguing system for investigating the interconnection between leaf gas exchange, water relations, and leaf functional traits with respect to leaf phenology (Fajardo, and Siefert 2016). Leaf physiological traits significantly change with phenological phases. During the growing leaf age, leaf physiological traits change as a result of the in seasonal environmental conditions and leaf age (Escudero et al. 2003; Chvaana et al. 2017; Chvaana et al. 2019). Leaf phenological cycle and leaf economic spectrum (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). Previous research has revealed that with leaf age, leaf mass area (LMA) rises while leaf nitrogen and phosphorus (N and P) are decline (Niinemets and Lukjanova 2003; Niinemets et al. 2006; Athokpam and Garkoti 2015). Previous workers also revealed the leaf and tree age-related decline in photosynthesis traits which could be associated with the change in leaf morphology and nutrient 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 decreased with tree and leaf age (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 the photosynthesis processes and also decreases with leaf and tree age (Wright et al. 2006, Mediavilla et al. 2011, Chavana-Bryant et al. 2017, 2019). Likewise stomatal conductance and stomatal control (i.e, stomatal opening and closing) also decrease with leaf and tree age (Reich and Borchet 1988) and affect photosynthesis.

Water (H_2O), 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. The water use efficiency (WUE) and photosynthetic N and P use efficiency (PNUE and PPUE) is an essential characteristic of different species and determines the leaf physiology, leaf economics, strategy, and competition expected to change with plant age and leaf age (Robinson et al. 2001; Wright et al. 2004; Nabeshima and Hiura 2004; Bai et al. 2015). The WUE, PNUE and PPUE are excellent ecological indicator of species performance in different environmental conditions expected to change with palnt age, and leaf age (Funk and Vitousek 2007). The 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 (WUE_i) is the quick ratio between net CO_2 assimilation and stomatal conductance. The PNUE, PPUE, and WUE predict how the 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, leaf phenological cycle and resource allocation strategies are physiologically related (Ackerly and Bazzaz 1995; Hikosaka 2005), making leaf lifespan a crucial characteristic controlling plant carbon and nutrient economies, which eventually result in adaptive modification in response to ecological heterogeneity.

Nitrogen-fixing *Alnus nepalensis* (D. Don) is one of the fast-growing early successional tree species that often forms pure stands in areas affected by landslide/ slip stages over 1400 m.a.s.l. but also occurred in a forest chronosequence in the central Himalaya (Joshi and Garkoti 2021b). *A. nepalensis* is an important economic and reforestation tree species in central Himalaya. Previous research has demonstrated that *A. nepalensis* is an extremely important for soil restoration and degraded forest management (Joshi and Garkoti 2021b). Most studies of *A. nepalensis* have focused on ecosystem carbon dynamics (Joshi and Garkoti 2021b), soil physicochemical properties, below ground biomass and litter dynamics (Joshi and Garkoti 2020; Joshi and Garkoti 2021a). However, it is still not understood how the leaves of *A. nepalensis* response (photosynthetically, chemically and morphologically) along with the leaf age, and tree age. Therefore, we investigated the effect of leaf age, and tree age on leaf ecophysiology traits in *A. nepalensis* . In this study, we proposed two questions 1) how do the leaf ecophysiological and morphological traits change with leaf

and plant age? We expect a shift from a more acquisitive physiological strategy in the young stage to a conservative physiological strategy in the older stage, and 2) A resource conservation strategy adopted by the older stage may have high WUE, tough leaf construction, and low leaf N and P concentration, low photosynthetic N-, and P-use efficiency and low rates of carbon assimilation when compared to the young stage.

Material and Methods

Study site

The study was undertaken at 30°31'36.7" N and 79deg6'42.0" E, 1612 m. a. s. l in the proximity of Kedarnath Wildlife Sanctuary, in the western part of the central Himalaya (Table 1). Cold temperatures and seasonal characterize the climate of the study area. The leaf age temperatures (maximum and minimum, mean) and precipitation during the study time, assessed by the meteorological station near the study region. The mean minimum and a maximum temperature range from -1.1 degC in January to 13.4 degC in July and the mean maximum temperature was recorded from 11.6 degC in January to 24. 4 degC in June. During the study, the period means annual 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 leaf age (July- September) and moderate to heavy snowfall during the December-February months (Joshi and Garkoti 2020). The soil type of the study area was sandy loam, brown podzolic mixed with pebbles and gravel (Joshi and Garkoti 2021b).

Experimental design

In December 2020, when a growing stage of the current year was completed, we selected tree *A. nepalensis* forest stands across a wide range of tree age ranges. Our field experiments were conducted in 0.1 ha permanent plots in three age groups, i.e., the age group I: young stage (5-8 years), age group II: mature stage (35- 40 years), and group III: old stage (130-145 years) forest sites. All of the study sites were separated by at least 500-800 m, and their topography (slope gradient, slope aspect), micro-environments, and soil conditions were similar (Table 1). The detailed information for the *A. nepalensis* individuals in the different tree age classes is given in Table 1.

The leaf lifespan of *A. nepalensis* was between 9-10 months, having leaflessness during the winter leaf age. Leaf budding starts during spring (March to April), and leaf production completes in two to four months. Leaf get fully expanded during the summer. We sampled five healthy individuals of *A. nepalensis* of each age stage for a total of 15 individuals. To measure the tree and leaf age-specific photosynthetic physiology, we conducted gas exchange and potential water measurements for all 15 individuals across the three-leaf age periods of varying ambient moisture and temperature conditions. The investigation dates for each sampling leaf age were 20-30 March 2021 (spring; leaf bud burst), 20-30 June 2021 (early monsoon summer; fully expended leaf) and 20-30 October (fall; leaf senescence).

Area-based physiological traits e.g., photosynthetic rate (A_{area} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($g_{\text{sw}_{\text{area}}}$; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (E_{area} ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured using an open-flow, portable measurement infrared gas analyzer (IRGA) (Li-6800, Li-Cor, Lincoln, NE, USA) (Evans & Santiago 2014) under ambient conditions and air temperature (T_{air} , °C), the leaf temperature (T_{leaf} , °C) and photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{ s}^{-1}$) were recorded at each measurement by the IRGA using a 6-cm² chamber with red-blue light-emitting diodes on normal cloudless days. To avoid the influence of fluctuating environmental condition, Photosynthetically Active Radiation (PAR) was set to 1200 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, while concentration CO₂, temperature, and humidity was set as per the ambient condition of the study site. Specific leaf area (SLA; $\text{cm}^2 \text{ g}^{-1}$) 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, Inc). Mass-based assimilation rate (A_{mass} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); mass-based stomatal conductance ($g_{\text{sw}_{\text{mass}}}$; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (E_{mass} ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were calculated as $A_{\text{mass}} = A_{\text{area}} \times \text{SLA}$; $g_{\text{sw}_{\text{mass}}} = g_{\text{sw}_{\text{area}}} \times \text{SLA}$ and $E_{\text{mass}} = E_{\text{area}} \times \text{SLA}$, respectively. Leaf functional traits measured included specific leaf area (SLA; $\text{cm}^2 \text{ g}^{-1}$), total nitrogen (leaf N; g kg^{-1}),

total phosphorus (leaf P; g kg^{-1}) concentrations, and total chlorophyll (Chl; mg g^{-1}) concentrations. For chemical analysis (N_m and P_m $\text{mg nutrient g}^{-1}$), eight to ten leaf discs of definite area (1.60 cm^2) were excised from the leaf (leaf without petiole), dried at 64 degC to constant weight, and weighted for each species. During the analysis, all samples were triplicated and averaged. Leaf N, and leaf P concentrations were calculated by $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ oxidation, Kjeldahl method, and modified $\text{H}_2\text{O}_2\text{-H}_2\text{SO}_4$ method (Rapp et al. 1999) respectively. The concentration of P were determined at 725 nm using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). Leaf chlorophyll concentration (mg g^{-1}) was measured on fresh leaf discs which were extracted by using 5 ml of dimethylsulfoxide (DMSO), with three replicates for each tree and leaf age. After the sample test tube was preheated to 64 degC in the water bath for 4 hrs and sample tissues were decolorized, and cooled at room temperature, the absorbance of the supernatant was measured using the spectrophotometer (Shimadzu UV-1201, Kyoto, Japan). Chlorophyll a, and b concentrations (mg g^{-1}) were calculated using the reading from 665 nm and 645 nm . Area-bases N and P concentrations and total chlorophyll (N_a , P_a mg m^{-2} , and $\mu \text{g cm}^{-2}$), were calculated based on N, and P concentrations and multiplied by the specific leaf area (i.e., N_a and $P_a = N_m$ and P_m / SLA). Leaf N and P concentrations were measured because they are important nutrient elements for photosynthesis, namely RUBISCO and ATP. The photosynthetic N – and P use efficiency were measured by calculating nitrogen-phosphorus use efficiency (PNUE or PPUE = $A_{\text{area}}/N_{\text{area}}$ or $A_{\text{area}}/P_{\text{area}}$ $\mu \text{mol CO}_2 \text{ N and P s}^{-1} \text{ g}^{-1}$). Intrinsic water use efficiency (WUE_i ; $\mu \text{mol CO}_2 \mu \text{mol}^{-1} \text{ H}_2\text{O}$) was measured as the ratio of $A_{\text{area}}/\text{gs}_{\text{w area}}$ and water use efficiency (WUE; $\mu \text{mol CO}_2 \mu \text{mol}^{-1} \text{ H}_2\text{O}$) was derived as the ratio of $A_{\text{area}}/E_{\text{area}}$ (Farquhar & Sharkey 1982). We coupled leaf physiological traits with the midday 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 predawn water potential on each individual tree before leaf physiological traits measurement.

Statistical analysis

Repeated measures analysis of variance (ANOVA) with Tukey's honest significant different (Tukey 's HSD) test was used to estimate the significant variation among treatment combinations of the leaf physiological, morphological, and chemical traits among the tree age stages. Data was tested for normality and homogeneity of variance, and, if necessary, \log_{10} transformation of the data was done before analysis. The relationship between physiological, leaf morphological, and chemical traits was determined using multiple linear regression with regression equations. All analyzed correlations were considered significantly different when $p < 0.05$. All the statistical analysis was performed using R 3.4.4 (R Development Core Team).

Results

Variation in leaf ecophysiological traits along with the leaf and tree age

Though the influence of tree age and leaf age manifested in significant physiological trait differences in *A. nepalensis*. The fully expanded leaf have highest values of all the physiological traits than leaf bud burst and leaf senescence stage significantly, regardless of the tree age gradient (Fig. 1). The effect of leaf and tree age was significant and evident for most of the leaf traits. Most of the leaf physiological trait values were highest in the young stage than in the mature and old stages (Fig. 1 and Table 2). Leaf age recorded patterns of most of the leaf trait values were in the descending order of summer (fully expanded stage) > spring (leaf bud burst stage) > autumn (leaf senescence stage). Fully expanded leaves had a greater capacity for photosynthesis compared to either young (leaf bud burst stage) or old leaves (senescence stage). Like the physiological traits, SLA, total chlorophyll concentrations, N and P concentration per unit leaf mass followed a similar leaf age-related pattern (higher for the fully expanded stage than leaf bud burst stage and senescence stage subsequently) (Fig.2 and Table 2).

A_{area} and A_{mass} , E_{area} and E_{mass} , $\text{gs}_{\text{w area}}$ and $\text{gs}_{\text{w mass}}$, PNUE and PPUE tend to decrease with tree age. WUE_i and WUE tend to increase with tree age. Despite high physiological traits in the fully expanded stage (summer leaf age), predawn (Ψ_{pd}) and midday (Ψ_{md}) water potential were more negative (indicating higher potential stress) during the fully expanded stage and leaf senescence stage in the old stage (Fig.3 and Table

2). However, SLA, N, and P per unit area did not change significantly with tree age. The total chlorophyll concentrations peaked when the leaf was fully expanded stage and decreased with tree age.

Relationship between leaf traits

In the present study, there was a positive correlation between E_{mass} , gsw_{mass} , PNUE, and PPUE with A_{mass} ; E_{area} , gsw_{area} with A_{area} ; and E_{area} with gsw_{area} . There was a negative correlation between WUE_i and WUE with A_{area} ; gsw_{area} with WUE_i and E_{area} with WUE in all three tree age stages (Fig.4). Intrinsic water use efficiency (WUE_i) and water use efficiency were significantly negatively correlated with PNUE in all three tree age stages. There was a positive correlation between midday water potential (Ψ_{md}) with A_{area} , E_{area} and gsw_{area} (Fig.5). The specific leaf area (SLA) was positively correlated with A_{mass} , E_{mass} , PNUE, and PPUE in all three tree age stages but negatively related to N concentration per unit leaf mass across all three tree age stages. In all three tree stages, the PNUE and PPUE were negatively correlated with N and P concentration per unit of leaf mass. The total chlorophyll concentration was positively correlated with A_{area} (Fig.6).

Discussion Variation in leaf ecophysiological traits along with the leaf age and tree age

Our first objective was to understand how key ecophysiological traits changing from resource acquisition to resource conservation with plant age and leaf age. As expected, we noticed a shift toward resource-conservative traits with tree age. Thus, our findings reinforce the idea that older tree have more conservative traits (Manson et al. 2013; Damián et al. 2018; Dayrell et al. 2018; Funk et al. 2021). Older tree had higher SLA, lower leaf N_{mass} and reduced photosynthetic traits compared with young trees. The modifications in photosynthetic rate were strongly dependent on E, gsw, PNUE, WUE, and SLA (Fig.4). Our results demonstrated that leaf age and tree age had a significant effect on gsw, which may be due the tree age-related changes in hydraulic conductance and differences in Ψ_{leaf} . The lower gsw of old tree than young and mature trees would be constant with the hydraulic limitation hypothesis and the influence of the gravitational hydrostatic gradient (Ryan and Yoder 1997). It is quite well recognised that stomatal closure is linked to decreased soil to leaf hydraulic conductance and variations in Ψ_{leaf} (Hubbard et al. 1999; Kolb and Stone 2000). As a result, plant strategies gradually shifted from more resource-acquisitive to more resource-conservative (Guariguata and Ostertag, 2001). This was also reflected in higher leaf chlorophyll concentrations and greater PNUE and PPUE in the young tree, which triggered a shift of leaf traits towards faster growth strategies involving greater photosynthesis and light capture to enhance the growth rate.

At the leaf scale, leaf age-driven differences in chemical and morphological traits have contributed significantly to the change in physiological traits along with the leaf age. The total chlorophyll, N_{mass} , and P_{mass} was higher in young tree which supports the higher growth rate, and productivity. This is because the combination of optimum morphological and chemical traits with more favorable environmental factors (light and temperature) of fully expanded leaf posed a positive impact on nutrient and water cycling. This supported optimum productivity during the summer season when the leaf was fully expanded than the leaf bud burst (spring) and leaf senescence stage (autumn). Previous studies also suggested the similar leaf age trends of the physiological traits related to leaf age; they showed peak values during fully expanded leaf (Wilson et al. 2000, 2001; Bauerle et al. 2004; Grassi et al. 2005). In the summer, day length was longer, highest photosynthetically active radiation, optimum air temperature and higher leaf N_{mass} , and P_{mass} combined to highest physiological traits (Grassi et al. 2005, Hikosaka et al. 2007, Wright et al. 2006). Leaf nitrogen and chlorophyll dropped to their lowest level in the leaf senescence stage due to the nutrient remobilization process after leaf senescence, associated with lowest physiological traits. Such leaf age and tree age-dependent decline in leaf morphological and chemical traits are significantly associated with changes in leaf physiological traits over time (i.e., photosynthetic rate) (Reich et al. 1991). In addition, the nutrient resorption process at the time of leaf senescence negatively affected the overall productivity during the leaf senescence stage (Crous et al. 2019). Photosynthetic rate in 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. 2005b). This indicated that, at a given photosynthesis rate, transpiration rate, stomatal conductance, and photosynthesis nutrient use efficiency were higher

in the young stage than mature and old stages. The tight connection between the levels of photosynthesis, chlorophyll, and N_{mass} reflected contribution of nitrogen to the Calvin-Benson cycle enzyme (in particular, ribulose-1,5-biphosphate carboxylase/oxygenase—Rubisco) and chlorophyll for better light harvesting. The high chlorophyll concentrations in the young stage indicated supported the high light-harvesting and higher carbon assimilation per unit leaf area.

Acquisitive vs. conservative resource-use strategy

In the present study, we found a significant PNUE-WUE trade-off for the young, mature, and old stage. Young stage 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 young stage. The PNUE for the young stage was 12 % higher than the mature stage and 18 % higher than the old stage. Young stage exhibited higher water potential suggesting a trade-off between leaves with greater photosynthetic rates in young stage and leave are water-stressed (low water potential) in the old stage. The PNUE and PPUE were higher in the young stage, favoring low investment and quick return resource strategy, than mature and old stages, which favored slow resource return strategy (Wright et al. 2004; Reich and Flores- Moreno 2017). In the present study, the young tree showed greater N assimilation efficiency and more focused allocation of N to chlorophyll tissues than other non-photosynthetic tissue to ensure optimum growth. This was supported by the morphological traits, as SLA showed comparatively lower values in the young stage than the older one (Abdul and Mencuccini 2009), to reduce the recourse investment in non-photosynthetic tissues in leaves of young stage. The trade-off N partitioning between photosynthetic and non-photosynthetic tissues in varying tree age has also been reported by previous studies (Hikosaka and Hirose, 2000; Hikosaka, 2004). In the present study, the majority of mass based-physiological traits (i.e A_{mass} , E_{mass} , and g_{swmass}) and photosynthesis nutrient-use efficiency, showed a positive correlation with SLA, which is similar to many previous studies (Wright et al. 2005b; Bahar et al. 2017; 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. Our results indicated that young trees allocate more nutrients to photosynthetic tissues to support their rapid growth than mature and old trees. This reveals the differences between leaf size and weight (Shipley et al. 2006). The old trees had higher WUE than young and mature trees, leading to significantly lower physiological traits. In general, the high WUE indicates a more conservative resource use pattern (Lambers et al. 1998). The midday water potential (Ψ_{md}) was more negative (indicating higher potential stress) during the summer in old tree, because of the enhanced water supply pressure and higher hydraulic pressure. Consequently, to adapt to the pressure and balance of demand and supply, the old tree acquires the more conservative approach to shift toward higher WUE.

Ecosystem implication

Leaf age and tree age leads to adaptive changes in ecophysiological process, such as variation in water relation, gas exchange, and growth rate among varying tree age stages (Sala et al., 2010). Physiological responses, including evolution and adaptation to environmental shifts, characterize phenotypic plasticity, which is believed to be the dominant underlying process with implications for ecological processes (Hovenden and Vander Schoor, 2003; Thomas, 2011). A better comprehension of the physiological traits of major native trees is required for introducing resilient forest management strategies to minimize the expected effects of climatic change on plant development and water stress. The stage-specific physiological traits of *A. nepalensis* appear to have important for the ecosystem process. The WUE, PNUE, and PPUE apparently play an important regulatory role in the functioning of *A. nepalensis* -dominated ecosystem. The PNUE and PPUE was significantly greater in the young stage, providing additional evidence that *A. nepalensis* has developed a mechanism for efficient use of N and P nutrient and the modifications in physiological traits among the *A. nepalensis* dominated forest stages accordingly. Ishida et al. (2005) have also explained the 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 gigantean* . All the stage-specific changes of physiological traits of *A. nepalensis* had important implications with respect to nutrient cycling, carbon assimilation, and feedback interaction between leaf, stand, and ecosystem-level

process of degraded forests in central Himalaya.

Conclusion

In conclusion, our findings indicate that most of the physiological traits of *A. nepalensis* decreased with a natural age gradient, indicating that the ecological strategy of *A. nepalensis* changed from a resource acquisitive approach to conservative resource approach with a change in age gradient with time. The relationship between the leaf traits and the structural and chemical traits changed during the age gradients, indicating different trade-off strategies across the age gradient. High photosynthetic nitrogen- and phosphorus use efficiency in the young stage could support the rapid growth of *A. nepalensis*. Specific leaf area and total chlorophyll concentration strongly influenced most of the physiological traits, being one of the 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. We also anticipated ecophysiological response to varying age gradients through the *A. nepalensis* forest stands. Additional studies are needed to understand the interaction with soil physicochemical properties and soil moisture concentration along the age gradient in the *A. nepalensis* forest stand in central Himalaya.

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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

Ethics approval

Ethics approval was not required for this study as no humans or animals were involved.

Consent to participate

Not applicable.

Consent for publication

Not applicable

Data availability statements

The authors confirm that the data supporting the findings of this study are available within the article.

Author contributions-

SCG, 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.

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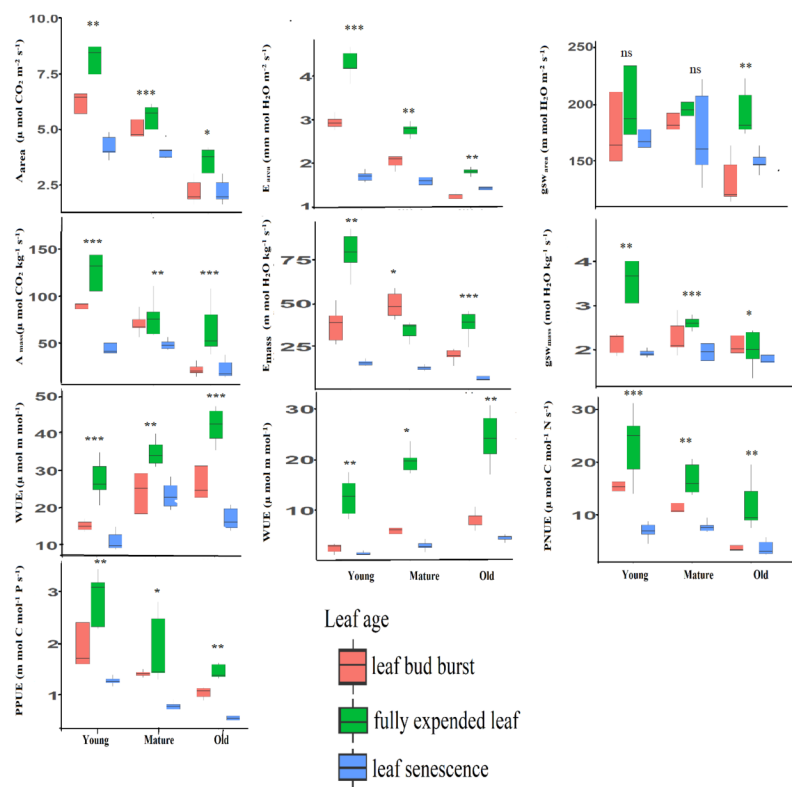


Fig. 1. Seasonal variation in different leaf physiological traits in the seedling, sapling and tree (young, mature, and old stages) of *A. nepalensis* tree in central Himalaya. Lower and upper box boundaries represent the 25% and 75% quantiles, respectively; the solid lines across each box are the median. Asterisks indicate significant seasonal differences within the same tree age based on independent sample *t-test* ; ns = not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

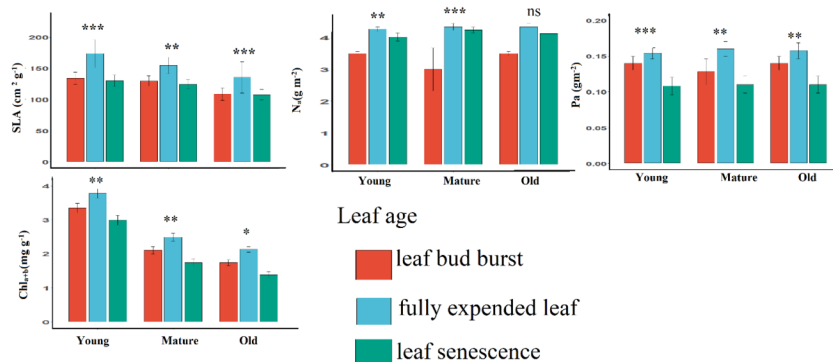


Fig.2. Seasonal variation in different leaf morphological and chemical traits in the seedling, sapling and tree (young, mature, and old stages) of *A. nepalensis* tree in central Himalaya. Asterisks indicate significant

seasonal differences within the same tree age based on independent sample *t-test* .; ns = not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

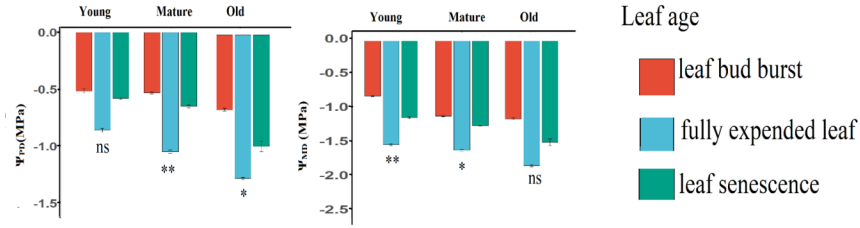


Fig.3. Seasonal variation in leaf water in the seedling, sapling and tree (young, mature, and old stages) of *A. nepalensis* tree in central Himalaya. (Ψ_{PD}) and (Ψ_{MD}) refer to pre-dawn and mid-day water potential. Asterisks indicate significant seasonal differences within the same tree age based on independent sample *t-test*.; ns = not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

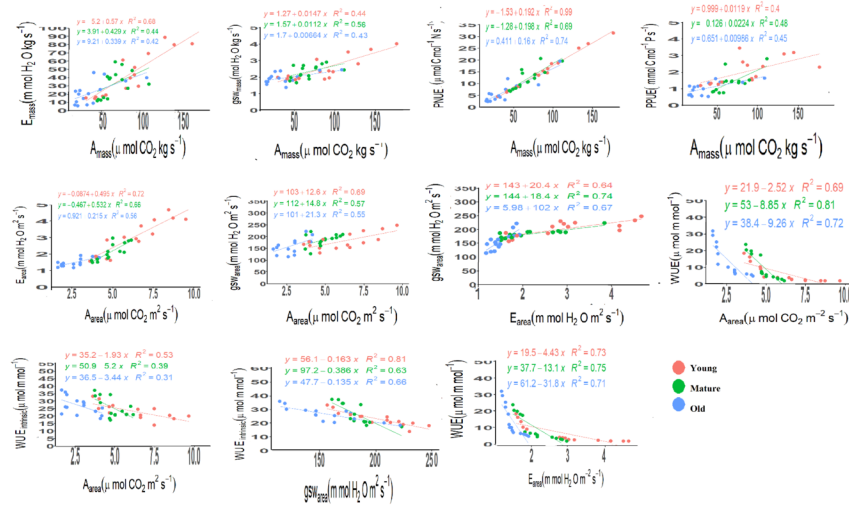


Fig. 4. Variation in relationship among the leaf physiological traits in the seedling, sapling and tree (young, mature, and old stages) of *A. nepalensis* tree in central Himalaya.

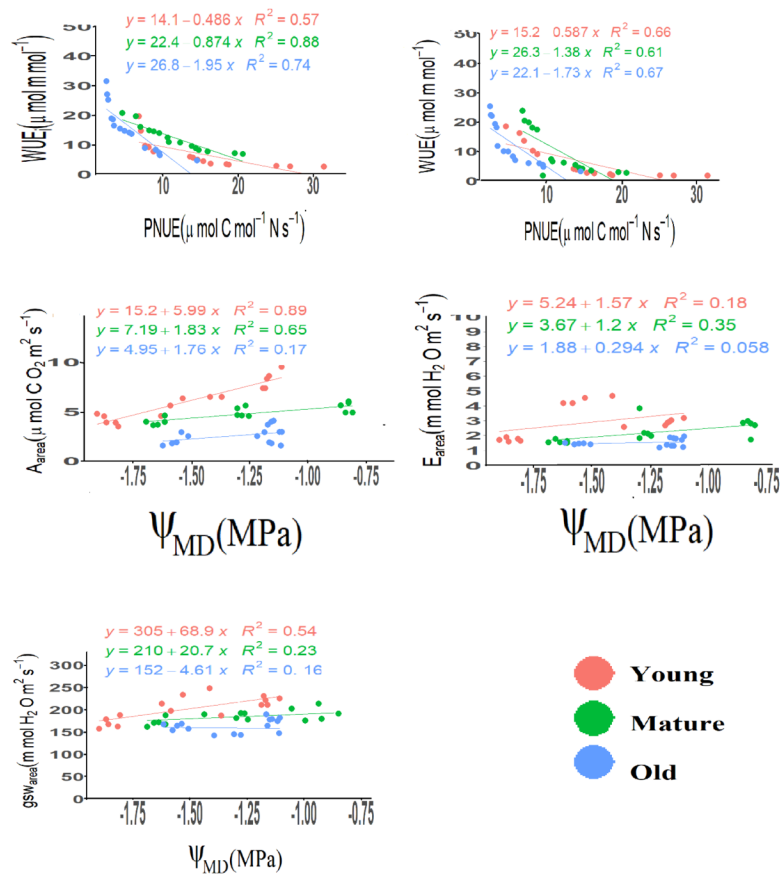


Fig.5. Variation in the relationship among the physiological, photosynthetic nitrogen use efficiency and photosynthetic phosphorus use efficiency and mid-day water potential in the seedling, sapling and tree (young, mature and old stages) of *A. nepalensis* tree in central Himalaya.

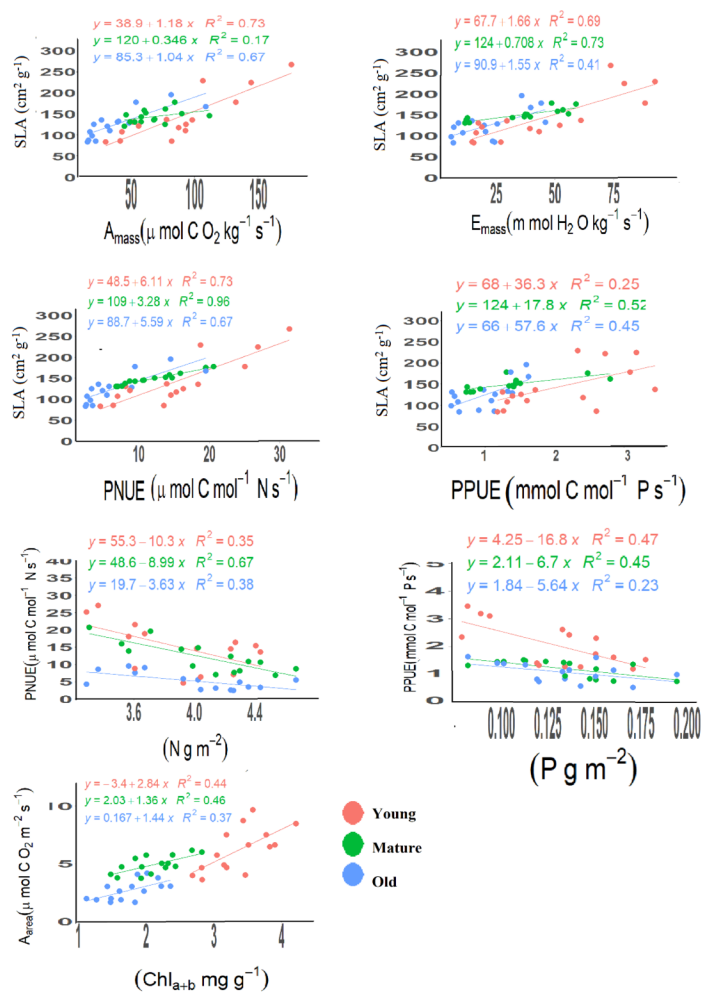


Fig.6. Variation in the relationship among selected leaf morphological, chemical, and physiological traits in the seedling, sapling and tree (young, mature, and old stages) of *A. nepalensis* tree in central Himalaya.

Forest sites	Latitude (N)	Longitude (E)	Altitude (m)	Aspect	Slope (°)	Tree age (years)	Tree height (m)	Mean D (cm)
Table								
1.								
Forest sites and geographical characteristics across in the central Himalaya.								
Forest sites								
Latitude (N)								
Longitude (E)								
Altitude (m)								
Aspect								
Slope (°)								
Tree age (years)								
Tree height (m)								
Mean DBH (cm)								
Young (Seedling stage)								
	30°31'51.61"							
	79°06'12.44"							
	1,540							
	NE 25							
	5-8							
	3-5							
	8-15							
Mature (Sapling stage)								
	30°31'36.39"							
	79°06'16.08"							
	1,609							
	NS 28							
	40-55							
	8-12							
	35-40							
Old (Tree stage)								
	30°31'35.46"							
	79°06'19.71"							

Forest sites	Latitude (N)	Longitude (E)	Altitude (m)	Aspect	Slope (°)	Tree age (years)	Tree height (m)	Mean D (cm)
	Leaf age	Leaf age	Tree age	Tree age	Leaf age × Tree age	Leaf age × Tree age		
Traits	F value	P value	F value	P value	F value	P value		
A _{area}	293.2	<0.001	78.3	<0.001	43.2	<0.001		
E _{area}	227.1	<0.001	148.9	<0.001	56.2	<0.001		
gsw _{area}	167.3	<0.001	67.2	<0.001	32.4	<0.001		
SLA	32	<0.001	3.1	0.054	0.34	0.956		
A _{mass}	99.2	<0.001	6.4	0.032	3.5	0.0367		
E _{mass}	67.2	<0.001	5.43	0.041	5.8	0.034		
gsw _{mass}	109.8	<0.001	13.4	<0.001	5.4	0.004		
N _{concentration}	145.2	<0.001	4.5	0.007	1.2	0.0425		
P _{concentration}	156.4	<0.001	8.1	0.004	0.1	0.768		
Ψ _{PD}	39.2	<0.001	7.4	0.0032	3.21	0.021		
Ψ _{MD}	67	<0.001	11.1	0.008	5.1	0.012		
WUE	145.5	<0.001	78.2	<0.001	7.2	0.054		
WUE _i	56.7	<0.001	63.2	<0.001	8.2	0.003		
PNUE	189.3	<0.001	45.1	<0.001	5.6	<0.100		
PPUE	36.7	<0.001	55.6	<0.002	2.2	0.004		
N _{area}	7.6	0.021	3.2	0.02	0.7	0.987		
P _{area}	5.3	0.031	4.1	0.034	1.2	0.356		
Chl _{area}	10.6	<0.001	5.2	0.054	3.9	0.809		

List of symbols A_{area} = area-based photosynthetic rate, A_{mass} = mass-based photosynthetic rate, gsw_{area} = area-based sto