RELATIONSHIP OF CHANGES IN THE STEM AND LEAF MORPHOLOGY, NUTRIENT AND ENDOGENOUS HORMONE CONTENTS AND FLOWER BUD NUMBER OF *POPULUS EUPHRATICA* OLIV.

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Abstract. During *Populus euphratica* ontogeny, the morphological changes of stems and leaves, nutrient and endogenous hormone contents were significantly different, but the relationship between these changes and the number of flower buds was not clear. We counted the number of *P. euphratica* flower buds, and measured the morphological of the stems and leaves nutrient and endogenous hormone contents at different ontogeny to clarify the change trends in the morphology and nutrient and endogenous hormone contents of stems and leaves and mutual relationships in the flower bud number throughout the ontogeny. The results showed that in diameter 4, flower buds and broad-ovate leaves appeared meanwhile, indicating that *P. euphratica* had entered the reproductive growth stage. Between diameter 4 to 12, stem length, leaf number and leaf C/N gradually decreased, and leaf area, leaf nitrogen, organic carbon, and ZR contents gradually increased, indicating diameters 4-12 represent the stage during which vegetative growth and reproductive growth cooccur in *P. euphratica*. In diameter 14, flower bud number increased significantly, while leaf shape index and stem diameter decreased. *P. euphratica* began to enter the vigorous reproductive growth stage. In addition, correlations were found between leaf and stem nitrogen, phosphorus, organic carbon, GA_3, ABA, IAA contents, leaf C/N and leave and stem morphology was closely related to the ontogeny; the change in coordination significantly affected the flower bud number. The synergistic changes in the nutrient and endogenous hormone contents in *P. euphratica* stems and leaves regulate the morphology of stems and leaves and flower bud number and directly or indirectly aaffect the transition from vegetative growth to reproductive growth.

Keywords: *leaf shape index, nitrogen, organic carbon, ABA, phase change*

Introduction

The transition from vegetative growth to reproductive growth is marked by flower bud differentiation; initiation of flowering is the standard for dividing juvenile from adult (Guo et al., 2008; Hao et al., 2017). The morphology of stems and leaves can also be used to measure the growth and development of trees (Besford et al., 1996; Zhang et al., 1997; Jin et al., 1998; Du et al., 2018). Research shows that petiole roughness and the leaf shape index have been considered for use as markers of the juvenile stage in peach trees (Zhang et al., 1997). *Malus hupehensis* leaves in the juvenile stage have deep cracks and straight lateral veins; in the adult stage, the leaf crack disappears, and the lateral veins become pliable and parabolic (Jin et al., 1998). In the model plant *Arabidopsis thaliana*, the shift to maturity is marked by a decrease in leaf hairs on the leaf blade after a certain age. The development of early flowers is often accompanied by

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a reduction in leaf hairs (Besford et al., 1996). During this transition, the nutrient and hormone content of leaves and stems also changed significantly. Xu et al. (2018) measured the nutrient contents in overwintering stems of *Styrax tonkinensis* Craib ex Hartw. and established a relationship between nutrient levels and blossom number. The results showed that a high carbohydrate content was beneficial to the flowering of *Styrax tonkinensis* Craib ex Hartw. Changes in the contents and equilibrium values of endogenous hormones such as gibberellin (GA_3), indole acetic acid (IAA), abscisic acid (ABA) and zeatin (ZR) regulate the amount of flowering on each branch and the stage transitions of trees (Munoz et al., 2012; Gao et al., 2012; Feng et al., 2014; Zhu et al., 2015; Hassankhah et al., 2018; Yu et al., 2019). GA increased the number of flowers per branch and significantly changed the vegetative branch to flowering branch ratio in the next year (Munoz et al., 2012; Hassankhah et al., 2018).

P. euphratica exhibits heteromorphism, showing only strip leaves in the seedling stage, followed by lanceolate ovate and broad-ovate leaves that gradually appear with ontogeny (Li et al., 2021). Previous studies have shown that there is a certain relationship between leaf morphology and flowering of *P. euphratica* (Huang et al., 2010a,b). The appearance of broad-ovoid leaves, stems length, leaves number, total nitrogen content in leaves, stems and leaves C/N ratio and ZR content in leaves could be used as indicators of the transition from juvenile to the adult stage of *P. euphratica* (Li et al., 2015b). Feng et al. (2014) showed that the total N content of *P. euphratica* varied with the location and growth stage of leaves, and the leaf shape index decreased with the increase of total N content. Li et al. (2017) showed that the leaf shape index of *P. euphratica* decreased gradually from the bottom to the top of the crown, and the broad-ovoid leaves were mostly distributed at the top of the crown, while GA_3 content showed a significant positive correlation with the leaf shape index, indicating that GA_3 content was closely related to the emergence of broad-ovoid leaves. Most studies showed that there was a certain correlation between the morphological and nutrients and endogenous hormone contents in stems and leaves. But the relationship between the morphological, nutrients, endogenous hormones contents in stems and leaves and the number of flower buds was not clear.

This study hypothesized that there was a correlation between leaf morphology, nutrient and endogenous hormone content and the number of flower buds. At the different stages of the investigation, individual stems of *P. euphratica* were assessed for their flower bud number, stem and leaf morphological characteristics, stem and leaf nutrients and endogenous hormone contents. The changes in stem and leaf morphology as well as in flower bud number and nutrient and endogenous hormone contents with tree development and their mutual relationships were analyzed to reveal the roles of the morphological and physiological characteristics of the stems and leaves of *P. euphratica* in the reproductive growth.

Materials and methods

Study site, plant material and experimental design

The study area is located on the northwest edge of the Tarim Basin, Alar, Xinjiang, China (81°17′56.52″E，40°32′36.90″N). The area of the artificial *P. euphratica* forest was 180.6 hectares, the spacing of plants and rows was 1.20 m×4.20 m, and the average tree height was 6.41 m. The forest included 355 strains of *P. euphratica* in different stages of development (different ages). This region experiences hot, dry weather and little rainfall throughout the year. The average temperature is 10.8°C, the average annual precipitation is 50 mm, and the potential evaporation is 1900 mm, with average annual sunshine hours of 2900 h, making it a typical temperate desert climate.

In the area of the artificial *P. euphratica* forest located on the northwest edge of the Tarim Basin, Xinjiang, China (81°17′56.52″E，40°32′36.90″N), all *Populus euphratica* trees with DBHs above 2 cm were investigated. The mean DBH (diameter at breast height, D) and mean age (A) of each class diameter of *Populus euphratica* fit the following relationship: $A = 13.679 / (1 + 3.3476\times\exp(-0.2099\ D))$ (Gu et al., 2013). The trees were sorted into age diameters as determined by their diameter. The DBH of each plant was rounded to the nearest 2 cm, and nine class diameters were established: 2, 4, 6, 8, 10, 12, 14, 16 and 18 cm. A total of 27 *P. euphratica* plants were selected from the 2 cm diameter to the 18 cm diameter, with 3 plants in each diameter taken for sampling; of these, 21 were flowering plants, sampling at year level (*Table 1*).

Table 1. *Distribution of diameter class, DBH and age*

Diameter class	2	4	6	8	10	12	14	16	18
Average DBH	2.44	3.99	5.95	7.85	9.79	11.99	14.08	15.66	17.40
Average age	4.13	5.20	6.48	7.72	8.65	10.33	10.52	11.63	11.33

The crown of the sample tree is divided into 5 layers from base to top. One-year-old stems were randomly selected from each layer from the east, south, west and north directions, and 20 stems were collected from each sample tree. All leaves from the base to the end of each stem were taken as the sample leaves. The collected stems were brought back to the laboratory, and the number of leaves and flowers per stem were counted. All nodal leaves from the base to the end of each stem were selected for stem and leaf morphological measurement and nutrient and endogenous hormone content measurement.

Determination of morphological indicators of flower buds, stems and leaves

Leaves (with petioles) were taken from the same stem and arranged in the order in which they grew on the stem. The stems, leaves and flower buds were scanned by an MRS-9600TFU2 scanner (made in Microtek). The measurements of stems (length and roughness) and leaves (leaf length, leaf width, leaf area, petiole length and leaf perimeter) were obtained by an LA-S plant image analyzer (made in Hangzhou wseen), and the leaf shape index (length/width) was calculated, the leaf shape index larger, the leaf shape tends to be round.

Determination of nutrient contents of flower buds, stems and leaves

All the stems of the same grade were combined, and all the leaves taken from the stems of each grade were combined. The mixed samples were rinsed with tap water and then rinsed with deionized water twice. They were dried in the shade and then placed in an oven. The samples were subjected to enzymolysis at 105°C for 10 min and then dried to a constant weight at 65°C. After the dried samples were removed from the oven, they were quickly ground through a 100-mesh sieve with a plant grinder for the determination of their total nitrogen, total phosphorus, total potassium and organic carbon contents. The total nitrogen content was determined by the Kjeldahl method. The total phosphorus content was determined by the molybdenum antimony colorimetric method. The total potassium content was determined by ammonium acetate extraction-flame photometry. The low-temperature external thermal potassium dichromate oxidation method determined the organic carbon content.

Determination of the endogenous hormone contents of flower buds, stems and leaves

All the stems of the same grade were combined, and all the leaves taken from the stems of each grade were combined. And in the day, approximately 0.2 g of the fresh mixed sample was weighed, quickly frozen with liquid nitrogen and stored in an ultralow-temperature refrigerator at -80°C for later use. The contents of indole acetic acid (IAA), zeatin (ZR), gibberellin (GA_3) and abscisic acid (ABA) were determined by an enzyme-linked immunosorbent assay. This part of the testing work was performed by China Agricultural University.

Statistical analyses

SPSS 25.0 was used to perform one-way ANOVA, correlation analysis, path analysis and stepwise regression analysis. The differences in leaf morphology, nutrient and endogenous hormone content and number of flower buds among different class diameters were analyzed, and the factors that had direct or indirect influences on the morphology of stems and leaves and the number of flower buds were identified. And differences were considered significant at $\alpha = 0.05$ by Tukey’s test.

Results

The morphology of stems and leaves and the flower bud number changed with developmental stage

With increasing class diameter, the leaf shape index, stem length and leaf number (*Figure 1b*) gradually decreased, while the stem diameter, flower bud number, leaf area, leaf perimeter (*Figure 1f*) and petiole length gradually increased (*Figure 1a-h*). From 4 diameter, flower buds and broad ovate leaves appeared at the same time, and stems became significantly shorter, indicating that *P. euphratica* individuals began to enter reproductive growth (*Figure S1*). From 4 diameter to 12 diameter, flower buds number gradually increased, the differences were not significant (*Figure 1a*). While the leaf shape index gradually decreased (*Figure 1d*), stems became thicker (*Figure 1g*), leaf area (*Figure 1c*), petiole length (*Figure 1e*) and leaf length (*Figure 1h*) increased, and it was a significant difference. When the flower buds number increased significantly to 1.36, *P. euphratica* individuals entered the vigorous reproductive growth stage and stems reached the thickest state, 0.42 cm.

Characteristic changes in stem and leaf nutrient contents with developmental stage

As shown in *Figure 2a-j*, with increasing class diameter, the contents of total nitrogen, phosphorus, potassium (*Figure 2e,f*) and organic carbon in stems and leaves increased gradually, while the C/N ratio of stems and leaves decreased gradually. The results showed that the total nitrogen content of leaves (*Figure 2b*) and the organic carbon content of stems (*Figure 2g*) increased significantly, and the C/N ratio of stems and leaves (*Figure 2i,j*) decreased significantly when flower buds appeared at the beginning of 4 diameter. From 4 to 12 diameter, the number of flower buds gradually increased (*Figure 1a*), the leaf shape index gradually decreased (*Figure 1d*), the leaf area gradually increased (*Figure 1c*), and the stems became thicker and shorter (*Figure 1g,h*), and the total nitrogen contents in stems and leaves (*Figure 2a,b*), total phosphorus in stems and leaf (*Figure 2c,d*) organic carbon (*Figure 2g,h*) gradually increased, the C/N ratio of stems and leaves (*Figure 2i,j*) unchanged, and the differences were not significant. When the flower buds' number of 14 diameter increased significantly, the total nitrogen content and total phosphorus content of leaves also increased significantly and reached the maximum value of 1.38%, 0.62%, 46.20% and 39.77%, respectively.

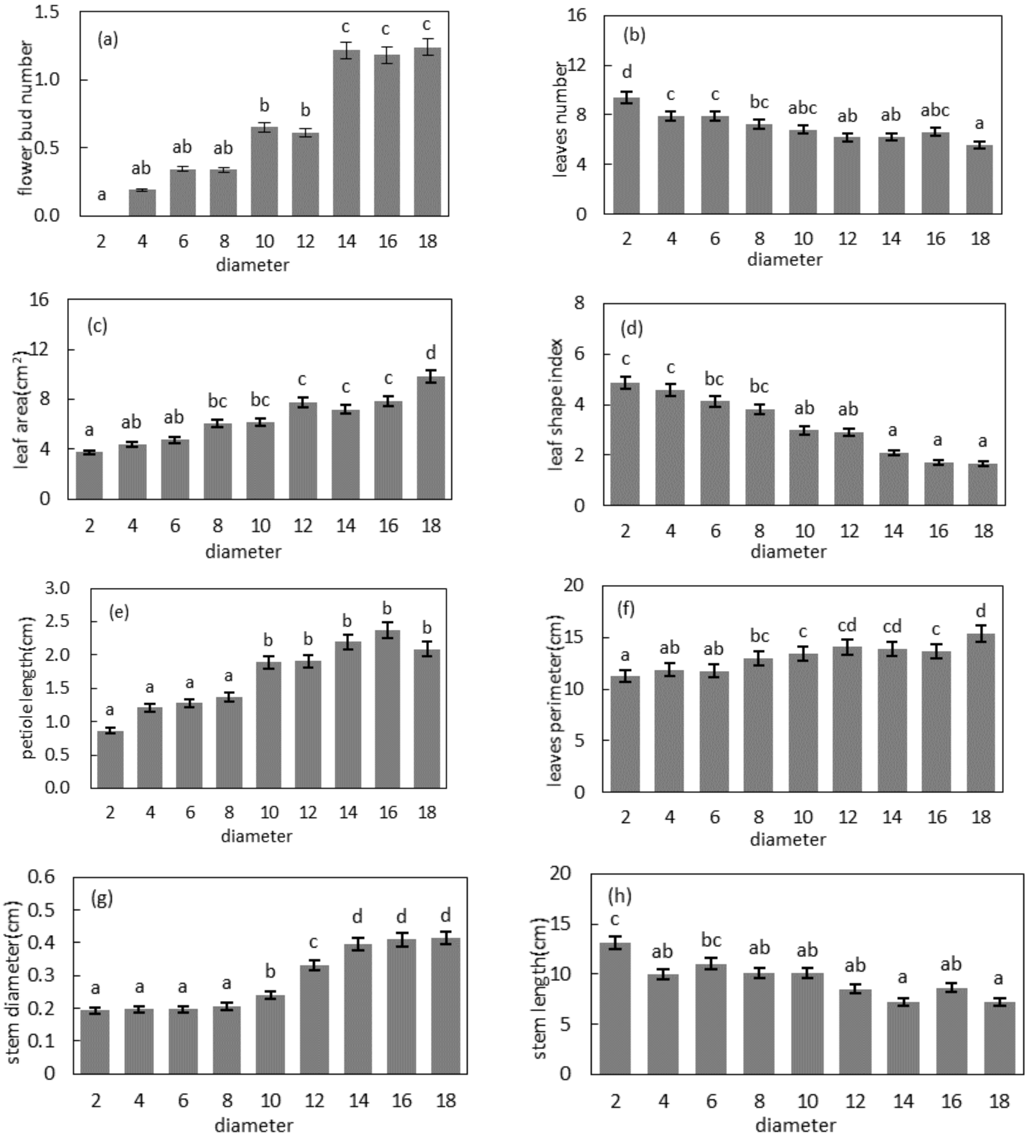

Figure 1. *Variation of leaf and stem morphology and flower bud number with ontogenetic stage of Populus euphratica. (a) flower bud number, (b) leaves number, (c) leaf area, (d) leaf shape index, (e) petiole length, (f) leaves perimeter, (g) stem diameter, (h) stem length, Different lowercase letters indicate significant, according to Tukey's test after one-way ANOVA at a significance level of $P < 0.05$, the bar chart represents the mean, the error line represents ± standard deviation*

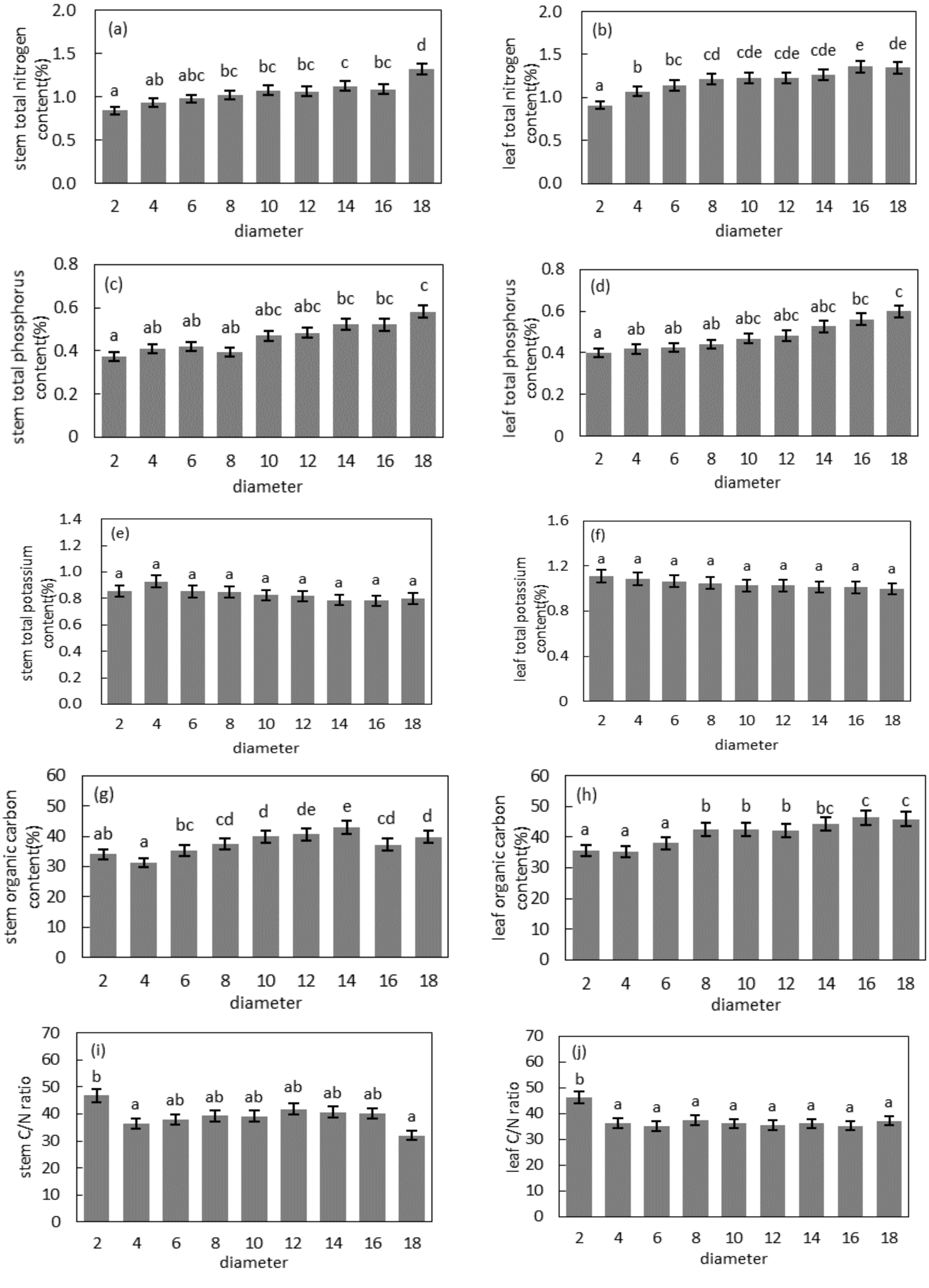

***Figure 2.** Variation of leaf and stem nutrient content with individual development stages of Populus euphratica. (a) stem total nitrogen content, (b) leaf total nitrogen content, (c) stem total phosphorus content, (d) leaf total phosphorus content, (e) stem total potassium content, (f) leaf total potassium content, (g) stem organic carbon content, (h) leaf organic carbon content, (i) stem C/N ratio, (j) leaf C/N ratio, Different lowercase letters indicate significant, according to Tukey's test after one-way ANOVA at a significance level of P < 0.05, the bar chart represents the mean, the error line represents ± standard deviation*

Characteristic changes in stem and leaf endogenous hormone content with developmental stage

The contents of endogenous hormones in the stems and leaves changed among the different class diameters. The analysis showed that the content of GA_3 in stems (*Figure 3c*) and the contents of GA_3 and IAA in leaves (*Figure 3b,d*) decreased with increasing class diameter, while the content of ABA in stems (*Figure 3e*) increased with increasing class diameter. The contents of IAA and ZR in stems (*Figure 3a,g*) first decreased and then increased with the increasing class diameter, and the content of ZR in leaves (*Figure 3h*) first increased and then decreased with increasing class diameter (*Figure 3a-h*). However, ZR content in leaves (*Figure 3h*) increased significantly with the appearance of 4 diameter flower buds, while 4-12 diameter flower buds gradually increased (*Figure 1a*), leaf shape index gradually decreased (*Figure 1d*), leaf area gradually increased (*Figure 1c*), stems became thicker and shorter (*Figure 1g,h*), and the contents of GA_3, IAA and ABA in stems (*Figure 3c,a,e*) decreased significantly. ZR content in leaves (*Figure 3h*) decreased significantly when the number of 14 diameter flower buds increased significantly.

Correlation analysis of leaf morphology, nutrient and endogenous hormone content with DBH and flower buds number

Correlation analysis showed (*Tables 2, 3*) that the morphology of stems and leaves, nutrient and endogenous hormone content was closely related to DBH (diameter at breast height) of *P. euphratica*. The leaf number, leaf shape index and stem length were negatively correlated with DBH of *P. euphratica*. Leaf area, petiole length, leaf circumference, stem diameter, flower buds number, total nitrogen, total phosphorus and organic carbon in stems and leaves were positively correlated with DBH of *P. euphratica*. There was a significant positive correlation between leaf ZR and DBH of *P. euphratica*, and a significant negative correlation between stem ABA and DBH of *P. euphratica*.

Correlation analysis showed (*Table 4*) that morphological nutrients and endogenous hormone contents of stems and leaves were closely related to the number of flower buds. However, there was a significant negative correlation between leaves number, leaf shape index, stem length and flower bud number, leaf area, leaf perimeter, petiole length and stem diameter were positively correlated with the number of flower buds, suggesting that the increase of leaf area, leaf perimeter, petiole length, stem diameter and the decrease of leaf number, leaf shape index and stem length were phenotypic characteristics of the increase of flower bud number. The contents of total nitrogen, total phosphorus and organic carbon in stems and leaves were positively correlated with the flower buds number, while the C/N ratio in leaves was negatively correlated with the flower buds number. The total nitrogen content and organic carbon content in stems and leaves increased with the growth of *P. euphratica* and the decrease of leaf C/N ratio promoted the increase of flower bud quantity. The results showed that total nitrogen in stems and leaves, organic carbon content and leaf C/N ratio synergistically promoted the reproductive growth of *P. euphratica*. There was a significant negative correlation between ABA and the number of flower buds, and a significant positive correlation between ZR and the number of flower buds. The results showed that the decrease of ABA content in stems and the increase of ZR content in leaves with the ontogeny of *P. euphratica* promoted the increase of the number of flower buds and the reproductive growth of *P. euphratica*. Four endogenous hormones played a synergistic role in the reproductive growth of *P. euphratica*.

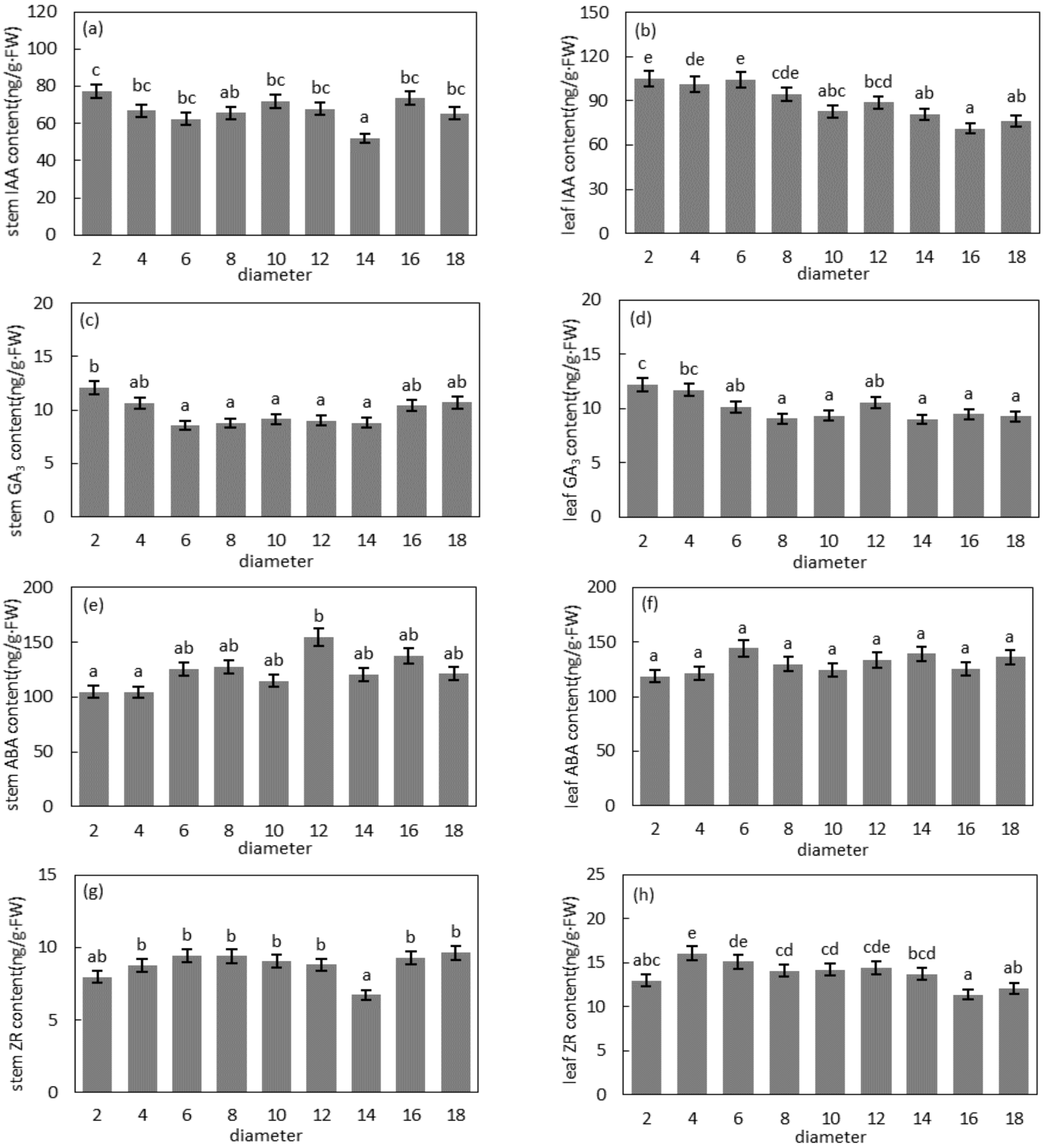

Figure 3. *Variation of leaf and stem endogenous hormone content with individual development stages of Populus euphratica. (a) stem IAA content, (b) leaf IAA content, (c) stem GA_3 content, (d) leaf GA_3 content, (e) stem ABA content, (f) leaf ABA content, (g) stem ZR content, (h) leaf ZR content, Different lowercase letters indicate significant, according to Tukey's test after one-way ANOVA at a significance level of $P < 0.05$, the bar chart represents the mean, the error line represents ± standard deviation*

Main factors affecting stem and leaf morphology and flower bud number

There was a certain correlation between the flower bud number and the morphology, nutrient contents, and endogenous hormone contents of stems and leaves. To screen for the factors that had a significant effect on the flower bud number, path analysis was conducted on the morphology, nutrient contents, and endogenous hormone contents of stems and leaves and the flower bud number (*Table S1*). The results showed that the magnitude of the direct effects of the different factors on the flower bud number could be ranked as follows: stem total nitrogen >leaf total nitrogen >leaf number >leaf organic carbon >leaf ABA >stem length >leaf C/N ratio >stem total phosphorus >leaf total phosphorus >petiole length >leaf area >leaf perimeter >stem diameter >leaf ZR >leaf shape index > stem C/N ratio >stem total potassium >stem ABA >leaf total potassium >leaf GA_3 >stem ZR >stem organic carbon >leaves IAA >stem IAA >stem GA_3. Among them, the contents of endogenous hormones and nutrients in stems and leaves also indirectly affected the number of flower buds by affecting the morphology of stems and leaves.

Table 2. *Pearson correlated with the stem and leaf morphology and diameter of Populus euphratica*

Coefficient of association	DBH	leaves number	leaf shape index	leaf area	petiole length	leaves perimeter	stem length	stem diameter
DBH	1							
crown height	0.95**							
leaves number	-0.66**	1						
leaf shape index	-0.81**	0.57**	1					
leaf area	0.75**	-0.57**	-0.82**	1				
petiole length	0.62**	-0.30**	-0.48**	0.44**	1			
leaves perimeter	0.55**	-0.34**	-0.45**	0.47**	0.51**	1		
stem length	-0.61**	0.65**	0.48**	-0.49**	-0.28*	-0.40**	1	
stem diameter	0.92**	-0.60**	-0.79**	0.73**	0.53**	0.50**	-0.67**	1

The asterisks **P <0.01; *P < 0.05

Table 3. *Pearson correlated with the stem and leaf nutrient, endogenous hormones content and diameter of Populus euphratica*

Coefficient of association	**DBH**	**Coefficient of association**	**DBH**	**Coefficient of association**	**DBH**
leaf N	0.73**	stem P	0.41**	stem ZR	-0.08
leaf P	0.50**	stem K	-0.05	stem ABA	-0.37*
leaf K	-0.27	stem Organic carbon	0.63**	leaf GA_3	-0.12
leaf Organic carbon	0.82**	stem C/N	-0.04	leaf IAA	0.01
leaf C/N	-0.22	stem GA_3	-0.21	leaf ZR	0.31*
stem N	0.48**	stem IAA	-0.17	leaf ABA	-0.16

The asterisks **P <0.01; *P < 0.05

In the stepwise regression analysis, the contents of endogenous hormones and nutrients in stems and leaves were taken as independent variables, and the morphology of stems and leaves and the flower bud number were taken as the dependent variables. The results showed that there was a very significant linear relationship between the dependent variable Y and the independent variable X (*Table 5*). Leaf total nitrogen, stem total phosphorus and stem IAA content directly affected flower bud number, and leaf total nitrogen also directly affected leaf number and petiole length. In addition, the stem and leaf total nitrogen contents, organic carbon content, and GA_3 content, stem ABA content and leaf C/N ratio indirectly affected the flower bud number by directly affecting the morphology of stems and leaves, promoting reproductive growth.

***Table 4.** Pearson correlated with the stem and leaf morphology, nutrient, endogenous hormones content and flower bud number of Populus euphratica*

coefficient of association	**flower bud number**	**coefficient of association**	**flower bud number**	**coefficient of association**	**flower bud number**	**coefficient of association**	**flower bud number**	**coefficient of association**	**flower bud number**
leaves number	-0.53**	stem length	-0.46**	leaf Organic carbon	0.59**	stem Organic carbon	0.45**	stem ABA	-0.46**
leaf shape index	-0.54**	stem roughness	0.58**	leaf C/N	-0.28*	stem C/N	-0.19	leaf GA_3	-0.27
leaf area	0.51**	leaf N	0.54**	stem N	0.48**	stem GA_3	-0.38**	leaf IAA	-0.04
petiole length	0.48**	leaf P	0.40**	stem P	0.34**	stem IAA	-0.02	leaf ZR	0.27
leaves perimeter	0.41**	leaf K	-0.17	stem K	-0.19	stem ZR	-0.01	leaf ABA	-0.28

The asterisks $^{**}P < 0.01$; $^{*}P < 0.05$. gibberellin (GA_3), indole acetic acid (IAA), abscisic acid (ABA), zeatin (ZR), total nitrogen (N), total phosphorus (P), total potassium (K), carbon/ nitrogen (C/N)

***Table 5.** Optimum regression models for prediction of stem and leaf morphology and flower bud number of Populus euphratica*

Dependent variable (Y)	**Regression equation**	**R**	**R^2**	**F**	**Sig.**
Y_1	$Y_1=-0.62+1.29X_1+1.31X_2-0.02X_3$	0.75	0.56	17.11	0.00
Y_2	$Y_2=16.63-0.14X_4-3.28X_1$	0.61	0.38	12.63	0.00
Y_3	$Y_3=6.71-0.21X_5+0.10X_6+0.10X_7$	0.82	0.67	27.88	0.00
Y_4	$Y_4=-354.16+23.51X_5+473.25X_8-60.24X_9$	0.75	0.57	17.77	0.00
Y_5	$Y_5=7.07+15.26X_1-0.06X_7$	0.65	0.43	15.57	0.00
Y_6	$Y_6=178.15-2.27X_5+6.79X_{10}-43.99X_8$	0.66	0.43	10.47	0.00
Y_7	$Y_7=-1.43+0.14X_5-0.30X_9+0.95X_8$	0.87	0.76	43.06	0.00

Y_1,flower bud number; Y_2,leaves number; Y_3,leaf shape index; Y_4,leaf area; Y_5,petiole length; Y_6,stem length; Y_7,stem diameter ; X_1,leaf N; X_2,stem P; X_3,stem IAA; X_4,stem organic carbon; X_5,leaf organic carbon; X_6,leaf C/N; X_7,stem ABA; X_8,stem N; X_9,stem GA_3; X_{10},leaf GA_3.Sig=Significant at $p< 0.05$, R=coefficient of association R^2 = coefficient of determination

Discussion

Relationship of morphological changes of stems and leaves on the flower buds number

There were significant differences in leaf morphology, nutrient and endogenous hormone contents and flower bud number at different diameters (Feng et al., 2014; Li et al., 2015b; Han et al., 2021). Zheng et al. (2015) showed that with the increase of tree age and canopy level (from base to top), the length of new stems, the number of stems and leaf shape index decreased gradually, while the leaf area and leaf dry weight increased gradually. Our results were consistent with that. The increase in flower bud

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number, leaf area, leaf perimeter, petiole length and stem diameter was closely related to the increase in diameter rank. At the same time, the increase in leaf area, leaf perimeter, petiole length, stem diameter and leaf shape index was closely related to the increase in flower bud number. Consistent with the results of De et al. (2016), traits of different plant organs (leaves, stems and roots) have a high degree of functional coordination and are highly correlated with physiological key traits. Leaf shape and leaf area can be used as markers for growth and development (England et al., 2006). The results of this study showed that flower buds and broad-ovoid leaves appeared at the same time in the 4-diameter order (5.20 years of age) (*Figure. S1*), and the stems length and the number of leaves per branch decreased significantly in this diameter order, suggesting that the emergence of broad-ovoid leaves and the shortening of stems were indicators of the reproductive growth of *P. euphratica*. Wang et al. (2019) showed that to cope with energy demand at the breeding stage, leaves of *P. euphratica* adopted an adaptive strategy of gradually changing from strip leaves to oval leaves with the increase of tree age and realized the transition from vegetative growth to reproductive growth through coordinated changes of stems and leaves. When the leaf shape index decreased and the leaf area increased during the growth and development, most of the leaves were broad ovate. The broad oval leaves of *P. euphratica* have a stronger photosynthetic capacity (Zhai et al., 2020), which can fix more carbon, and the structural basis of leaf area and branch thickness can effectively improve photosynthetic efficiency, material transport and storage capacity (Runion et al., 2017; Han et al., 2019), provides material and energy for the reproductive growth of *P. euphratica* (Liu et al., 2016).

Relationship of changes in the nutrient contents of stems and leaves on the flower buds number

Morphological manifestations lag behind their physiological and biochemical triggers. When obvious morphological changes such as flowering are observed, the internal physiological and biochemical environment has already undergone great changes (Guo et al., 2008). N, P and K are three essential nutrients for plant growth, and N and P are important components of macromolecules (proteins, nucleic acids, etc.) (Krapp et al., 2012). K can activate enzymes related to energy metabolism, protein synthesis and solute transport. High N, P and K levels can promote cell division and size and have certain regulatory effects on flower bud differentiation and morphological changes in stems and leaves (Kirkby et al., 2010). Feng et al. (2014) showed that the total N content of *P. euphratica* promoted the increase of leaf area and the decrease of leaf shape index, thus promoting the emergence of broad-ovoid leaves. Zhang et al. (2017) showed that the nutrient contents of N, P and K from dormancy to flowering in flower buds increased significantly at the flowering stage. The results of this study showed that the growth of flower buds and broad ovoid leaves was promoted by the significant increase of total N content in leaves and organic carbon content in stems.

Carbon and nitrogen metabolism plays an important role in plant growth and development. Carbon and nitrogen interact and restrict each other to coordinate the process of plant growth and development (Barney et al., 1989). According to the C/N theory, flowering is controlled by the vegetative state, and a high carbohydrate to nitrogen ratio is necessary for flowering (Corbesier et al., 2002). It was found that C/N of *Dimocarpus longan* Lour., *Syzygium samarangense* (Bl.) Merr. et Perry and *Litchi chinensis* Sonn. increased significantly during the initiation of flower buds (Matsumoto et al., 2007; Sritontip et al., 2008). This suggests that an appropriate C/N balance can promote the reproductive transition in plants (Liu et al., 2015). C/N promoted the differentiation of flower buds but also regulated the morphological changes in stems and leaves. The high level of carbohydrates in the shoot tip promoted an increase in the leaf number and flower bud height as well as the ratio of leaf width to flower bud height; these changes were conducive to the initiation of flower bud differentiation (Li et al., 2019). Li et al. (2015a) showed that starch metabolism in the leaves of *P. euphratica* played a regulatory role in the changes in leaf length and width. Starch is a high molecular-weight carbohydrate, and the higher the content of organic carbon, the higher the content of starch. In this study, *P. euphratica* ontogenesis exhibited a significant correlation with the total P, organic carbon, and total N contents in leaves; the leaf total N significantly influenced stem morphology, and the number of flower buds and the stem and leaf organic carbon contents significantly influenced stem and leaf morphology. These results indicate that the synergistic effects of total N, total P and organic carbon in leaves and stems promoted the transition from vegetative growth to reproductive growth.

Relationship of changes in the endogenous hormone contents of stems and leaves on the flower buds number

The physiological effects of different hormones promote or antagonize each other, and their effects involve various processes, such as synthesis, transportation, and metabolism. Plant growth and development are often the results of the comprehensive action of the balance of multiple hormones (Hsu et al., 1999). A certain level of IAA in flower buds is conducive to nutrient input and differentiation (Zhao et al., 2020). Mo et al. (2020) found that low levels of IAA and GA_3 and high levels of ABA were conducive to flower bud differentiation and could accelerate the transition from the physiological differentiation stage to the morphological differentiation stage. ABA and ZR are a diameter of hormones that promote the transition of the plant body into maturity. The transition from the seedling to mature stages in trees requires a lower GA level and a higher ABA level (Chen et al., 2020). Wang et al. (2020) studied the changes in endogenous hormones during flower bud differentiation in female *Ginkgo biloba* L. and showed that the GA_3 content reached a peak at the beginning of flower bud differentiation. According to the study by Ma et al. (2021), ABA reached its peak at the flowering stage of *Phalaenopsis aphrodite* H. G. Reichenbach, and the content of ABA in leaves was one of the main factors affecting the number of flower buds. A low IAA content before flower bud differentiation can reduce sugar loss, increase starch accumulation and prepare the plant for flower formation. After entering the physiological differentiation stage of flower buds, increased IAA content is conducive to nutrient input and promotes flower bud differentiation (Du et al., 2021). Meanwhile, an exogenous IAA treatment also showed that a low concentration of IAA was necessary for flower bud initiation, while a high concentration of IAA inhibited flower bud initiation (Zhao et al., 2020). It was further proved that IAA content was the main factor promoting the increase of flower buds per shoot. In this study, the decrease of IAA content in branches and leaves was closely related to the significant increase in the number of flower buds, and the IAA content in branches directly affected the number of flower buds. The results showed that IAA content in branches and leaves increased the reproductive capacity of *P. euphratica*.

Differences in the GA content in the leaf primordium in aquatic and terrestrial environments determine the *Rorippa indica* L. changes in their leaf shape (Bengera et al., 2012; Nakayama et al., 2014; Nakayama et al., 2017). GA_3 not only terminates vegetative growth but also promotes flower bud formation in woody plants (Goldberg-Moeller et al., 2013). ABA is involved mainly in the establishment and maintenance of the terrestrial morphological characteristics of heteromorphic plants (Wanke et al., 2011). Studies have shown that *P. euphratica* regulates the dormancy and germination of winter buds through the interaction of ABA, GA_3, and ZR, and promotes stem and leaf growth through the interaction of IAA, ABA, and ZR (Xu et al., 2007). The content of IAA in leaves decreased gradually with increasing tree age, while the contents of ABA and ZR in leaves showed an overall increasing trend, and the content of GA_3 in leaves showed a trend of first increasing and then decreasing (Li et al., 2017). Zheng et al. (2015) showed that the leaf shape index of *P. euphratica* gradually decreased from the bottom to the top of the crown and that broad-ovate leaves were distributed mostly at the top of the crown; the GA_3 content was significantly positively correlated with the leaf shape index, indicating that the GA_3 content was closely related to the emergence of broad-ovate leaves. In this study, the GA_3 content of stems and leaves had a direct and significant effect on the morphology of stems and leaves. Flower buds appeared in the 4 cm class diameter, when *P. euphratica* entered the reproductive growth stage, and the content of ZR in leaves increased significantly. In the 4-12 cm class diameteres, the number of flower buds increased gradually, the leaf area increased, the leaf shape index gradually decreased, *P. euphratica* entered the stage of vegetative growth and reproductive growth, and the GA_3 content of stems and leaves decreased gradually. The results indicated that leaf ZR content promotes the reproductive growth of *P. euphratica* and that the GA_3 content of stems and leaves promotes reproductive growth mainly by affecting vegetative growth in the stage during which vegetative growth and reproductive growth cooccur.

Cells transmit light signals through the cytokinin signal transduction pathway, and lightly regulates leaf initiation by activating cytokinin signals and affecting the efflux-dependent IAA gradient (Li et al., 2020). In this study, only the ABA and ZR contents in the stems and leaves were significantly correlated with the ontogenetic development of *P. euphratica*. It was inferred that endogenous hormones in the stems and leaves did not affect the ontogenetic stage of *P. euphratica* because IAA induces changes in the balance of other hormones (Nakayama et al., 2017). Therefore, the effect of IAA on the flower bud number during the phase change in *P. euphratica* may be due to the signaling function of IAA. The hormone balance of *P. euphratica* in the juvenile stage was changed, to make the transition to adulthood; at the same time, the hormones distributed nutrients to meet the needs of *P. euphratica* during its stage transition.

Conclusions

The flower buds and broad ovoid leaves of 4 diameter (5.20 years old) appeared simultaneously, indicating that P. euphratica had entered the reproductive growth stage, and 14 diameter (10.52 years old) had entered the vigorous reproductive growth stage. The leaf and stem morphology and the total nitrogen, total phosphorus and organic carbon contents changed synergistically with developmental stages. The leaf total nitrogen, stem total phosphorus and stem IAA contents significantly affected the number of flower buds, and the stem total nitrogen, organic carbon, GA_3 content, stem

ABA content and leaf C/N ratio significantly affected the morphology of stems and leaves and indirectly affected the number of flower buds. We believe that the synergistic changes in the nutrient and endogenous hormone contents of *P. euphratica* stems and leaves regulate the morphology of stems and leaves as well as the flower bud number and directly or indirectly affect the transition from vegetative growth to reproductive growth. Therefore, the synergistic changes among the influencing factors should be considered in the study of the transition between plant growth and development stages.

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Conflict of interests. The authors declare that they have no conflict of interests.

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APPENDIX

Table S1. *Path coefficient analysis of stem and leaf morphology,nutrients,endogenous hormones and flower bud number of Populus euphratica*

Effect of factors	**Direct effect**	**Indirect effects**																								
		leaves number	**leaf shape index**	**leaf area**	**petiole length**	**Leaves perimeter**	**stem length**	**stem diameter**	**leaf N**	**leaf P**	**leaf K**	**leaf Organic carbon**	**leaf C/N**	**stem N**	**stem P**	**stem K**	**stem Organic carbon**	**stem C/N**	**stem GA_3**	**stem IAA**	**stem ZR**	**stem ABA**	**leaf GA_3**	**leaf IAA**	**leaf ZR**	**leaf ABA**
leaves number	-0.411		-0.090	0.119	-0.072	-0.049	0.213	-0.107	0.265	-0.042	0.013	-0.164	-0.057	-0.149	0.046	-0.025	-0.026	-0.015	-0.006	-0.006	-0.003	-0.021	0.004	0.004	-0.032	0.042
leaf shape index	-0.157	-0.235		0.152	-0.107	-0.080	0.163	-0.148	0.363	-0.090	0.017	-0.225	-0.062	-0.197	0.060	-0.006	-0.031	-0.010	-0.006	-0.004	0.007	-0.030	0.010	-0.005	-0.052	0.069
leaf area	-0.201	0.244	0.119		0.109	0.094	-0.161	0.132	-0.291	0.080	-0.018	0.187	0.049	0.274	-0.103	0.022	0.027	-0.019	0.009	0	-0.008	0.036	-0.006	0	0.045	-0.108
petiole length	0.215	0.138	0.078	-0.102		0.103	-0.102	0.102	-0.281	0.096	-0.014	0.157	0.066	0.165	-0.074	0.013	0.019	-0.009	0.003	0	-0.012	0.036	-0.024	0.011	0.023	-0.080
leaves perimeter	0.179	0.113	0.071	-0.105	0.124		-0.120	0.092	-0.269	0.091	-0.010	0.118	0.074	0.089	-0.032	-0.001	0.011	0.010	0.005	-0.001	-0.013	0.013	-0.024	0.003	0.038	-0.008
stem length	0.279	-0.314	-0.092	0.116	-0.079	-0.077		-0.125	0.220	-0.038	0.011	-0.146	-0.050	-0.220	0.069	-0.028	-0.021	0.009	-0.009	0.003	0.007	-0.024	0.025	0	-0.039	0.043
stem diameter	0.175	0.250	0.133	-0.151	0.126	0.094	-0.198		-0.319	0.097	-0.021	0.244	0.027	0.208	-0.093	0.014	0.029	0	0.010	0.001	0.001	0.037	-0.018	0.002	0.042	-0.083
leaf N	-0.519	0.210	0.110	-0.113	0.117	0.093	-0.118	0.108		0.095	-0.006	0.188	0.160	0.156	-0.016	-0.003	0.022	0.009	0.003	0.004	-0.007	0.014	-0.005	-0.004	0.055	-0.011
leaf P	0.224	0.078	0.063	-0.072	0.092	0.072	-0.048	0.076	-0.219		-0.029	0.170	-0.004	0.132	-0.112	0.017	0.006	-0.027	0.001	0	-0.007	0.021	-0.004	0.007	0.004	-0.030
leaf K	0.068	-0.080	-0.039	0.054	-0.045	-0.026	0.044	-0.053	0.047	-0.097		-0.101	0.038	-0.007	0.046	-0.050	-0.004	-0.002	-0.002	-0.004	0.013	-0.012	0.009	-0.006	0.015	0.032
leaf Organic carbon	0.312	0.216	0.114	-0.120	0.108	0.068	-0.130	0.137	-0.313	0.122	-0.022		-0.027	0.126	-0.067	0.025	0.032	0.020	0.001	0.005	0.001	0.021	0	0	0.054	-0.055
leaf C/N	-0.243	-0.097	-0.040	0.040	-0.059	-0.055	0.057	-0.019	0.341	0.004	-0.011	0.035		-0.111	-0.025	0.026	-0.002	0.018	-0.003	-0.002	0.012	0.001	0.010	0.002	-0.018	-0.042
stem N	0.547	0.112	0.057	-0.101	0.065	0.029	-0.112	0.067	-0.148	0.054	-0.001	0.072	0.049		-0.158	0.031	0.016	-0.098	0.003	0.002	-0.002	0.024	-0.006	0.011	0.011	-0.060
stem P	-0.225	0.085	0.042	-0.092	0.070	0.026	-0.085	0.072	-0.037	0.111	-0.014	0.092	-0.027	0.383		0.058	0.010	-0.083	0.005	0	0.003	0.036	-0.008	0.008	-0.018	-0.078
stem K	-0.128	-0.079	-0.007	0.034	-0.022	0.001	0.060	-0.019	-0.013	-0.029	0.027	-0.060	0.049	-0.132	0.102		-0.007	0.026	0.001	0.004	0.003	-0.008	0.007	-0.003	0.035	0.068
stem Organic carbon	0.050	0.218	0.097	-0.109	0.081	0.041	-0.116	0.104	-0.234	0.028	-0.005	0.200	0.009	0.173	-0.047	0.018		0.055	0	0	0.019	0.031	-0.007	0.005	0.040	-0.077
stem C/N	0.145	0.043	0.011	0.027	-0.014	0.012	0.017	0.000	-0.032	-0.042	-0.001	0.044	-0.030	-0.367	0.129	-0.023	0.019		-0.003	-0.002	0.014	-0.003	0.001	-0.007	0.027	0.020
stem GA_3	-0.027	-0.092	-0.035	0.066	-0.025	-0.032	0.097	-0.067	0.052	-0.004	0.004	-0.017	-0.022	-0.060	0.043	0.003	-0.001	0.015		-0.001	0.001	-0.034	0.024	0	0.012	0.121
stem IAA	-0.042	-0.059	-0.014	0.002	-0.001	0.002	-0.018	-0.004	0.049	0.001	0.007	-0.039	-0.010	-0.030	-0.001	0.013	0	0.006	-0.001		-0.015	-0.001	-0.012	-0.009	-0.036	0.014
stem ZR	-0.064	-0.020	0.017	-0.024	0.040	0.036	-0.030	-0.002	-0.053	0.024	-0.014	-0.006	0.044	0.018	0.012	0.006	-0.015	-0.033	0.001	-0.010		0.005	-0.012	0.015	0.020	-0.002
stem ABA	-0.080	-0.110	-0.059	0.091	-0.097	-0.030	0.083	-0.080	0.089	-0.058	0.010	-0.081	0.003	-0.166	0.103	-0.012	-0.020	0.005	-0.011	0.000	0.004		0.027	-0.022	0.029	0.222
leaf GA_3	0.066	-0.026	-0.024	0.019	-0.077	-0.066	0.105	-0.047	0.042	-0.014	0.009	0	-0.037	-0.050	0.027	-0.014	-0.005	0.001	-0.010	0.008	0.011	-0.032		-0.017	0.029	0.046
leaf IAA	-0.047	0.036	-0.018	0.000	-0.051	-0.010	0.002	-0.006	-0.039	-0.033	0.008	0.001	0.009	-0.125	0.038	-0.008	-0.005	0.020	0	-0.008	0.021	-0.038	0.024		0.008	0.113
leaf ZR	0.159	0.083	0.051	-0.057	0.032	0.043	-0.068	0.047	-0.181	0.005	0.007	0.106	0.027	0.039	0.025	-0.029	0.013	0.025	-0.002	0.010	-0.008	-0.015	0.012	-0.003		0.066
leaf ABA	0.296	-0.058	-0.037	0.074	-0.058	-0.005	0.040	-0.049	0.020	-0.023	0.007	-0.059	0.035	-0.111	0.060	-0.029	-0.013	0.010	-0.011	-0.002	0	-0.060	0.010	-0.018	0.036	

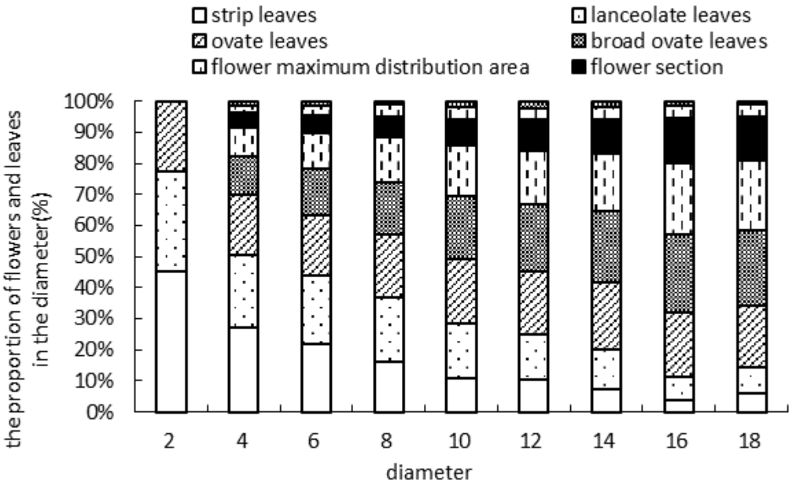

Figure S1. *Overlaps of spatiotemporal distribution of heteromorphic leaves and flower buds in Populus euphratica. X-axis is the ontogenetic stage and Y-axis represent the proportion of flowers and leaves in the diameter*