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Changes in Biomass and Root Architecture During Axillary Bud Development of *Pinus yunnanensis*

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Abstract: *Pinus yunnanensis*, a member of the genus *Pinus*, a dominant coniferous species on the Yunnan-Guizhou Plateau, shows great potential for asexual propagation owing to the difficulty of retaining desirable traits in afforestation. Eight month old *P. yunnanensis* seedlings were used to divide axillary bud development into four stages. Seedling growth traits, biomass allocation and root architecture were investigated from axillary bud dormancy to elongation. The main findings were that seedling height and basal diameter followed a logistic growth pattern throughout axillary bud development, with the rapid growth phase occurring during bud dormancy, and both height and basal diameter were highly correlated with axillary bud number. Seedling roots were dominated by 0 - 2 mm fine roots. Most root morphological indices peaked at the axillary-bud elongation stage except average root diameter, which kept increasing. Mantel test revealed a significant correlation between axillary bud elongation and root architecture. With axillary bud development, plant organ biomass increased continuously, and the root-stem ratio first increased and then decreased, reaching the maximum value in the development stage. Biomass was mainly allocated to roots and needles with a low stem proportion. During axillary bud formation, resources are preferentially allocated to roots, while axillary bud elongation shifts growth priority to above ground needles. In summary, axillary bud development is closely linked to seedling growth, root-architecture formation and biomass allocation strategies. These findings provide a theoretical basis for scion cultivation of elite *P. yunnanensis* clones.

Key words: *Pinus yunnanensis*, Axillary bud development, Growth characteristics, Biomass, Root architecture

1. Introduction

Pinus yunnanensis, an evergreen coniferous tree of the genus *Pinus yunnanensis*, is one of the main coniferous species in the Yunnan-Guizhou Plateau. It has the characteristics of liking light and tolerating barren conditions, so it has become the pioneer tree species for alpine afforestation in the suitable areas of *P. yunnanensis* and an important tree species for controlling soil erosion[1]. At the same time, it is also the main vegetation type and one of the important materials and ecological public welfare forest species in Southwest China. Its wood is widely used in civil construction, agricultural tools, and living appliances[2]. In recent years, most of the pine stands in Yunnan are pure stands of the same age, which leads to poor stability, poor quality, and low biodiversity of the pine stands in Yunnan[3]. Moreover, due to anthropogenic deforestation, deforestation for land reclamation, environmental climate change, biological invasion, and pests and diseases, most of the *P.yunnanensis* stands have shown varying degrees of degradation, resulting in the reduction of excellent gene resources and the occurrence of variation types in *P.yunnanensis* stands[4-6].

Owing to extensive management practices and the cumulative genetic effects of *P. yunnanensis*[7]. Undesirable individuals with short, curved, and twisted trunks emerge in *P. yunnanensis* stands, and superior genetic resources gradually decrease[8]. Problems including short, twisted trunks and poor timber quality commonly exist[9]. It seriously affects tree growth and the utilization value of timber[10]. Asexual propagation can completely preserve the genotype of straight-stem trees. Asexual reproduction of Pinus yunnanensis can stably retain desirable genotypes such as straight trunks of superior trees and avoid the defect of abundant twisted trunks in seedling progenies. Moreover, cutting, grafting, and tissue culture can shorten the breeding cycle and mass-produce improved varieties with uniform traits. Therefore, asexual propagation acts as an important way to improve stand quality[11]. Asexual propagation generates offspring directly from part of the parent plant and can maintain desirable parental traits. Superior varieties can be selected for asexual propagation, which not only shortens the seedling nursery period but also effectively preserves parental traits[12]. Asexual propagation includes cuttage, grafting, and tissue culture. Among these methods, cuttage is simple to operate, efficient and low-cost, serving as the main form of asexual propagation[13]. Cuttage propagation helps maintain the stability of superior traits of mother trees and greatly shortens the breeding cycle[14]. To carry out cuttage propagation of *P. yunnanensis*, a large number of high-quality rejuvenated cuttings should be obtained first. The cutting orchard ensures a continuous supply of superior cuttings. In the management of cutting orchards, stumping can stimulate plants to produce many new sprout shoots, which serves as one of the primary sources to provide abundant high-quality cuttings for the cuttage of *P. yunnanensis*[15].

Stumping can remove the above-ground parts as required, break apical dominance, and promote sprouting and branch growth[16]. Thus, sufficient cuttings can be provided to create favorable

conditions for cuttage propagation. Stumping promotes the sprouting of cuttings, rapidly restores population quantity and structure, improves tree growth and reproductive capacity, and achieves the goal of regeneration and rejuvenation[17]. After stumping of *P. yunnanensis* seedlings, their sprouting capacity is relatively weak, which limits the acquisition of superior materials for asexual propagation[18]. For the propagation of superior *P. yunnanensis* clones, obtaining high-quality cuttings is the basis of clonal reproduction, and branch development is the prerequisite for acquiring high-quality cuttings. Branch development is critical for plant morphogenesis, and it is directly related to plant biomass and yield[19]. For the propagation of superior *P. yunnanensis* clones, obtaining high-quality cuttings is the basis of clonal reproduction, and branch development is the prerequisite for acquiring high-quality cuttings. Branch development is critical for plant morphogenesis, and it is directly related to plant biomass and yield[20].

Plant structure plays an important role in determining photosynthetic efficiency, water use, and yield, and the ideal plant structure plays a crucial role in achieving maximum resource capture per unit area. Branching is an important feature of plant structure, which is a complex developmental trait of plants. It is the result of the influence of terminal buds and the growth and development of axillary buds[20]. The character, location, and number of branches affect the diversity and productivity of plant types[21]. More branches are conducive to improving biomass, adjusting crown shape, windbreak and sand fixation, which not only have huge economic benefits, but also have strong ecological and social benefits. The character, location,n and number of branches affect the diversification of plant types and their productivity[22]. Among crops, the axillary bud is a key factor affecting yield. In the study of axillary buds of Oryza sativa, it was found that axillary bud germination ability is an important agricultural trait of rice. When the germination ability is strong, rice can have the breeding characteristics of excellent rice quality, low pollution, low energy consumption, high multiple planting index, and large yield[23]. In addition, the axillary bud can also be used as one of the important standards to measure the quality of fruit tree seedlings[24]. High-quality fruit tree seedlings not only require root development, seedling height, ground diameter, and other indicators, but also need to observe whether the axillary bud development state is good[25]. Good axillary bud development not only refers to the number and growth quality of axillary buds, but also needs to meet the appropriate branch length and branch Angle in the subsequent growth and development[26].

Plants continuously produce new organs or alter their growth patterns throughout their life cycle to adapt to changes in their environment[27]. Current research indicates that lateral branch development involves several stages, including the formation of axillary meristems and lateral buds, the activation of these buds, and their continued growth to form lateral branches. These various developmental processes are regulated by a combination of internal genetic factors, hormonal signals, sugar signals, and external environmental cues[28]. The branches that develop from the axillary buds

of gymnosperms, whether serving as vegetative or reproductive organs, have a certain impact on the plant’ s morphology, yield, and biomass[29, 30].

At present, for *P. yunnanensis*, most stands originate from seedling propagation, which causes stand degradation year by year. In addition, *P. yunnanensis* seedlings exhibit seedling stagnation at the seedling stage. Therefore, it is necessary to promote the formation of more high-quality axillary buds, so that they can develop into branches with strong reproductive capacity in the later growth stage. Superior branches of *P. yunnanensis*, they can guarantee the quality of clonal propagation and further improve the productivity of afforestation. Meanwhile, for *P. yunnanensis*, axillary buds are not only important reproductive organs but also serve as materials for its clonal propagation. Their growth and development exert vital influences on the variant types, morphological architecture, seed yield, and clonal propagation of *P. yunnanensis*. However, the rules governing axillary bud germination and growth and development of *P. yunnanensis* remain unclear to date. Therefore, taking axillary buds of *P. yunnanensis* as the main research object, this study measured seedling height, ground diameter, and biomass at four developmental stages of axillary buds. Root scanning was also carried out to explore the growth and development patterns of axillary buds in *P. yunnanensis*. This study lays an important theoretical foundation for the genetic improvement of *P. yunnanensis* and provides scientific guidance for the management of cutting orchards.

2. Materials and Methods

2.1 Overview of the test site

The experiment was carried out in the greenhouse of Southwest Forestry University, located in the north of Kunming City, at 25°04′00″north latitude, 102°45′41″ east longitude, and 1945 m above sea level. The annual average temperature is 14.7℃; the hottest month (July) the average temperature is 19.7℃; the coldest month (January) the average temperature is 7.5℃; the annual temperature difference is 12~13℃, the annual precipitation is 700~1100 mm, the relative humidity is 74%, the annual frost-free period is more than 240 days, the average annual sunshine is 2445.6 h. Sunshine rate 56%; Belongs to the north subtropical subhumid plateau monsoon climate, dry and wet season is distinct, May to October for the rainy season, precipitation accounted for about 85 % of the year, November to the next April for the dry season, precipitation accounted for only about 15 % of the year[31].

2.2 Test material

The seeds of the experimental seedling cultivation were collected from "Midu *P. yunnanensis* Clonal Seed Garden (Yun S-CSO-PY-001-2016)", the National *P. yunnanensis* Seed Base, Midu County, Dali Prefecture, Yunnan Province. This experiment was carried out in the greenhouse of Southwest Forestry University in the middle of January 2023. The seeds were soaked in potassium permanganate for 1 h in advance, washed with clean water, and soaked in clean water for 2 days. When sown, the soil and sowing plates were disinfected with potassium permanganate, and 2 to 3 seeds per hole were used. After planting, pine needles were applied, a greenhouse with arch frames was used, and water was applied regularly. Then, according to the "100 days seedling" method, the time of transplanting seedlings. After transplanting the seedlings into the cultivation substrate (that is, humus soil and red soil with a 3:1 allocation), the growth of seedlings was regularly observed, and water and fertilizer management was normal. According to the results of the previous pre-experiment, when *P. yunnanensis* seedlings in the natural growth state grow and develop to 8 months, the axillary buds will gradually break the dormancy state and become activated buds. Therefore, pot seedlings of *P. yunnanensis* at 8 months of age (16 cm diameter ×20 cm height) were selected for the test seedlings, and 600 seedlings with uniform growth and healthy and disease-free were selected for the axillary bud growth and development observation test. Because most of the axillary buds of *P. yunnanensis* seedlings were still in the dormant state when they were 8 months old, this time was regarded as the first stage of axillary bud development (axillary bud pre-initiation). When the seedlings were 10 months old, most of the axillary buds of *P. yunnanensis* seedlings had broken the dormancy state and become activated buds. Therefore, this time was regarded as the second period of axillary bud development (axillary bud activation). At the age of 12 months, most of the activated buds of *P.*

yunnanensis seedlings had developed into axillary buds, so this time was regarded as the third period of axillary bud development (namely axillary bud formation). Due to the decrease in winter temperature, most of the axillary buds of *P.yunnanensis* seedlings did not elongate and grow into side branches until 15 months of seedling age, and this time was defined as the fourth period of axillary bud development of *P.yunnanensis* (axillary bud elongation).

2.3 Determination and method of growth index

（1）Determination of seedling height and ground diameter

The height, ground diameter, and the number of axillary buds of *P. yunnanensis* seedlings were measured when the seedlings reached 8 months of age (when the axillary buds were dormant). Determinations were made at 30-d intervals for a total of nine assays. A ruler (accuracy 0.1 cm) and an electronic vernier caliper (accuracy 0.01 cm) were used to measure the height and ground diameter of *P. yunnanensis* seedlings. Taking the growth time x of *P. yunnanensis* seedlings as the independent variable and the cumulative growth amount as the dependent variable y, the Logistic equation $y=k/(1+e^{a-bt})$ was used to fit the seedling growth process on the DPS7.05 statistical analysis platform, and the parameters k, a, and b in the Logistic equation were estimated by the McQuat algorithm. Through the derivation of the equation, the time point corresponding to the growth peak, namely the rapid growth point, as well as the beginning and end points of the rapid growth period, can be obtained. Among them, the point of rapid growth =a/b, the start points of the rapid growth period =(a-1.317)/b, and the end point of the rapid growth period =(a+1.317)/b.

（2）Determination of biomass

According to the proposed four periods, the biomass of roots, stems, needles, and other organs was measured once in each period. Ten *P. yunnanensis* seedlings were randomly selected in each period and repeated three times for a total of 30 seedlings. The total excavation method (CAI Nianhui et al., 2024) was used to sample *P. yunnanensis* seedlings. Each *P. yunnanensis* seedling was divided into three organs: root, stem, and needle, and the fresh weight of the root, stem, and needle of each plant was weighed. Then, it was cleaned in an oven at 105 ° C for 30 min and then dried at 80 ° C until the mass was constant. The dry weight of roots, stems, and needles (biomass, accuracy 0.0001g) was weighed and placed in a dry and cool place and then used as the samples for subsequent nutrient content and glucose metabolism to be measured.

Aboveground biomass (g) = Stem biomass + Needle biomass

Belowground biomass (g) = Root biomass

Individual biomass (g) = Aboveground biomass + Belowground biomass

Stem biomass ratio (%) = (Stem biomass / Individual biomass) × 100%

Needle biomass ratio (%) = (Needle biomass / Individual biomass) × 100%

Root biomass ratio (%) = (Root biomass / Individual biomass) × 100%

Root-stem ratio = Belowground biomass / Aboveground biomass

（3）Root scan

The Phantom 9900XL Plus scanner was used to scan the roots at four stages of axillary bud growth and development. After scanning, LA-S Ten Thousand Deep Root Analysis software was used to obtain data.

2.4 Allometric growth analysis

The power function ($y=ax^b$) or its logarithmic distortion ($\lg t=\lg a+b\lg x$) is the most widely used model of relative biomass growth[32].The resulting in a linear relationship: y =a+bx. y=a+bx . Here's a detailed explanation: In this context, y and x each denote the biomasses of two distinct plant parts. The intercept of the trait relationship is denoted by a; The allometric growth index is represented by b, that is, the gradient of the linear relationship. Data analysis was carried out using standardized major axis (SMA) regression analysis to calculate the allometric growth constant and allometric growth index of the relationship between biomass[33].

2.5 Data statistics and analysis

The obtained data were preliminarily classified and analyzed, and Excel 2016, SPSS26.0, DPS7.05, and other software were used for statistical analysis. The growth, biomass, nutrient content, sugar content, and endogenous hormone content of seedlings were analyzed by one-way ANOVA using SPSS21.0, and the significance test was performed by Duncan's method (α=0.05). Origin2021 and GraphPad Prism 9 software were used for plotting, and all test data results were expressed in the form of Mean± standard error (Mean±SE).

3. Results

3.1 Growth of *Pinus yunnanensis* at different stages of axillary bud development

As shown in Table 1 and Figure 1-a, logistic growth fitting was performed on *P. yunnanensis* seedlings in the natural growth state. The fitting results showed that the fitting degree of seedling height and ground diameter under the natural growth state with time was extremely significant (P<0.01), seedling height showed a slow-fast-slow growth trend with time in the natural growth state, but the rapid growth point of seedling height was 170d, which was later than the rapid growth point of ground diameter of 137d, but the rapid growth period of seedling height lasted longer than that of ground diameter. However, among the four stages of axillary bud development of *P. yunnanensis*, only the resting stage of axillary bud (the first stage of axillary bud development) was in the rapid growth stage of seedling height, and the other stages were not in the rapid growth stage of seedling height and ground diameter. It can be seen from Figure 1-b that the change of activated buds is a trend of increasing and decreasing, and the first germination peak is in September, and the second germination peak is in February of the following year. This change trend may be caused by changes in temperature. However, the change of axillary buds showed a different trend. The number of axillary buds continued to increase from August to December, and the increase trend was stagnant in January of the following year. Although there was a small increase in February, there was no upward trend in the number of axillary buds in the subsequent growth of *P. yunnanensis*. The reason for the increase in the number of axillary buds may be the effect of temperature and the further development of some activated buds into axillary buds, while the reason for the decrease may be the further growth and development of some axillary buds into lateral branches or needles. In the process of growth and development of *P. yunnanensis*, seedling height and ground diameter have a high correlation with the number of axillary buds (Figure 1-c, d), and the correlation between the number of axillary buds and seedling height is higher than that of ground diameter, indicating that the growth of *P. yunnanensis* seedling height can also indirectly affect the number of axillary buds.

The needle length of *P. yunnanensis* showed a significant upward trend with the development of axillary buds. According to Figure 1-c, the length of needles is the shortest in the dormant stage of axillary bud development. With the development of axillary buds into the activation stage, the needles elongate rapidly, and the length increases significantly. The needle length further increases and reaches a maximum value during the developmental period and the elongation period. Therefore, the needle length increased step by step from the early stage to the end of bud development, and there were significant differences between each stage (P<0.05). This indicates that in the activation and development of axillary buds, the pre-formed needle primordia in axillary buds begin to elongate rapidly, and the bud scales gradually split, so that the needle tips are exposed and continuously extended. With the rise of temperature and the increase of light in spring, the dormancy of axillary

buds is broken, and new shoots and new needles begin to grow almost at the same time, and the elongation of needles is linearly correlated with the growth of new shoots until axillary buds are fully expanded.

Table 1 Fitting results of the logistic model for growth of *P. yunnanensis* seedlings under natural conditions

Metrics	Equation	Point of rapid growth	Rapid growth period	*P*	R^2
Height of seedling	$y=5.9752/(1+e^{(2.5541-0.015000x)})$	170	82-258	0.0002	0.9690
Ground Diameter	$y=4.9914/(1+e^{(3.0216-0.022112x)})$	137	77-196	0.0001	0.9930

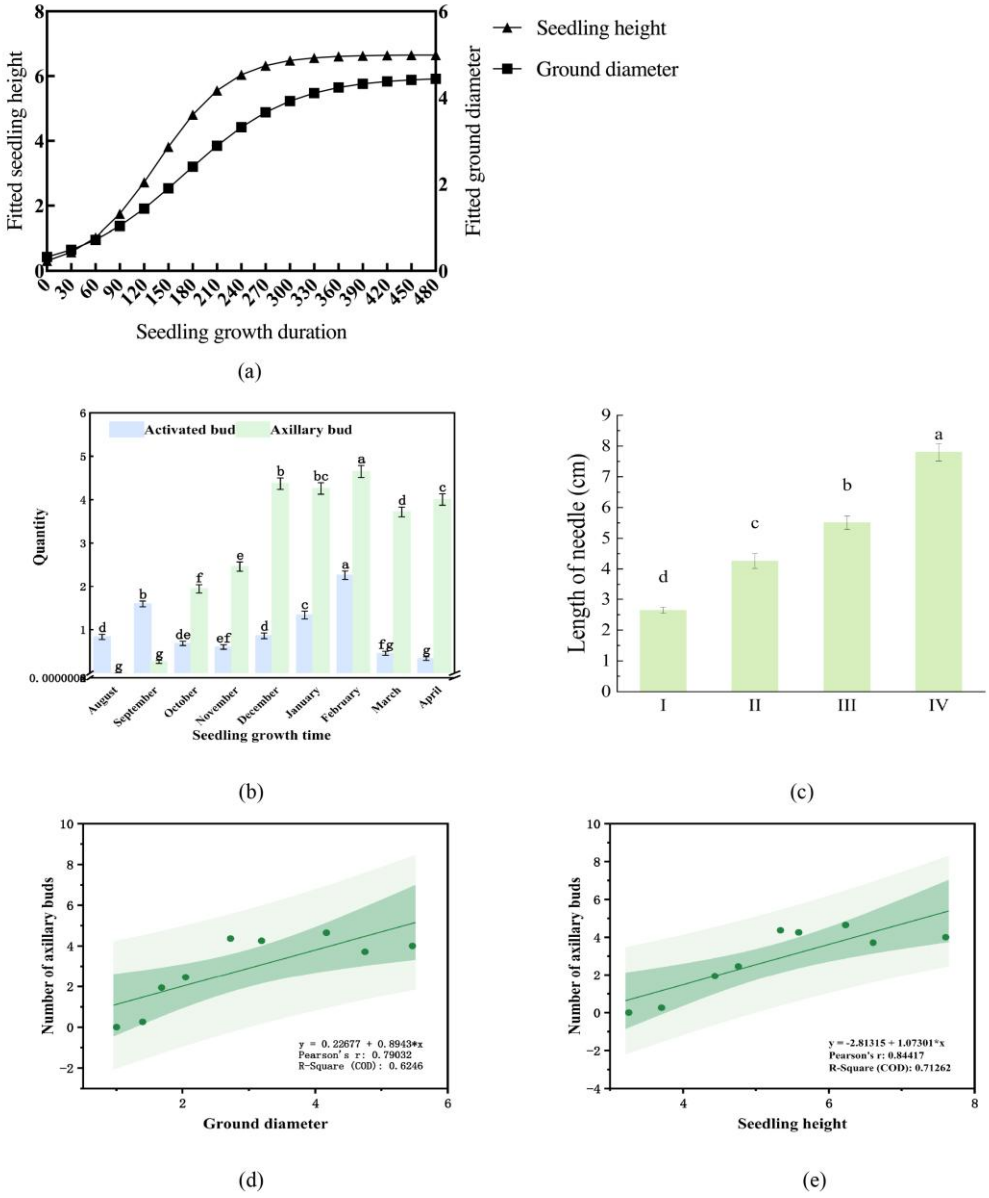


Figure 1 Growth of *P. yunnanensis* seedlings at different developmental stages of axillary buds

Note: Figure (a) shows the Logistic model curve of seedling height and ground diameter of Pinus yunnanensis under the natural growth state. The four development stages of axillary buds were 240d (I), 300d (II), 360d (III) and 450d (IV), respectively. Figure (b) shows the changes of activated buds and axillary buds from August to April of the next year. Different lowercase letters represent significant differences between different months. The data in the figure are mean ± standard error; the same below. Figure (c) shows the needle growth of *Pinus yunnanensis* seedlings at

different developmental stages of axillary buds; I: Axillary bud pre-initiation, II: Axillary bud activation, III: Axillary bud formation, IV: Axillary bud elongation, the same below. Figures (d)-(e) show the linear relationship between seedling height and ground diameter and the number of axillary buds, respectively.

3.2 Changes in root configurations of different grades at different axillary bud development stages of *P. yunnanensis*

The length, surface area, projected area, volume, number of root tips, and length of root tips of different diameter classes were measured in four stages: axillary bud pre-initiation, axillary bud activation, axillary bud formation, and axillary bud elongation.

The results in Figure 2 show that in the four stages of axillary bud development of *P. yunnensis*, roots with diameters of 0- 0.5 mm and 0.5 mm-2 mm accounted for the main part of the total root length, followed by roots with diameters of 2 mm-3 mm and 3 mm-5 mm, and roots with diameters of more than 5mm were the rarest. This indicates that *P. yunnensis* seedlings mainly develop fine roots (0- 2 mm), which are essential for nutrient uptake and growth. Further analysis shows that the growth trend of each index is similar under the same diameter class. In the axillary bud pre-initiation, the values of all indicators were almost close to 0, and the root growth was stagnant. From axillary bud pre-initiation to axillary bud activation, it increased slightly and slowly, and the root system started weak growth after axillary bud activation. The growth rate of axillary bud activation to axillary bud formation was significantly accelerated; the axillary bud entered rapid differentiation, and the root system expanded synchronously. The growth from axillary bud formation to axillary bud elongation showed an explosive steep increase. In the stage of rapid axillary bud elongation, root morphological indicators at all levels increased significantly, which was the most vigorous stage of root growth.

From the perspective of root diameter class differences, the values of all morphological indices of 0 mm-0.5 mm and 0.5 mm-2 mm roots were much higher than those of 2mm-3mm, 3mm-5mm and more than 5mm roots, and the base of all indices of roots above 2mm was low, and the increase was gentle. Axillary bud pre-initiation root activity was almost stagnant; in the axillary bud activation, the root system grew slowly, and the root tip differentiated slightly. In the axillary bud formation, a large number of axillary bud cells differentiate, and the nutrient demand increases, which drives the rapid proliferation and expansion of fine roots. In the axillary bud elongation, the axillary bud grows rapidly longitudinally, and the plant distributes a large amount of photosynthate to supply the underground fine roots. There is a synergistic promotion relationship between the development of the axillary bud above ground and the growth of underground fine roots.

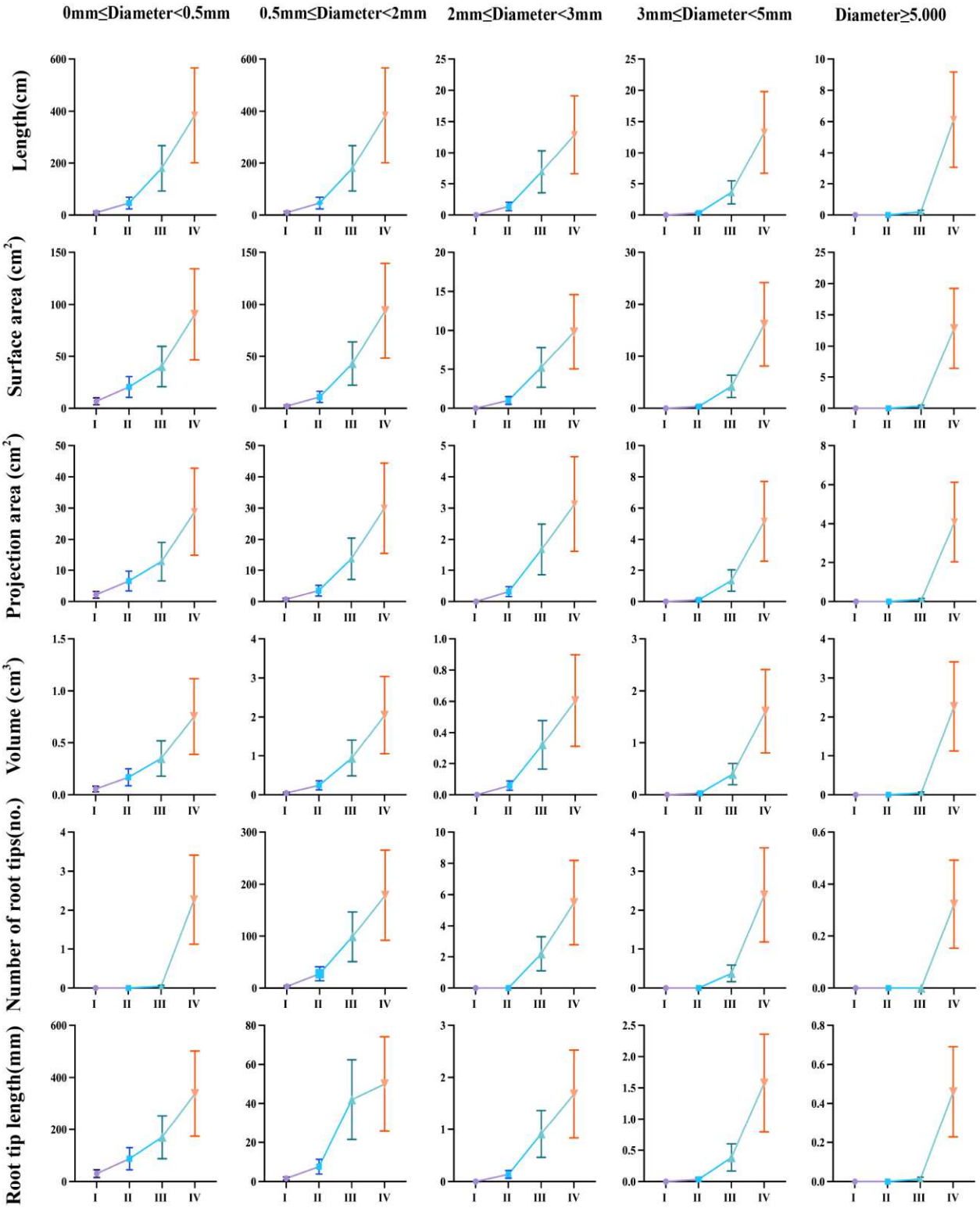


Figure 2 Changes of seedling root diameter grades at different developmental stages.

3.3 Changes of root architecture in different axillary bud development stages of *Pinus yunnanensis*

Four stages of axillary bud pre-initiation, axillary bud activation, axillary bud formation, and axillary bud development were analyzed for the root fractal dimension, average diameter, number of crossings, number of nodes, number of connections, root tip number, and pixel area, and the number of bifurcations were measured during the elongation period in Figure 3.

The results showed that all the root structural parameters except the average diameter showed a continuous upward trend with the development of axillary buds. Overall, axillary bud pre-initiation maintained the lowest level, and the period from axillary bud pre-initiation to axillary bud activation the period increased slowly and slightly, and the period from axillary bud activation to axillary bud formation increased steadily and the period from axillary bud development the axillary bud elongation increased sharply from period to period of axillary bud elongation, and all indicators reached the peak in period of axillary bud elongation. Other indicators, except average diameter were significantly higher than axillary bud pre-initiation, axillary bud activation, axillary bud formation, axillary bud pre-initiation, axillary bud activation, axillary bud formation, axillary bud pre-initiation. The first three stages of axillary bud elongation.

The results of fractal dimension showed that the spatial complexity of the root system increased significantly only during axillary bud elongation. There was no significant difference in the complexity of root architecture between axillary bud pre-initiation and axillary bud formation. The average diameter of the root system increased significantly with the development of the axillary bud. The number of nodes, connections, root tips, and bifurcations of root branch related indicators were highly synchronized with the changes of intersection points and pixel projection area of soil distribution characteristics indicators: The number of axillary bud pre-initiation was the least, and the underground spreading range was narrow; axillary bud activation, root growth started slowly and the number increased slightly; axillary bud formation leads to continuous branch expansion of root system. During the axillary bud elongation, the root system had a large number of multi-level branches with overlapping space, and the branch network and soil coverage area reached the maximum, and the number of root absorption sites increased significantly.

The overall results showed that there was a significant synergistic relationship between axillary bud development and underground root architecture construction. The rapid elongation stage of the axillary bud would drive the root system to form an absorption network with higher complexity, more developed branches, and a wider distribution range, so as to meet the nutrient demand of rapid growth

above ground.

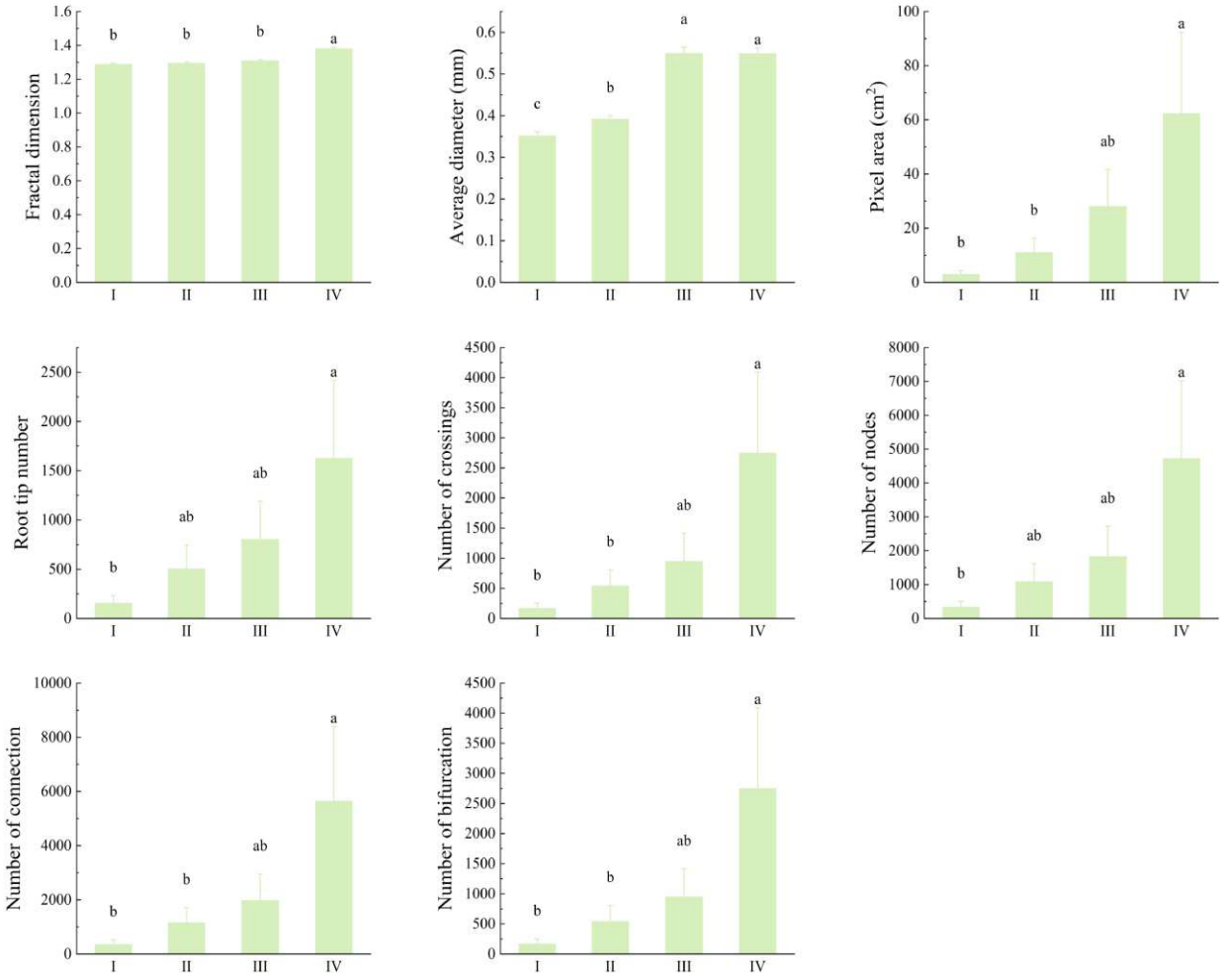


Figure 3 Changes in root architecture at different developmental stages of axillary buds

3.4 Variation analysis of root morphological traits under different axillary bud development stages

With axillary bud pre-initiation to axillary bud activation, axillary bud formation, as the axillary bud elongation progressed, the overall morphological indicators of the root system gradually increased, and the root growth level continued to increase with the axillary bud germination process (Figure 4). During the axillary bud pre-initiation, all root parameters were at a low level; only root tip number (RTN) and root length (RL) were relatively high, average diameter (MRD), volume (RV), and surface area (RSA) were low, and root growth was significantly inhibited. After entering the axillary bud activation stage, the root volume (RV) first increased significantly, and the other indicators maintained a high level except MRD. At this time, root growth and material accumulation were initiated. During the axillary bud formation, RV and root length (RL) increased significantly, while RPA and RSA increased synchronously, and root elongation and growth became the main characteristics. In the axillary bud elongation, the number of root tips (RTN) and RV reached the peak of the whole development stage, and the root branching ability was significantly enhanced. The

average root diameter (MRD) increased slowly during the whole development process, indicating that the root growth induced by axillary bud germination was mainly longitudinal elongation and new root tip occurrence, and the radial thickening of roots was weak. In each period, the trend of repeated polyline contours of T1, T2, and T3 groups was highly consistent. In conclusion, there was a sequential response of root morphology to axillary bud development, and the growth process showed the rule of volume accumulation first, length extension later, and a large number of differentiations of new root tips finally.

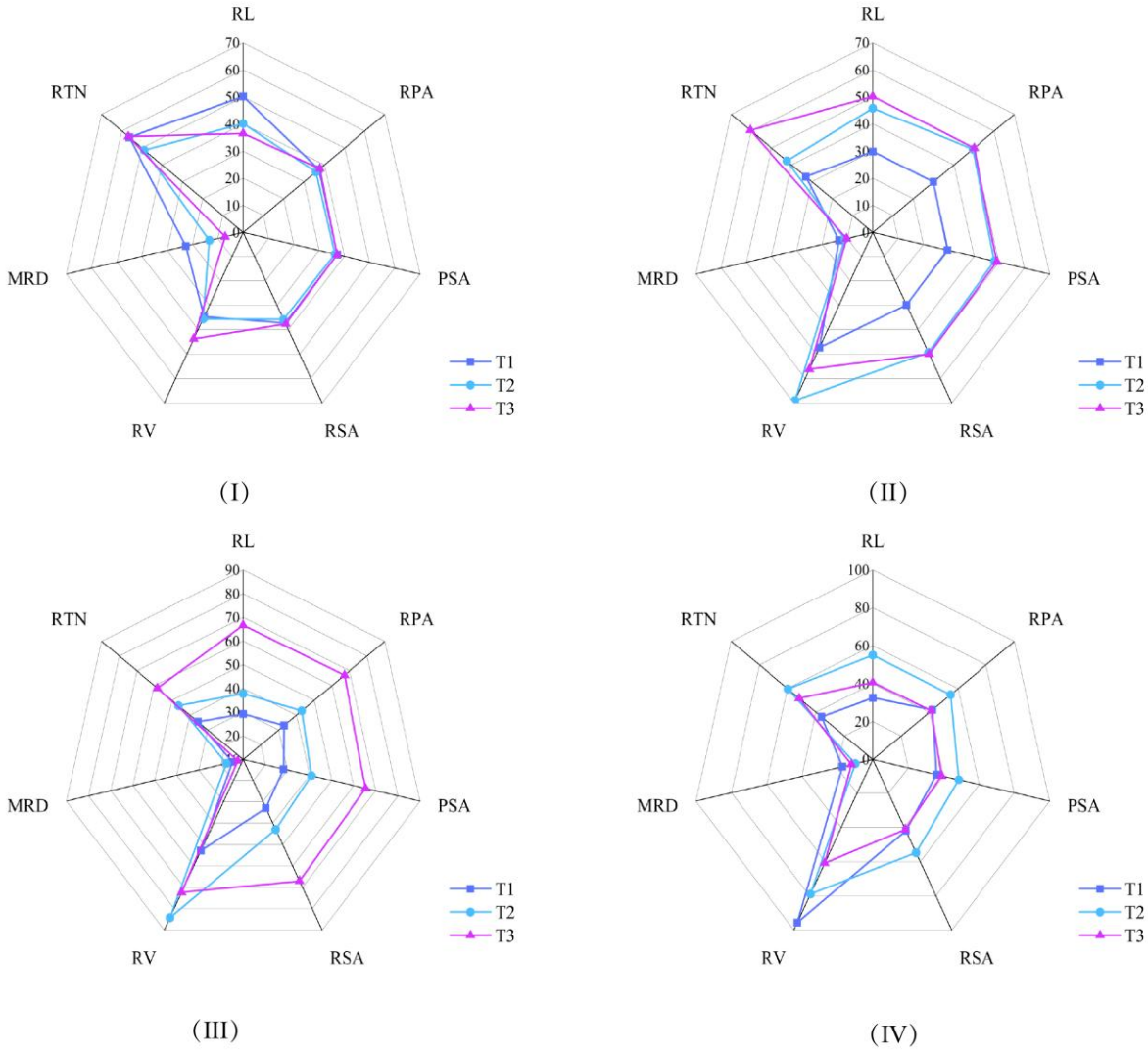


Figure 4 Variation analysis of root morphological traits under different axillary bud development stages

Note: Symbol description: Length (cm): RL; Projection area (cm^2): RPA; Pixel area (cm^2): PSA; Surface area (cm^2): RSA; Volume (cm^3): RV; Average diameter (mm): MRD; Number of root tips(no.): RTN, the same below.

3.5 Correlation analysis between different axillary bud development stages and root morphological traits

Mantel test was used to analyze the association between axillary bud development stage and root morphological characteristics (Figure 5). Root length (RL), projected area (RPA), pixel area (PSA),

surface area (RSA), and root tip number (RTN) were highly correlated with each other; root volume (V) was next. Mean root diameter (MRD) had the weakest correlation with other root indices, indicating that radial thick root growth was relatively independent of elongation and branching growth. The results of the Mantel test showed that there were significant differences in the strength of association between different stages of axillary bud development and root morphological indices. Axillary bud elongation and axillary bud activation were not significantly correlated with most root indices. Axillary bud formation was significantly correlated with root length (RL), root projection area (RPA), root surface area (RSA), root volume (RV), mean root diameter (MRD), and root tip number (RTN). Axillary bud pre-initiation and axillary bud formation were significantly correlated with mean root diameter (MRD), but axillary bud activation was not significantly correlated with root architecture. The results showed that axillary bud elongation was the most critical stage of root morphological remodeling, and the root length, surface area, and number of root tips were sensitive to the axillary bud development process. Root volume and average diameter of the root system can reflect the development status of axillary buds to a certain extent.

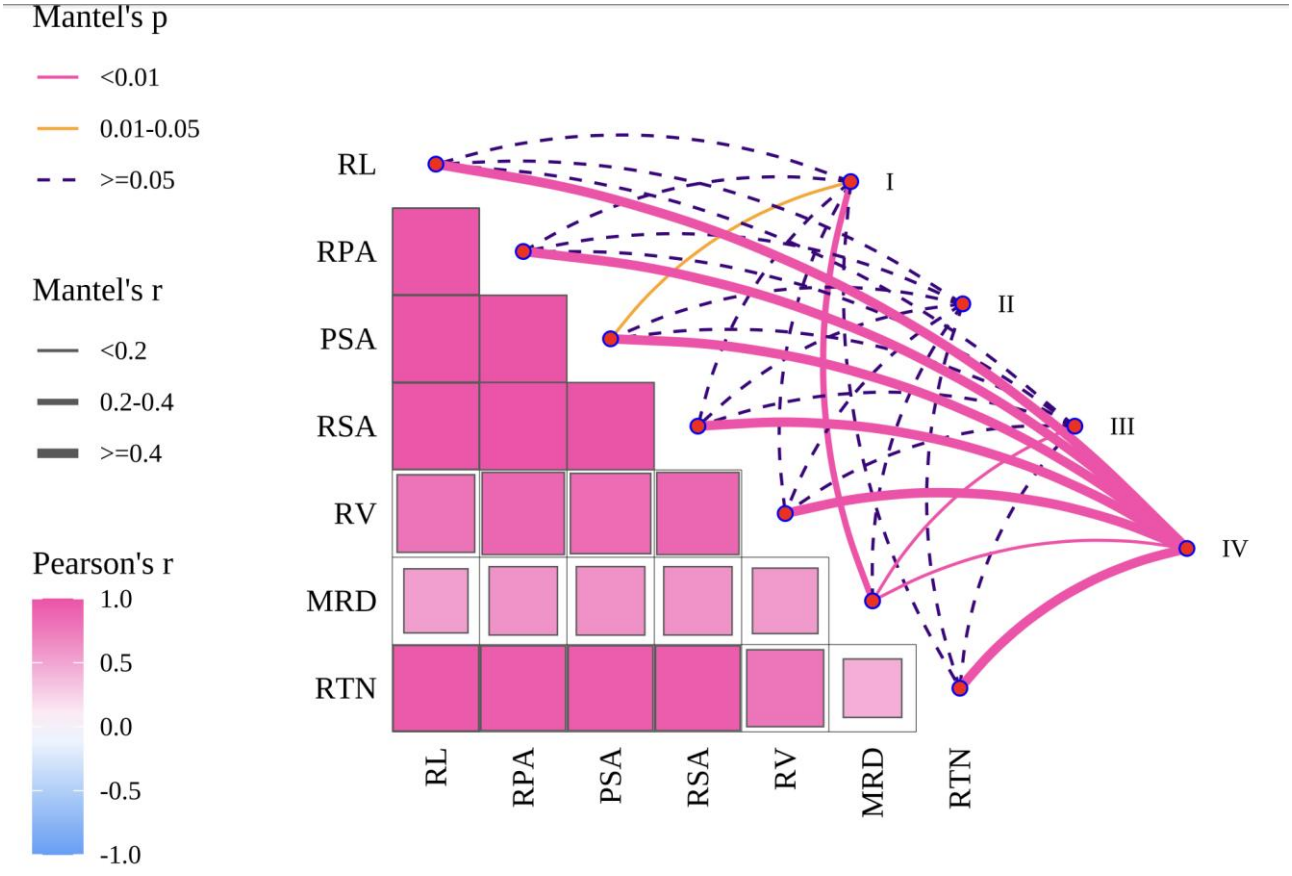


Figure 5 Correlation analysis between different axillary bud development stages and root morphological traits

Note: The bottom left matrix represents the Pearson correlation coefficient between the seven root morphological indicators (Env-cor; darker color and larger area represent higher correlation). The right line shows the Mantel test results. The color of the line represents the significance *P* value of the Mantel test, and pink represents the significant correlation. The thickness of the line represents the degree of association.

3.6 Biomass accumulation in different organs of *Pinus yunnanensis* seedlings at different development stages of the axillary bud

As the axillary bud goes from axillary bud pre-initiation to axillary bud activation, axillary bud formation, with the advancement of axillary bud elongation, the biomass of roots, stems, needles, aboveground biomass, and total biomass per plant showed a continuous and significant upward trend (Figure 6). The biomass of roots, stems, and needles during axillary bud pre-initiation and axillary bud activation was maintained at a low level, and there was no significant difference between them. After entering the axillary bud formation, the biomass of roots, stems, and needles increased significantly. All biomass indices reached the maximum value during the axillary bud elongation, which was significantly higher than the other three periods. The root-shoot ratio increased first and then decreased with axillary bud development: It increased significantly from axillary bud pre-initiation to axillary bud formation, and reached the maximum value in axillary bud formation. The root-shoot ratio decreased significantly after the axillary bud entered the stage of axillary bud elongation. The results showed that plants in the early stage of axillary bud development tended to allocate more biomass to roots. When axillary buds entered the stage of rapid elongation, the focus of plant resource allocation shifted to the aboveground organs, and the growth advantage of aboveground parts increased.

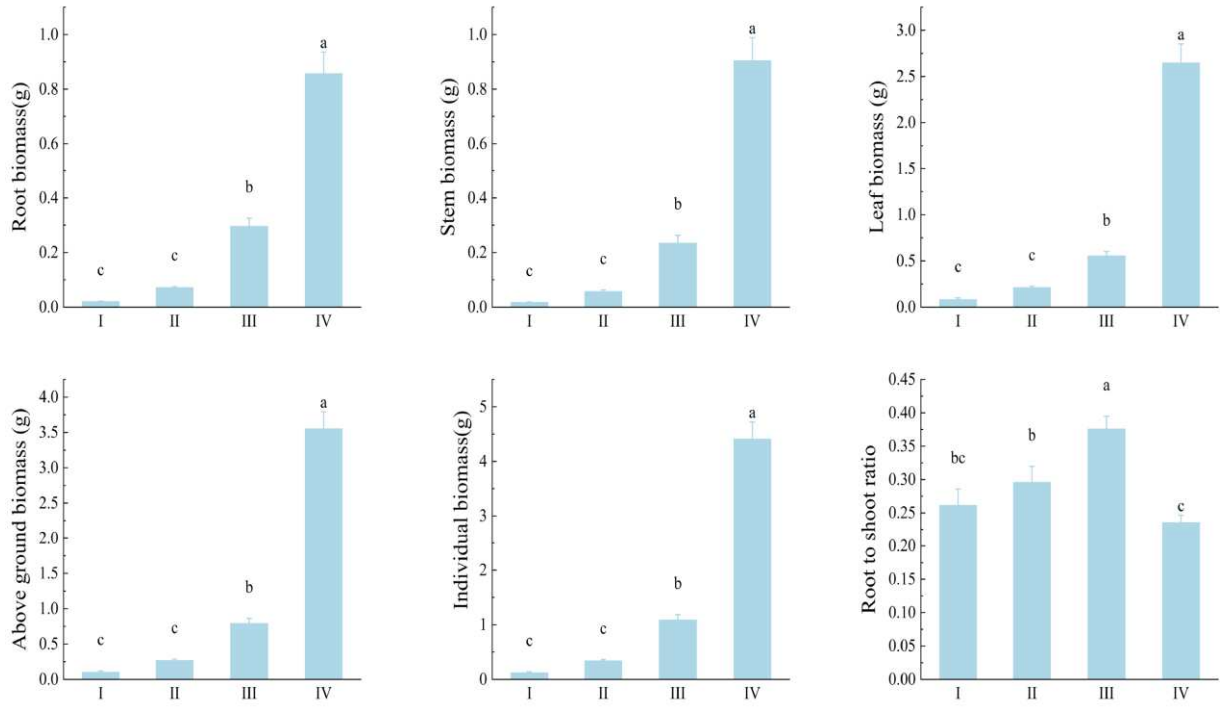


Figure 6 Biomass response of *P. yunnanensis* to different developmental stages of axillary buds

3.7 Biomass allocation of different organs of *Pinus yunnanensis* seedlings at different development stages of axillary buds

A ternary phase diagram was used to analyze the biomass allocation patterns of root biomass proportion (RBR), stem biomass proportion (SBR), and leaf biomass proportion (NBR) of plants at different stages of axillary bud development (Figure 7). In general, during the whole development process of axillary buds, all sample sites were close to the NBR and RBR axes, and the stem biomass allocation ratio (SBR) maintained a low level as a whole, and plant biomass was mainly allocated to needles and roots. The biomass proportion of axillary bud pre-initiation was concentrated in the area close to NBR, and the biomass proportion of needles was the highest, followed by that of roots. After entering the axillary bud activation, the proportion of biomass shifted slightly in the direction of RBR; the proportion of root biomass increased, and the proportion of stem allocation increased slightly. The RBR of the axillary bud formation increased further and reached the peak of the whole period. NBR decreased continuously compared with axillary bud pre-initiation and axillary bud activation. In the axillary bud elongation, the proportion of biomass returned to the NBR direction, the proportion of leaf biomass rose compared with that of axillary bud formation, the proportion of root distribution decreased, and the proportion of stem biomass did not show an overall increase.

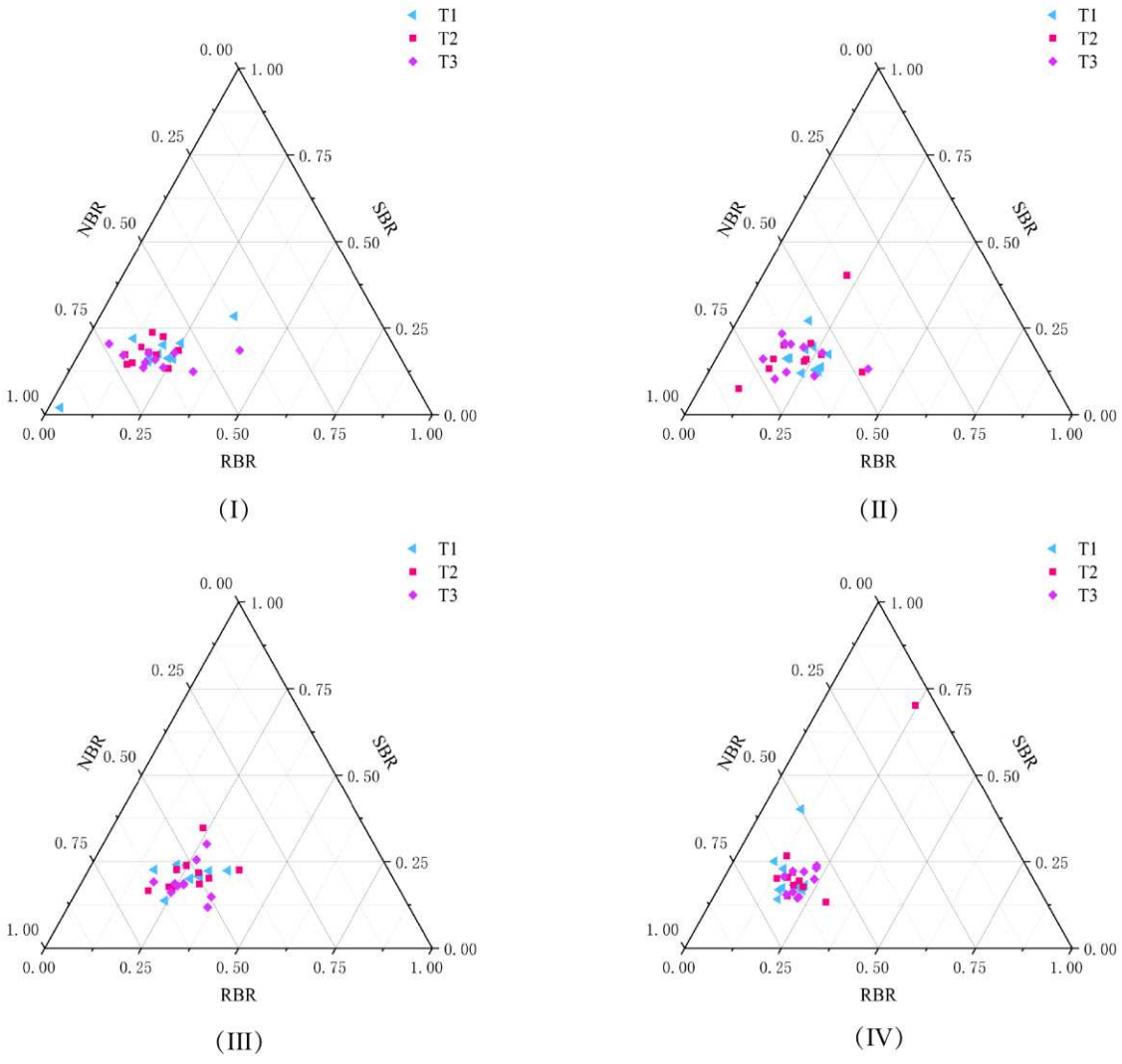


Figure 7 Biomass allocation of different organs in *P. yunnanensis* seedlings in response to different developmental

stages of axillary buds

Note: T1, T2, T3, for the three replicates of each epoch. Root biomass ratio: RBR, Stem biomass ratio: SBR, Needle biomass ratio: NBR

3.8 Allometric relationship of biomass allocation at different stages of axillary bud development

A logarithmic transformation allometric model was used to analyze the allometric relationship between root, stem, leaf, and aboveground biomass and total biomass per plant at different development stages of axillary buds (Fig. 8). Allometric slope significance test $P_{-1.0,}$ it was used to determine whether organ growth was isokinetic ($P_{-1.0}>0.05$, slope not significantly different from 1) or allometric ($P_{-1.0}<0.05$). The results showed that as the axillary bud progressed from pre-initiation to elongation, the growth trade-off pattern among root, stem, and leaf organs continued to be dynamically reshaped, and the combination characteristics of isokinetic and allometric growth differentiated at each developmental stage. axillary bud pre-initiation, the growth rhythm of root and shoot organs was isokinetic, but the growth rate of the shoot part, shoot, and leaf diverged into allometric. After the axillary bud was released from dormancy and entered axillary bud activation, only the growth of roots and stems first formed differences and showed allometric growth, while the other organs still maintained isokinetic growth. After entering axillary bud formation, allometric relationships were formed between root and leaf, stem and leaf, and shoot parts. During axillary bud elongation, the root and stem, needles, and shoot parts all showed isokinetic growth, and the rest showed allometric growth. In general, the axillary bud development process continuously regulates the biomass allocation tradeoff among plant organs, and the different isokinetic/allometric growth patterns at different developmental stages reflect the plastic growth strategy of plants to adapt to the axillary bud germination process.

The distribution characteristics of root, stem, needle, and aboveground biomass relative to the total biomass per plant were further analyzed. The results showed that the growth pattern of plant organs relative to the whole plant changed regularly with the development sequence of axillary buds. At the pre-initiation of the axillary bud, the biomass of root and stem grew isokinetically with the total biomass of the plant, and the biomass of needle and shoot grew allometrically with the total biomass of the plant. With the progress of development to axillary bud activation, only isokinetic growth was observed between root and stem biomass per plant, and the rest were allometric growth. Compared with axillary bud activation, axillary bud formation turned to an allometric growth mode,

and plant resources were invested toward the root system. After axillary bud elongation, the needles changed from isokinetic growth to allometric growth in axillary bud formation, the growth rate of photosynthetic organs accelerated, and the center of plant growth shifted from the root system to the shoot part.

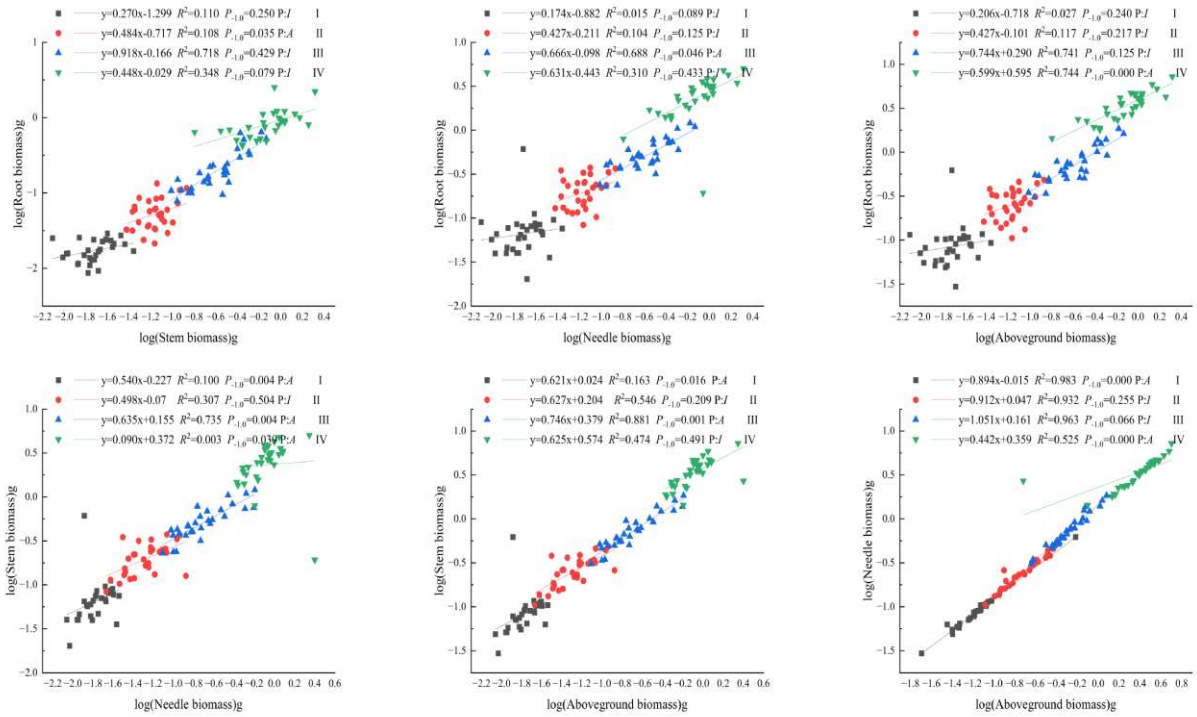


Figure 8 Allometric relationship of biomass between organs

Note: The fitting formula adopts the double-logarithmic linear equation; R^2 denotes the goodness of fit. (P-1.0) is applied to examine the significant difference between the allometric-growth slope and one. Equal-rate growth occurs when ($P_{-1.0}$>0.05), whereas allometric growth is defined as ($P_{-1.0}$<0.05), the same below.

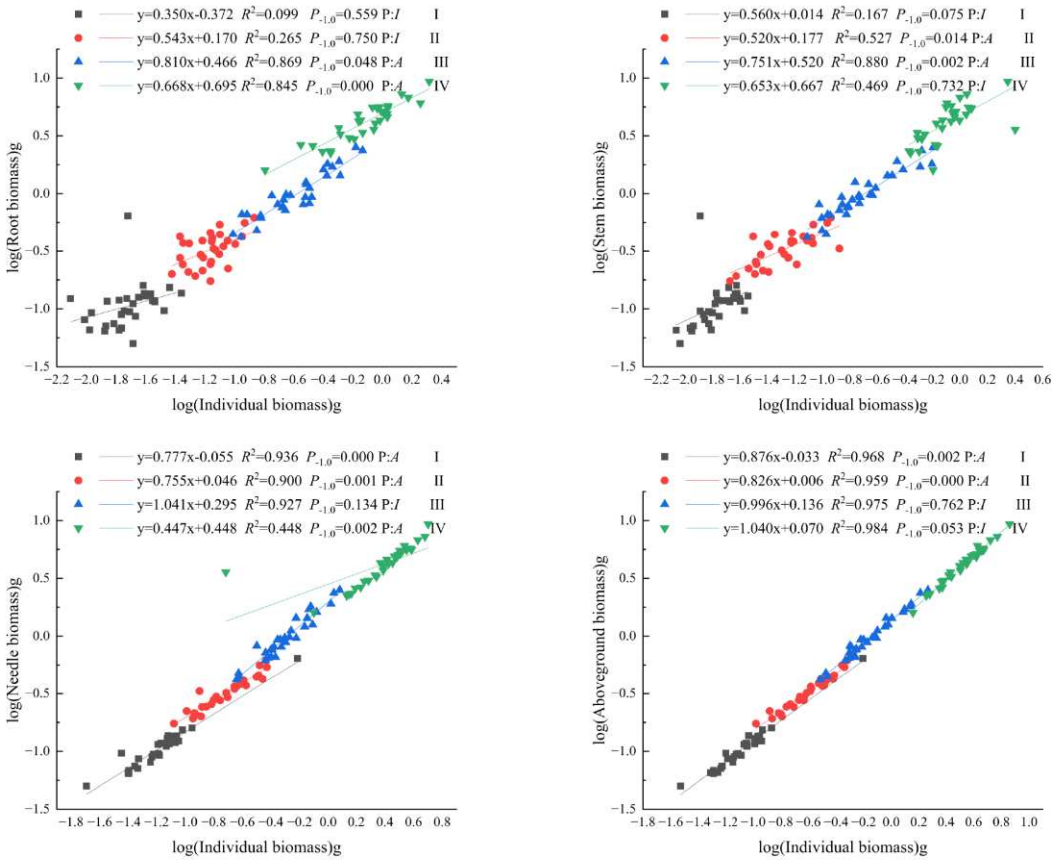


Figure 9 Allometric relationships between organs and individual plant biomass

4. Discussion

4.1 Seedling growth of *P. yunnanensis* along developmental stages of axillary buds

Axillary bud germination is a hallmark feature of plant growth and development, which not only diversifies plant architecture, but also changes plant biomass and productivity[34]. Seedling height and ground diameter are the most basic morphological indicators to reflect the growth state of plants. In this study, it was found that only the axillary bud dormancy stage was in the rapid growth stage of *P. yunnanensis* seedlings, and the other three stages were after the rapid growth stage of seedling height and ground diameter. It is generally believed that the vertical growth of plants sometimes weakens the lateral development of plants. This is mainly because when the plant grows vertically, it allocates more nutrients and energy, which indirectly reduces the ability to develop horizontally[35]. Therefore, in this study, after the rapid growth period of seedling height and ground diameter, the seedlings could have more nutrients allocated to break the dormancy of *P. yunnanensis* axillary buds, so that *P. yunnanensis* axillary buds could continue to grow and develop. Most plants today have evolved growth rhythms to keep their growth in line with environmental cycles on Earth. Most plant growth arrest and shoot dormancy are related to environmental rhythm changes, such as temperature, rainfall, and light[36]. *Crocus sativus* blooms and germinates in autumn, winter, and spring, but goes dormant in summer, while tulips bloom and grow in spring[37]. Therefore, different plants will improve their ability to reproduce in different ways to adapt to the environment. In this study, the growth status of axillary buds in autumn, winter and spring was observed, and it was found that the reason for the change in the number of axillary buds may be the influence of seasonal temperature plus the further development of some activated buds into axillary buds, and the reason for the decrease may be the further growth and development of some axillary buds into lateral branches or needles. Pine seedlings develop two different types of needles in the early ontogeny stage, namely primary needles and secondary needles[38]. The primary needles are short and flat, and each needle is arranged in a single helix on the stem. In contrast, secondary needles are longer and thicker and usually grow in clusters along the stem[39]. From the external morphology, it was observed that the axillary meristem of *P. yunnanensis* would promote the formation of leaf primordia and the growth of secondary needles during the development of axillary buds, so that the needle length of *P. yunnanensis* showed a significant upward trend with the development of axillary buds.

4.2 Changes of root architecture in different stages of axillary bud development of *P. yunnanensis*

Roots not only provide structural support for the aboveground parts of the plant but also transport nutrients and water. Thus, the survival of plants depends on their proper growth, development, and proper root function[40]. Root growth and configuration seriously affect seedling morphology and

survival rate[41]. Roots are not only the main organ for absorbing water and nutrients, but also an important place for synthesizing hormones, amino acids, and organic acids. In addition, root morphology and physiology are closely related to the aboveground parts, including seedling height, ground diameter, and tiller ability[42]. In this study, six morphological indicators of roots with different diameter classes were measured across four axillary bud developmental stages of Pinus yunnanensis. The results showed that seedlings were dominated by fine roots of 0–2 mm, which are critical for nutrient uptake. Root indicators gradually increased with axillary bud development: root growth nearly stagnated in the axillary bud pre-initiation, increased slowly in the axillary bud activation, accelerated in the axillary bud formation, and exhibited explosive growth in the axillary bud elongation. All indicators of 0-2 mm fine roots were significantly higher than those of coarse roots. The differentiation and elongation of aboveground axillary buds raise nutrient demands, driving the allocation of photosynthates to fine roots. There exists a synergistic promoting relationship between aboveground axillary bud development and underground fine root growth. Increasing root length is an important strategy for plants to improve root cover area in the soil layer and plant nutrient acquisition efficiency[43].

In order to maintain the continuous growth and development of axillary buds in the above part, the roots in the underground part have to expand the growth area to improve nutrient absorption efficiency. Since plants need to draw water and essential minerals from the soil, and these resources are not evenly distributed in the soil, seedling foraging efficiency and productivity in the soil fundamentally depend on dynamic root growth and root configuration[44]. And many root architecture properties have been shown to increase plant growth rates and change plant phenotypes, which are especially important in the seedling stage of plants[45, 46]. The three-dimensional geometric structure of roots is closely related to their ability to compete for nutrients, so this structure is often used to evaluate the root shape and distribution in soil. The characteristics of fractal dimension help us better understand the function and growth strategy of plant roots in soil. The topological structure of roots not only determines the spatial distribution of roots, but also has an important impact on their nutrient absorption and fixation ability[47]. In this study, nine root indexes of *P. yunnanensis* at the axillary bud elongation stage were measured. It was found that except for the average diameter, which continued to increase, the other indexes gradually increased with axillary bud development and reached the peak in axillary bud elongation. Axillary bud elongation and root branches developed and distributed more widely. The results indicate that axillary bud development is coordinated with root architecture construction, and an efficient absorption network is formed during axillary bud elongation to supply above-ground growth. With the advancement of axillary bud development, the root morphological index gradually increased. The root parameters of axillary bud pre-initiation were low. Root volume increased first during axillary bud activation. During axillary bud formation, root length and

projection area increased significantly. The number of root tips and root volume peaked during axillary bud elongation. The average diameter of the root system increased slowly, and the growth was mainly longitudinal elongation and new root tip formation. Mantel test was used to analyze the relationship between axillary bud development and root morphology. The results showed that root length (RL), projection area (RPA), pixel area (PSA), surface area (RSA), and root tip number (RTN) were highly correlated with each other. Root volume (V) was the second, average root diameter (MRD) had the weakest correlation with other root indicators, and axillary bud elongation was highly significantly correlated with root architecture indicators. Root surface area can reflect the nutrient absorption efficiency of plant fine roots in the soil. The larger the value is, the higher the nutrient utilization efficiency of the plant under the same external conditions[48]. In the study of roots of *P. yunnensis* at different seedling ages, it was found that with the increase of seedling age, the development of root tissue degenerated, the development of vascular bundles and xylem improved, and the rate of root metabolism and nutrient absorption decreased, thereby improving the ability of root transport and storage of nutrients[49]. In this study, with the continuous development of axillary buds, the demand for nutrients in the aerial part of *P. yunnensis* seedlings also increased. The length of needles also reflected that *P. yunnensis* seedlings needed more photosynthesis to improve nutrient supply during the four stages of axillary bud development. The aerial part is mainly responsible for the transport and storage of seedling nutrients, and the breaking of axillary bud dormancy and subsequent growth and development are all based on sufficient root development. This not only reflects the balanced growth of seedlings but also reflects the characteristics of above-underground cooperative growth of seedlings.

4.3 Biomass changes at different stages of axillary bud development of *P. yunnanensis*

Biomass is a key factor in the process of plant growth, reflecting the ability of plants to accumulate energy[50]. Seedling biomass can reflect the adaptability of seedlings to the external environment, which is of great significance for scientific evaluation of seedlings[51]. The allocation of biomass between plant parts is affected by a variety of factors, including environmental conditions, plant age, and individual plant size[52]. The allocation strategy of biomass in plants may be an optimization process in response to stress[53]. With the advancement of axillary bud development, the biomass of each organ, above-ground and total of *P. yunnensis,* continued to increase significantly. The biomass of axillary bud pre-initiation and axillary bud activation was lower, and most of them had no significant difference. The biomass of each axillary bud elongation reached the peak value. The root-shoot ratio first increased and then decreased, and the axillary bud formation was the maximum. In the early stage of axillary bud development, the biomass was preferentially allocated

to the root system, and the focus of resource allocation shifted to the aboveground organs during axillary bud elongation. The results showed that the biomass of *P. yunnanensis* was mainly allocated to needles and roots, and the proportion of stem was low. The proportion of leaf biomass in axillary bud pre-initiation was the highest. The proportion of root biomass increased slightly during axillary bud activation. During axillary bud formation, the proportion of root biomass reached the peak, and the proportion of leaf biomass decreased. The proportion of leaf biomass increased, and the proportion of root biomass decreased during axillary bud elongation. This result is similar to the study of *P. yunnensis* grading, which found that the proportion of needle biomass was the highest in the two extreme seedlings after grading treatment[54]. This phenomenon indicates that, from the perspective of axillary bud development or growth variation of *P. yunnensis* seedlings, seedlings will adjust the biomass allocation of each organ in stages to coordinate the utilization of seedling growth resources and overall photosynthetic efficiency, so as to realize the coordinated optimization of the seedling growth and development process. Root-stem ratio can reflect the growth correlation between underground and aboveground parts of plants. In this study, it was found that the root-stem ratio of *P. yunnensis* seedlings was higher in the stage of axillary bud development, indicating that in the stage of rapid axillary bud development, *P. yunnensis* seedlings had a stronger demand for nutrients and increased competitiveness for nutrients and energy, which led to a relatively weak root biomass reserve. Allometry is when two phenotypic traits of an organism exhibit a disproportionate growth rate during growth[55]. According to allometric theory, the allocation of resources by plants will be constrained by individual size: specifically, smaller plants will preferentially devote resources to leaf development, thereby maximizing light energy harvest[56]. The allometric model was used to explore the allometric relationship of plant biomass at the axillary bud development stage. Organ between isokinetic, allometric growth pattern with axillary bud development dynamic remodeling; Organ total plant biomass analysis showed that axillary bud formation resources were preferentially supplied to the root system, axillary bud elongation needles were allometric growth, the growth center was shifted to the ground, and the plant depended on biomass allocation plasticity to adapt to axillary bud germination.

5. Conclusion

In order to explore the growth of *P. yunnensis* seedlings in different axillary bud development stages, the seedling height and ground diameter of *P. yunnensis* seedlings conformed to the Logistic growth process in the whole process of axillary bud growth, and the seedling height of *P. yunnensis* was in the rapid growth stage during the axillary bud pre-initiation. In addition, during the growth and development of *P. yunnensis*, seedling height and ground diameter had a high correlation with the number of axillary buds. In order to provide sufficient energy for the development of axillary buds of *P. yunnensis*, the differentiation and elongation process of the above axillary buds, accompanied by continuous material demand, drove the adaptive changes in the morphology of the underground root system and the allocation pattern of plant biomass. Axillary bud development of *P. yunnensis* significantly regulated underground root growth and plant biomass allocation, with 0- 2 mm fine roots as the main root system. Root morphological indicators gradually increased with axillary bud development, and except for the average diameter, which continued to increase, other root structural indicators reached the peak at the elongation stage, at which time the root branches were developed, and the spatial configuration complexity was significantly increased. Mantel test confirmed that axillary bud elongation was the key stage of root structure remodeling, and root length, surface area, and root tip number were sensitive to axillary bud development. With the advancement of axillary bud development, the biomass of each organ of the plant continued to rise, the root-stem ratio first increased and then decreased, and the axillary bud formation reached the maximum value. Biomass was mainly allocated to roots and needles, and the proportion of stem was low. Allometric analysis showed that axillary bud development regulated the biomass allocation tradeoff of plant organs, axillary bud formation preferentially supplied roots, and axillary bud elongation shifted its growth focus to needles, thus generating a plastic strategy suitable for axillary bud germination. In conclusion, there was a synergistic relationship between aboveground axillary bud development and underground fine root growth, and plants dynamically adjusted root architecture and biomass allocation strategy to meet the nutrient requirements of axillary buds at different stages of development.

Declarations

Author Contributions: Writing—original draft, X.G.; data analysis, X.G., C.Z. and B.Y.; conceptualization, experimental design and funding acquisition, B.Y., Y.X. and N.C.; investigation, C.Z.; writing—review and editing, C.Z., N.C., X.Y., X.K. and B.Y. All authors have read and agreed to the published version of the manuscript.

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