Forest Ecology and Management 434 (2019) 63–75

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Coordination of intra and inter-species leaf traits according to leaf phenology and plant age for three temperate broadleaf species with different shade tolerances

Zhili Liu[a], Feng Jiang[a], Fengri Li[b], Guangze Jin[a,*]

[a] Center for Ecological Research, Northeast Forestry University, Harbin 150040, China
[b] School of Forestry, Northeast Forestry University, Harbin 150040, China

ARTICLE INFO

Keywords:
Chemical traits
Leaf development
Leaf economic spectrum (LES)
Intraspecific trait variation
Morphological traits
Shade tolerance

ABSTRACT

Intraspecific leaf economic spectrum (LES) traits vary considerably with leaf phenology and plant age, but whether these trait patterns significantly vary between species with different shade tolerances at local scales has rarely been examined. The presence of LES within species at local scales has recently been debated; however, the conclusions are far from universal, and whether the trait-trait relationships are modulated by leaf phenology and plant age needs to be tested. We measured six leaf traits (i.e., specific leaf area, leaf dry matter content, leaf thickness, mass-based leaf nitrogen content, leaf phosphorus content and ratio of nitrogen and phosphorus contents) among different leaf phenological stages (young, mature and old leaves) and plant ages (adult and sapling) for three temperate broadleaf species with different levels of shade tolerance. Intraspecific trait variation depended on the species and traits, and interspecies trait variation patterns were inconsistent with the LES prediction. Specific leaf area significantly decreased as the shade tolerance of a species increased, which was in contrast with the global LES prediction. Additionally, 38% and 28% of intraspecific trait variation was explained by leaf phenology and plant age, respectively, for the overall leaf traits of the three species. Generally, global LES held at the local scale, but trait-trait relationships were strongly modulated by leaf phenology and plant age. The intraspecific trait variation among the leaf phenology and plant age stages was driven by the LES axis and leaf thickness, respectively. We highlight the importance of leaf phenology and plant age on intraspecific trait variation and trait-trait relationships. We provide direct evidence of the LES at a local spatial scale and a seasonally temporal scale, but variations in LES traits are not always reliable for predicting the resource-use strategies of plants at local scales.

1. Introduction

Intraspecific trait variation is increasingly recognized as having important impacts on the assembly and functioning of communities, species coexistence and associated ecological processes (Bolnick et al., 2011; Siefert, 2012; Violle et al., 2012; Siefert et al., 2015; Hart et al., 2016; Wu et al., 2016; Griffiths et al., 2018). Furthermore, understanding species' trait variation and covariation is necessary to explain species' response strategies to environmental gradients and to develop ecological predictions (Lecerf and Chauvet, 2008; Chasco, 2010; Jung et al., 2014; Albert et al., 2015; Niinemets, 2015; Moran et al., 2016; Anderegg et al., 2018; Osnas et al., 2018; Poorter et al., 2018). However, constant trait values or fixed trait-trait relationships are commonly assigned in dynamic global vegetation models (DGVMs) (Pavlick et al., 2013; Prentice and Cowling, 2013). This assigning method has frequently been questioned mainly because much evidence has demonstrated that both traits and trait-trait relationships are not invariable within species through space or time (Reich et al., 1991; Wright et al., 2005a; Hulshof and Swenson, 2010; Siefert et al., 2015; Bloomfield et al., 2018); moreover, there is no consensus on this topic, and it requires additional discussion.

One of the most successful examples of exploring fundamental trait relationships is the global 'leaf economic spectrum' (LES), which reflects the trade-off between resource acquisition and resource conservation (Wright et al., 2004; Wright et al., 2005b; Shipley et al., 2006). One end of the spectrum represents quick investment-return species with high specific leaf area (SLA), high leaf nitrogen content, high photosynthetic rates and short lifespans, and the opposite end represents slow investment-return species with low SLA, low leaf nitrogen content, low photosynthetic rates and long lifespans (Wright

* Corresponding author.
E-mail address: taxus@126.com (G. Jin).

https://doi.org/10.1016/j.foreco.2018.12.008
Received 19 September 2018; Received in revised form 1 December 2018; Accepted 4 December 2018

et al., 2004; Onoda et al., 2011; Osnas et al., 2013; Reich, 2014). Although this method is a good way to explore plant responses to environmental changes at the global scale, the presence of the LES within species has been debated in recent years (Albert et al., 2010; Martin et al., 2017; Messier et al., 2017a; Anderegg et al., 2018). The potential drivers of the LES include two main viewpoints. First, if natural selection drives the LES, this should apply at the intraspecific as well as the interspecific levels (Cornwell et al., 2006; Siefert, 2012; Fajardo and Siefert, 2018), i.e., the key trait-trait relationships of the LES should be consistent across different levels of organization. Alternatively, if intraspecific trait variation is shaped by unique selection pressures and phenotypic plasticity, intraspecific trait-trait relationships may be decoupled from the LES (Siefert, 2012; Blonder et al., 2013; Messier et al., 2017a). Both views have supporters. For example, using compiled data on *Quercus ilex*, Niinemets (2015) found the coordination of LES traits despite there being a slight departure from the global spectrum. Across multiple scales, Martin et al. (2017) found consistent but weaker correlations between LES traits in coffee. Similarly, Hu et al. (2015) also found evidence of within-species LES across multiple spatial scales in *Phragmites australis*. In contrast, Messier et al. (2017a) demonstrated that LES relationships may not hold at local scales. Thus, the coordination of intraspecific LES traits still needs further testing across different scales (Anderegg et al., 2018; Bloomfield et al., 2018), especially at local scales.

It is worth noting that well-lit and fully mature leaves were sampled to initially assess the LES traits in early studies (e.g., Wright et al., 2004); thus, whether the LES applies for immature (e.g., young or old) leaves needs further assessment. Recently, leaf phenology, i.e., the seasonal dynamics of leaf development, maturity and senescence (also described as 'leaf development stages' here), has drawn more attention, partly due to its climate sensitivity, which potentially makes phenology useful in terms of modeling the response of species to a changing environment and the seasonality of gross ecosystem productivity (Mckown et al., 2013; Chavana-Bryant et al., 2016; Wu et al., 2016; Albert et al., 2018; Martinez et al., 2018). As leaves develop, the corresponding air temperature, relative humidity and gap fraction in a forest will also change (Fig. S1), which may lead to systematic intraspecific trait variation in many leaf traits (e.g., photosynthetic capacity, SLA, leaf nitrogen content or leaf dry matter content, LDMC) (Wright et al., 2005a; Mckown et al., 2013). However, there is no consensus on the trend between leaf traits and leaf development. Wu et al. (2016) and Albert et al. (2018) found that mature leaves generally had higher photosynthetic capacity than young and old leaves in Amazon evergreen forests. Wilson et al. (2000) reported that the photosynthetic capacity at the leaf level was highest during early summer (i.e., June 1-July 1) and then decreased during the growing season in a deciduous forest; in contrast, it was reported that photosynthetic capacity did not decline until a period much closer to senescence (Jurik, 1986; Sullivan et al., 1996). In this case, leaf traits that are closely related to photosynthetic capacity are likely to exhibit different trends with changes in leaf development, and this poses an interesting question worth verifying with field data. Furthermore, Reich et al. (1991) showed that the youngest leaves (sampled before June 22) generally had the greatest SLA and mass-based leaf nitrogen content (N_{mass}) when compared with mid-aged and old leaves (sampled after June 22) in three deciduous broadleaf species. Similarly, Chavana-Bryant et al. (2016) demonstrated that the SLA and mass-based leaf phosphorus content (P_{mass}) monotonically decreased with leaf development for 12 lowland Amazonian canopy trees. However, only mature individuals were sampled in the studies mentioned above.

Like leaf phenology, plant age (or plant size) characterization must consider the tremendous intraspecific variation in many leaf traits (Liu et al., 2010; Martin and Thomas, 2013; Mason et al., 2013; Price et al., 2014; Kuusk et al., 2018). In contrast to adult trees (i.e., canopy trees), saplings and seedlings are severely limited by light; thus, these understory trees often have higher shade tolerance (Valladares and Niinemets, 2008). Additionally, the carbon gain hypothesis suggests that shade-tolerant species have higher SLA and N_{mass} and lower leaf thickness (LT) in low light than do intolerant species (Valladares and Niinemets, 2008). Thus, saplings are expected to have higher SLA (Thomas, 2010), N_{mass} (Liu et al., 2010; Martin and Thomas, 2013) and lower LT than adult trees; however, whether these patterns vary with leaf phenology at local scales has rarely been tested. Specifically, the performance of SLA as an indicator of a species' shade tolerance has recently raised more debate. A positive correlation between SLA and shade tolerance was found in both evergreen species (Hallik et al., 2009) and deciduous species (Niinemets and Kull, 1994; Niinemets, 1997; Janse-ten et al., 2007), and these results generally support the carbon gain hypothesis. However, a negative correlation between SLA and shade tolerance was also found for evergreen (Walters and Reich, 1999; Lusk and Warton, 2007; Lusk et al., 2008) and deciduous species (Walters and Reich, 1999), and these results support the stress tolerance hypothesis (Kitajima, 1994). The stress tolerance hypothesis suggests that species with low SLA are more conservative in terms of resource acquisition, and thus, these species are generally more shade tolerant (Kitajima, 1994); this hypothesis is based on that fact that, generally, a high SLA indicates low plant survival because thin leaves with low construction costs are less likely to resist stressors induced by mechanical damage and attacks from herbivores and pathogens (Augspurger, 1984; Valladares and Niinemets, 2008). Therefore, the relationship between SLA and shade tolerance remains controversial, especially in deciduous trees. Additionally, the variation patterns of other LES traits (e.g., LDMC, LT and leaf chemical traits) in co-occurring species with different shade tolerances at the local scale deserve more attention. In addition, the ratio of leaf N and P content (N:P) is very useful for indicating plant productivity and nutrient limitation, and N:P $<$ 14 generally indicates N limitation, while N:P $>$ 16 suggests P limitation (Koerselman and Meuleman, 1996). Recently, many studies have found that the N:P varied with plant size (Liu et al., 2010; Li et al., 2018), but few studies have been conducted to explore the influence of leaf development stages on N:P for temperate broadleaf species. Moreover, leaf trait variations within individuals may be caused by the following factors: degrees of exposure to sunlight (White and Scott, 2006; Catoni et al., 2015; Niinemets et al., 2015; Hikosaka et al., 2016), leaf height (Niinemets et al., 2015; Coble et al., 2016) or leaf size (Niinemets et al., 2006; Niinemets et al., 2007; Milla et al., 2008; Legner et al., 2014). While many factors (e.g., leaf phenology or plant age) may influence intraspecific trait variation in leaves, the relative contributions of these factors in driving intraspecific trait variation has not been assessed at local scales; specifically, leaf phenology and plant age have not yet been simultaneously integrated in previous studies.

In this study, we measured six commonly assessed LES traits, including three leaf morphological traits (i.e., SLA, LDMC and LT) and three leaf chemical traits (i.e., N_{mass}, P_{mass} and N:P) (Wright et al., 2004; Li et al., 2015; Siefert et al., 2015; Mediavilla et al., 2018), in three deciduous broadleaf species with different shade tolerances in an old-growth temperate forest. The leaf traits were measured at three leaf development stages (young, mature and old) for each species during two plant ages (adult and sapling). We addressed the following hypotheses: (1) the SLA, LT, N_{mass} and P_{mass} would increase with the shade tolerance of the species according to the carbon gain hypothesis (Givnish, 1988; Valladares and Niinemets, 2008), and SLA and LT would be negatively correlated for three species; (2) leaf traits would vary with leaf phenology and plant age and show different responses with changes in the shade tolerance of the species (Reich et al., 1991; Ishida et al., 2005; Thomas, 2010; Mckown et al., 2013; Fajardo and Siefert, 2016; Bloomfield et al., 2018). Furthermore, we hypothesized that SLA, N_{mass} and P_{mass} would be higher for mature leaves than young and old leaves (Wu et al., 2016; Albert et al., 2018), and these traits would be higher for saplings than for adults of three species (Thomas and Winner, 2002; Valladares and Niinemets, 2008); and (3) the proportion of intraspecific trait variation is mainly caused by leaf

phenology and plant age for the three studied species at the local scale. Additionally, we tested whether well-established global trait-trait relationships (Wright et al., 2004; Wright et al., 2005b) held at the local scale; if they did, we then tested whether these relationships remained constant or were modified by leaf phenology and plant age.

2. Materials and methods

2.1. Study site

The study site was located within the Heilongjiang Liangshui National Nature Reserve in northeastern China (47°10′50″N, 128°53′20″E). This site is characterized by rolling mountainous terrain that ranges from 300 m to 707 m above sea level, and the typical slope is 10–15°. The mean annual air temperature is −0.3 °C, and the mean air temperature during the summer months (i.e., from June to August) is 17.5 °C. The mean annual rainfall is 676 mm, and 10–20% of this derives from snowfall; additionally, the area is covered by snowpack from December through April. The old-growth forest (about 300 years old) was mixed broadleaved-Korean pine (*Pinus koraiensis*) forest at the climax stage (Liu et al., 2015).

2.2. Sample design and leaf trait measurements

In the mixed forest (2.0 ha), the species *Betula platyphylla*, *Fraxinus mandshurica* and *Acer mono* were selected (the basal area of the three species accounted for 0.2%, 2.6% and 8.3% of total basal area of all species in this forest, respectively); these species represent a sequence from shade-intolerant to highly shade-tolerant species, and they have shade tolerance values of 1.25, 2.75 and 4.25, respectively (tolerance scales range from 0 (no tolerance) to 5 (maximum tolerance)) (Niinemets and Valladares, 2006). Leaf traits were measured using two scales, i.e., leaf phenology (leaf development) and plant age. The leaf development stages included young, mature and old leaves, and the classification principles generally referred to Chavana-Bryant et al. (2016) and Albert et al. (2018) (Figs. S2–3). In this study, young leaves refer to immature leaves (< 1 month old, leaf area lower than 80% of seasonal peak value, less than 80% of total leaves emerged, light green), which were sampled in early June of 2015. Mature leaves refer to leaves that had recently reached maturity (1–3 months old, leaf area larger than 80% of seasonal peak value or fully expanded, more than 80% of total leaves existed, darkened green), which were sampled in mid-July of 2015. Old leaves refer to leaves that started aging but have not dropped (> 3 months old, fully expanded, more than 20% of total leaves fell, green or faint yellow), and these leaves were sampled in mid-September of 2015. The plant age stages included adult and sapling, and the stage was mainly differentiated by evaluating the diameter at breast height (DBH). For three species, the DBH of adults ranged from 20 to 25 cm and tree height was in a scope of 17–23 m, and the DBH of saplings ranged from 1 to 2 cm and tree height was in a scope of 1–2 m.

For each species, six individuals (three adults and three saplings) were randomly selected in the forest for sampling. The same individuals were sampled across three leaf development stages, and all sampling was conducted in each stage using the same sample design. For each adult individual, the canopy position was divided into three height levels (top, middle and low); for each height level, two large sample branches in the south and north side (confirmed by compass) were first cut via tree climbing, then 40–50 sample leaves were collected from exterior sections of each branch, representing sunlit and shade leaves respectively. Similarly, 25–40 sunlit and shade leaves were collected from 2 to 3 branches of each sapling individual by directly cutting them with scissors. Totally, we separately collected 530–540 and 380–520 samples leaves of three adult and sapling individuals of three leaf development stages for each species to measure the morphological traits (i.e., SLA, LDMC and LT). After measuring the morphological traits, these sample leaves as well as the remaining sampled leaves were all used to measure the chemical traits (i.e., N_{mass}, P_{mass} and N:P).

For each sampled leaf, a micrometer was used to measure the LT (with precision of 0.01 mm), which was calculated as the mean of three replicate values taken across a given leaf while avoiding major veins. The leaf area (with precision of 0.01 cm^2) was measured using a BenQ-5560 image scanner (BenQ Corporation, China, 300 dpi resolution). To guarantee water saturation of the leaves, we placed the sampled leaves into distilled water for 12 h. Then, the water was carefully wiped off of each sample leaf with filter paper, and the water-saturated leaf was weighed. After these measurements were taken, each leaf was dried at 60 °C to constant mass and weighed (with precision of 0.0001 g). The SLA ($cm^2\ g^{-1}$) was then calculated by dividing leaf area by leaf dry mass. The LDMC ($g\ g^{-1}$) was determined by dividing leaf dry mass by saturated fresh mass.

All sampled leaves used to measure chemical traits were initially oven-dried at 60 °C for 48 h and were ground to fine powder by a pulverizer. The mass-based leaf N content (N_{mass}, $mg\ g^{-1}$) was measured using a Hanon K9840 auto-Kjeldahl analyzer (Jinan Hanon Instruments Co., Ltd., Jinan, China). The mass-based leaf P content (P_{mass}, $mg\ g^{-1}$) was measured using the molybdenum blue colorimetric method, using leaves digested in a H_2SO_4 + H_2O_2 solution. Finally, we calculated the leaf N:P ratio.

2.3. Statistical analysis

All statistical analyses were performed using R-3.2.5 (R Core Team, 2016). The difference in the mean leaf traits among the three species were determined using least significant differences (LSD), which was also used to assess differences in the leaf traits among the three leaf development stages. Independent sample T test was used to assess the differences in leaf traits between adults and saplings of each species. We used nested analysis of variance (ANOVA) coupled with variance partitioning techniques to evaluate the amount of variation in leaf traits that was explained by the different ecological scales. We first fit a linear mixed model to each leaf trait individually using the 'lme' function in the 'nlme' package. In these models, the nested level was employed, including four different hierarchical scales: (i) among leaves within an individual (as a result of responses to varying leaf height or light availability as well as unexplained variance), (ii) among individuals, (iii) between plant ages and (iv) among leaf development stages (leaf phenology). Although sunlit and shade leaves were sampled for adults and samplings, it was difficult to exactly distinguish sunlit and shade leaves for saplings in the understory. Thus, we evaluated intraspecific leaf variation not only between individuals, but also spatially within individuals, across season, and across ontogeny. We then calculated the variance components associated with the nested level using the 'varcomp' function in the 'ape' package (Paradis et al., 2004).

To evaluate patterns of trait covariation, we employed pairwise bivariate correlations among six leaf traits. Although three morphological traits (i.e., SLA, LDMC and LT) were measured at each leaf scale, the mean values of leaves in the sun-shade scale were used in this step, i.e., correlations among the six leaf traits were analyzed based on the sun-shade scale because one leaf was not enough to accurately measure the chemical traits (N_{mass} or P_{mass}). Our results showed that leaf phenology and plant age were the two most important factors that explained the intraspecific variation in most leaf traits for these three species (see Fig. 3 in Results); thus, we further evaluated whether the patterns of trait-trait relationships varied with leaf phenology and plant age. Then, we compared the slopes of these linear relationships during different leaf development stages (i.e., young, mature and old leaves) and plant ages (i.e., adult and sapling) using standardized major axis (SMA) regression analysis, which was implemented in the 'smatr' (standardized major axis tests and routines) package (Warton et al., 2006). If the slopes did not vary significantly with leaf phenology or plant age stage, then we calculated the common slope. If there was

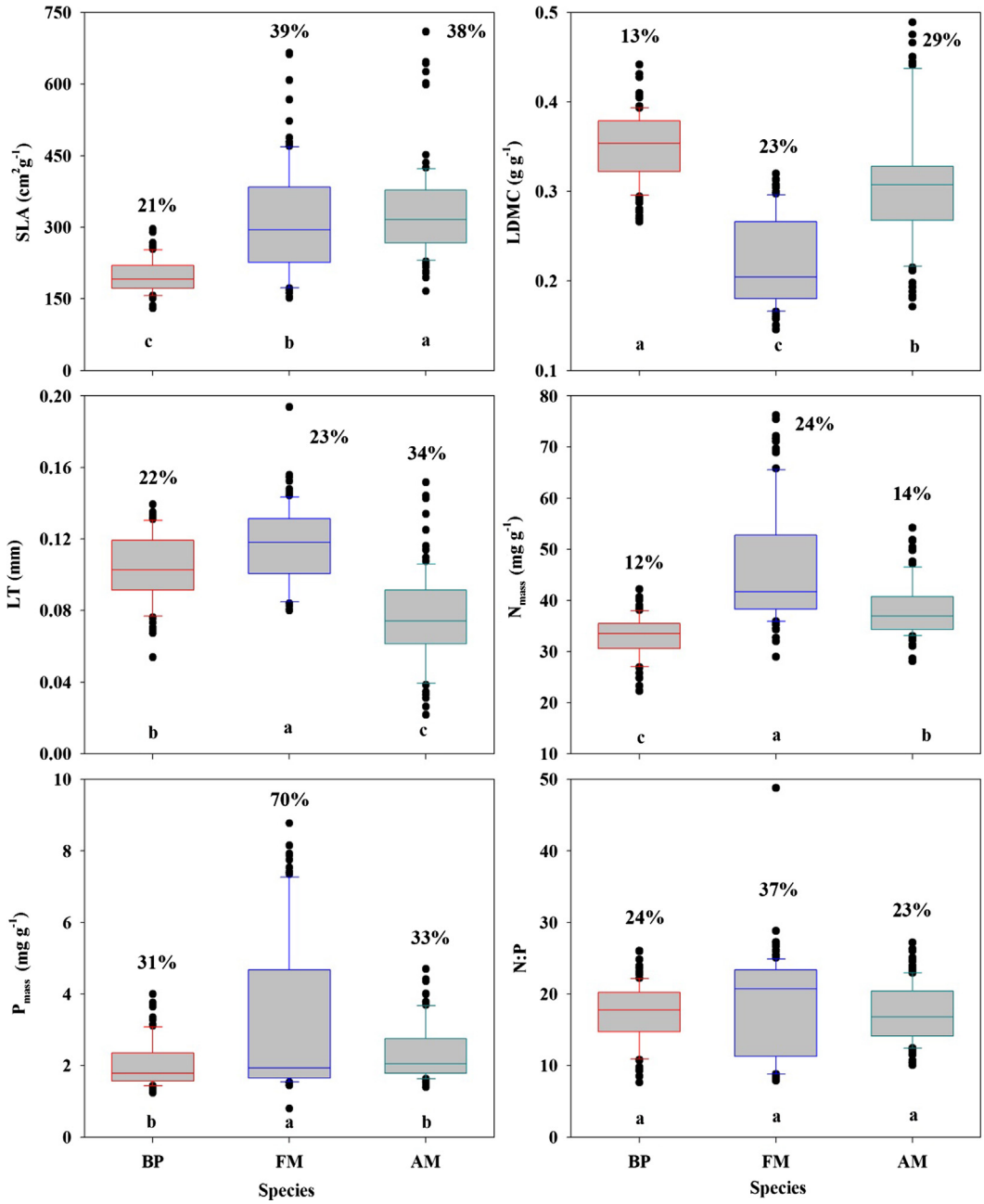

Fig. 1. Box plots of SLA (cm^2 g^{-1}), LDMC (g g^{-1}), LT (mm), N_{mass} (mg g^{-1}), P_{mass} (mg g^{-1}) and N:P for three temperate broadleaf species with different shade tolerances. Boxes indicate the interquartile range and median values. Whiskers extend to the largest or smallest observations that fall within 1.5 times the box size; any observations outside these values are depicted by the circular dots. Values in insets are the coefficient of variation for each leaf trait. Boxes that share the same letter correspond to species' mean trait values that were not significantly different (LSD) at the 0.05 level. BP: *Betula platyphylla*; FM: *Fraxinus mandshurica*; and AM: *Acer mono*.

significant variation, this suggested that the trait-trait relationships were significantly modified by these ecological scales. Finally, we performed principal component analysis (PCA) on all leaf traits for each species and all three species individually to test whether there was a within-species LES across these ecological scales. Finally, we evaluated the distributions of leaves across different leaf phenology and plant age stages along the spectrum. All analyses were based on log-transformed leaf trait values.

3. Results

3.1. Comparison of leaf traits among species

Most leaf traits (except N:P) significantly varied among different shade-tolerant species ($P < 0.05$) (Fig. 1). For example, the SLA increased as the shade tolerance of the species increased. *F. mandshurica* (mid-shade tolerant) had the lowest LDMC values and the highest values for LT, N_{mass} and P_{mass}. The leaf morphological traits had less variation (mean CV = 27%) than did the leaf chemical traits (mean CV = 30%) among the three species (Fig. 1). Additionally, the CV of the leaf morphological traits generally increased with species with higher shade tolerance (Fig. 1).

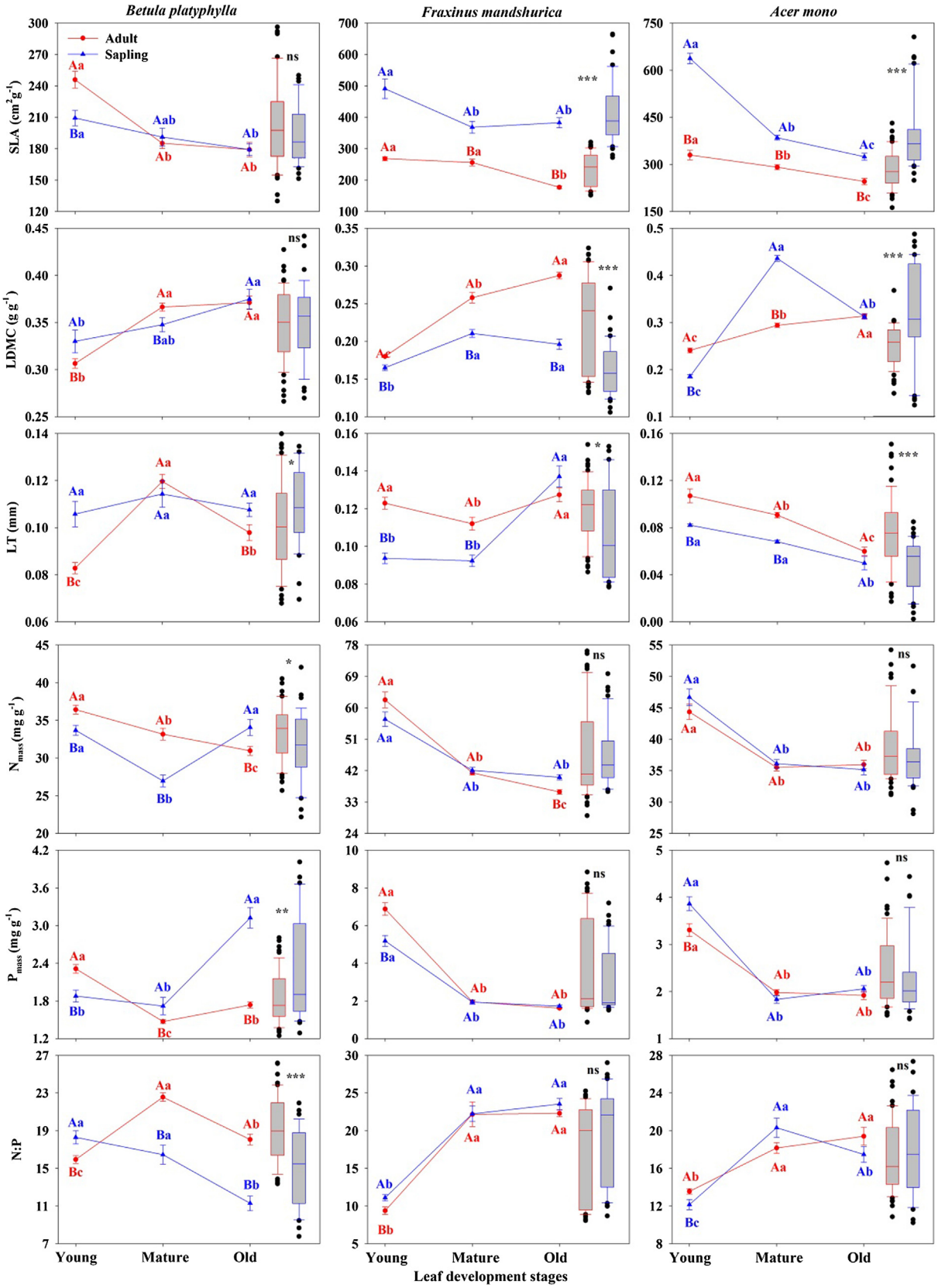

Fig. 2. Mean values and standard errors of SLA ($cm^2\ g^{-1}$), LDMC ($g\ g^{-1}$), LT (mm), N_{mass} ($mg\ g^{-1}$), P_{mass} ($mg\ g^{-1}$) and N:P across different leaf development stages (i.e., young, mature and old) and plant age stages (i.e., adult and sapling) for three temperate broadleaf species with different shade tolerances. Same lowercase letters for adults or saplings meant that leaf traits were not significantly different among leaf development stages for each species at the 0.05 level. Same capital letters in each leaf development stage meant that leaf traits were not significantly different between adults and saplings of each species at the 0.05 level. Inset boxes report ranges of each leaf trait in three leaf development stages for adult (left) and sapling (right) groups. Boxes indicate the interquartile range and median values. Whiskers extend to the largest or smallest observations that fall within 1.5 times the box size; any observations outside these values are depicted by the circular dots. Asterisks in insets indicate results of independent sample T test; ns, no significant difference, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

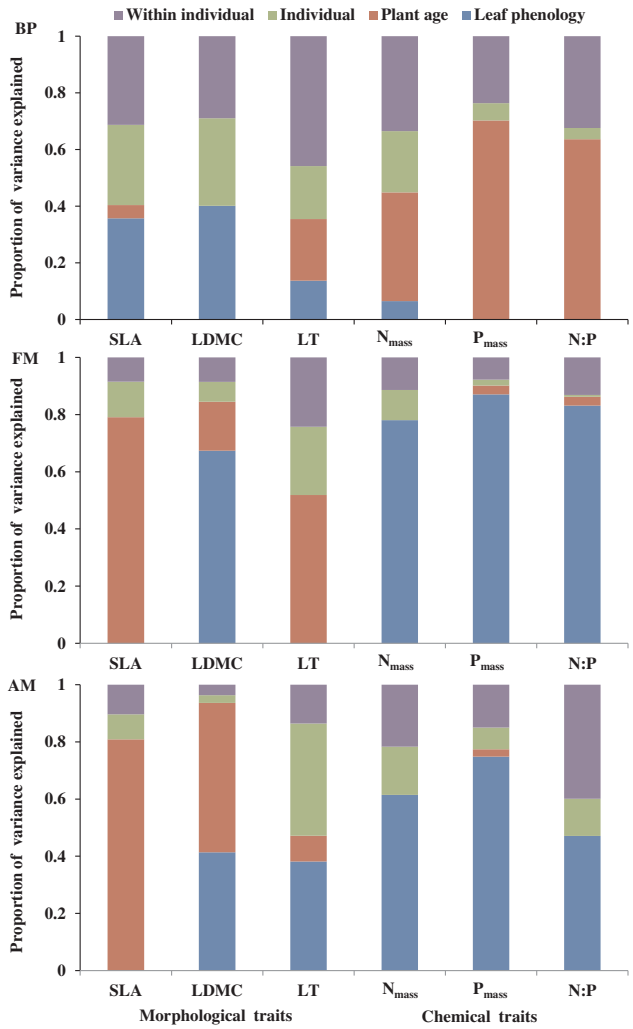

Fig. 3. Variance partitioning of six leaf traits for all trees (including adult and sapling samples) for three temperate broadleaf species across four nested levels: leaf phenology, plant age, individual and within individual. For any leaf trait-level combination, the proportion of variation explained here is based on the observed value derived by a nested ANOVA variance partitioning procedure. BP: *Betula platyphylla*; FM: *Fraxinus mandshurica*; and AM: *Acer mono*.

3.2. Variations in leaf traits among leaf phenology and plant age stages

The leaf traits significantly varied with leaf phenology for the three species; for example, the SLA and N_{mass} generally decreased with leaf development, and LDMC showed an opposite changing trend (Fig. 2). For shade-intolerant species (i.e., *B. platyphylla*), plant age had more influence on the leaf chemical traits than did the morphological traits; however, for young leaves, adults had higher SLA, N_{mass} and P_{mass} values than did saplings, and the other three leaf traits exhibited opposite trends (Fig. 2). As shade tolerance increased, the leaf morphological traits varied significantly with plant age, but this pattern was not observed for the chemical traits. Specifically, for each leaf development stage, saplings had higher SLA values than did adults of *F. mandshurica* and *A. mono*, but LT showed nearly opposite results (Fig. 2).

3.3. Variance partitioning

Leaf phenology explained the largest proportion (38%) of intraspecific trait variation in the six leaf traits for these three species; leaf phenology was followed by plant age, which explained 28% of the variance (Fig. 3). As the shade tolerance of the species increased, the decisive role of leaf phenology in explaining the variations in the leaf morphological traits was replaced by plant age. Similarly, the decisive role of plant age in explaining the variations in the leaf chemical traits was replaced by leaf phenology (Fig. 3). For example, leaf phenology explained 30% and 2% of the intraspecific trait variation in the leaf morphological and chemical traits, respectively, while the amount of intraspecific trait variation explained by plant age was 9% and 57%, respectively, for *B. platyphylla*. However, for the other two species, on average, leaf phenology explained 25% and 48% of the intraspecific trait variation in the leaf morphological and chemical traits, respectively, while the amount of intraspecific trait variation explained by plant age was 72% and 2%, respectively. Additionally, variations caused by the within individual scale accounted for 21% of the intraspecific trait variation in all leaf traits for these three species (Fig. 3).

3.4. Intraspecific trait-trait relationship

In a bivariate framework, many significant intraspecific trait-trait relationships were found at the local scale (Table 1). However, most covaried relationships were significantly affected by leaf phenology for the three species ($p < 0.05$) (Fig. 4, Figs. S4–6). For *B. platyphylla*, the slopes of all pairwise-trait (six leaf traits) relationships significantly varied with leaf phenology ($p < 0.05$), except for the relationships

Table 1
Bivariate relationships among six leaf traits in three temperate broadleaf species in northeast China.

Species	Leaf traits	Log SLA	Log LDMC	Log LT	Log N_{mass}	Log P_{mass}
Betula platyphylla	Log LDMC	−0.82***				
	Log LT	−0.52***	0.45***			
	Log N_{mass}	0.45***	−0.35**	−0.2		
	Log P_{mass}	0.30*	−0.26	−0.33*	0.37**	
	Log N:P	−0.1	0.11	0.26	0.08	−0.90***
Fraxinus mandshurica	Log LDMC	−0.78***				
	Log LT	−0.52***	0.29*			
	Log N_{mass}	0.30*	−0.64***	−0.15		
	Log P_{mass}	0.25	−0.64***	−0.15	0.92***	
	Log N:P	−0.21	0.59***	0.14	−0.81***	−0.98***
Acer mono	Log LDMC	−0.30*				
	Log LT	0.01	−0.31*			
	Log N_{mass}	0.43***	−0.64***	0.42***		
	Log P_{mass}	0.43***	−0.74***	0.32*	0.65***	
	Log N:P	−0.29*	0.58***	−0.17	−0.25*	−0.90***

All analyses were based on log-transformed leaf trait values.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

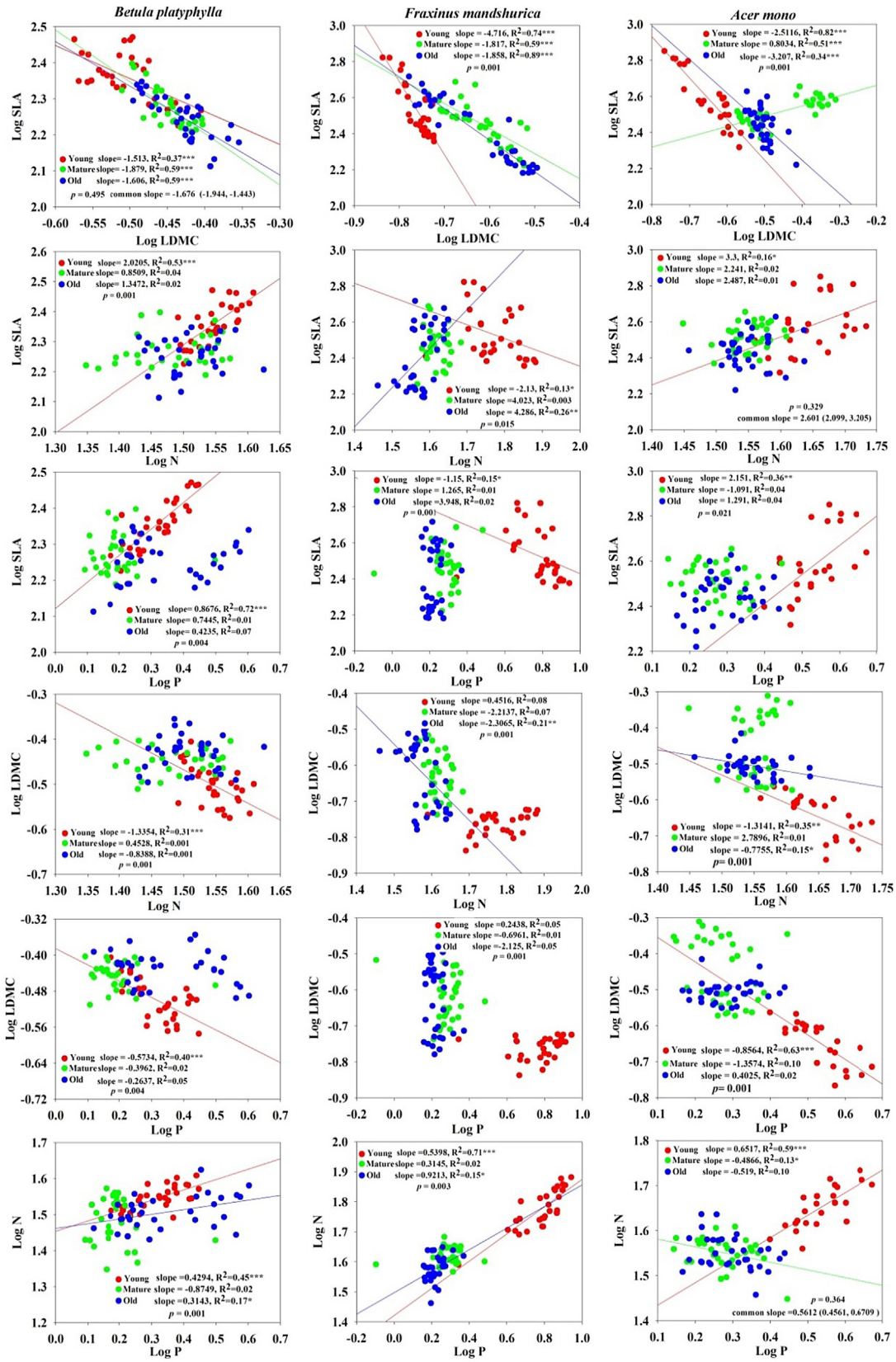

Fig. 4. Standardized major axis (SMA) bivariate relationships among SLA ($cm^2\ g^{-1}$), LDMC ($g\ g^{-1}$), N_{mass} ($mg\ g^{-1}$) and P_{mass} ($mg\ g^{-1}$) across different leaf development stages (i.e., young, mature and old) for three temperate broadleaf species with different shade tolerances. Each dot indicates the mean of each sun-shade level. Different colors denote the different leaf development stages. If there was no significant difference in the slopes of the relationships, the common slope and the 95% confidence intervals were shown. A line was not shown if there was no significant correlation between leaf traits. All analyses were based on log-transformed leaf trait values.

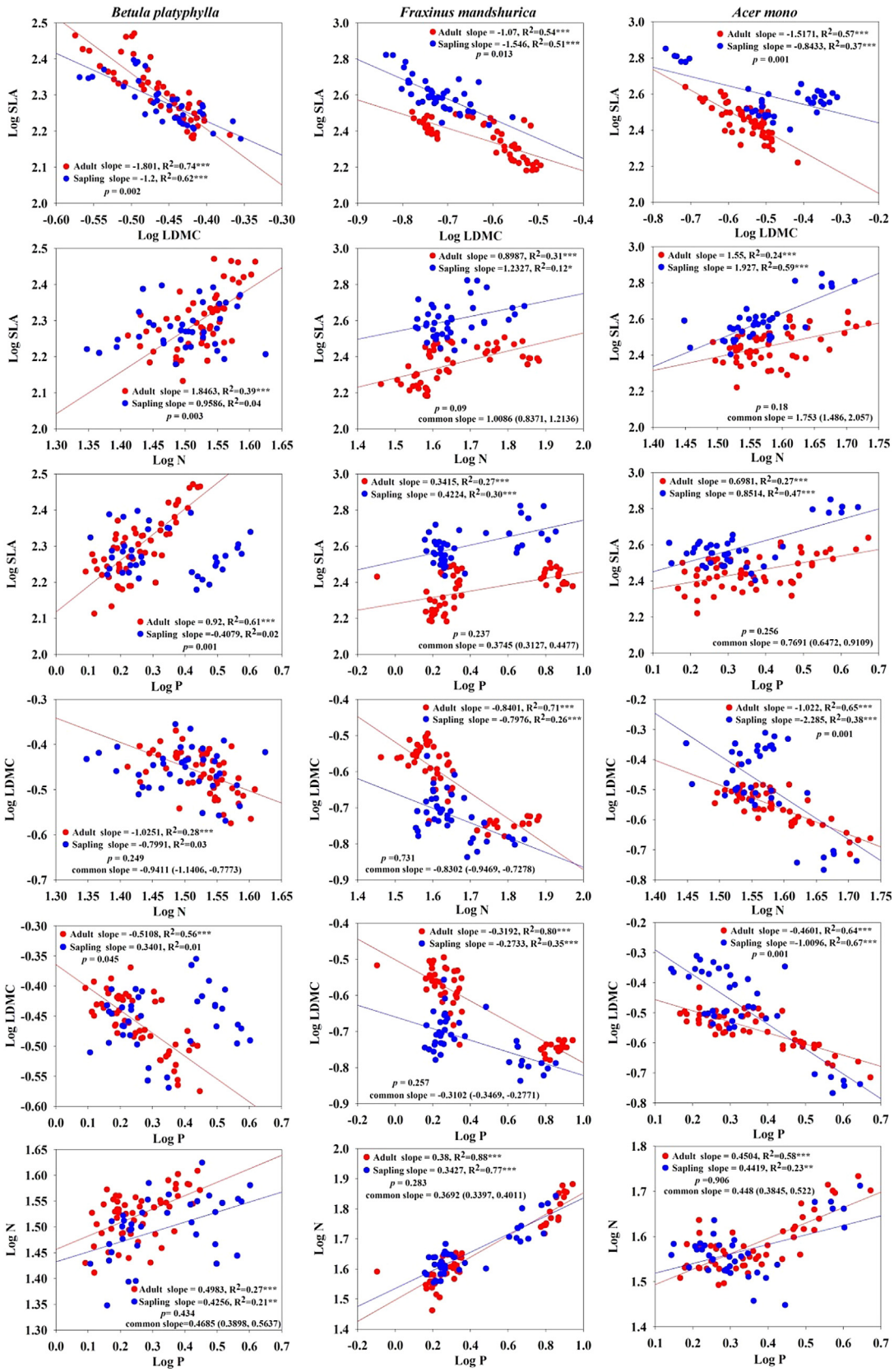

Fig. 5. Standardized major axis (SMA) bivariate relationships among SLA ($cm^2\ g^{-1}$), LDMC ($g\ g^{-1}$), N_{mass} ($mg\ g^{-1}$) and P_{mass} ($mg\ g^{-1}$) in different plant age stages (i.e., adult and sapling) for three temperate species with different shade tolerances. Each dot indicates the mean of each sun-shade level. Different colors denote the different plant age stages. If there was no significant difference in the slopes of the relationships, the common slope and the 95% confidence intervals were shown. The line was not shown if there was no significant correlation between leaf traits. All analyses were based on log-transformed leaf trait values.

between SLA and LDMC ($p = 0.495$ and common slope = −1.676), between SLA and LT ($p = 0.832$, and common slope = −0.8629), and between LDMC and LT ($p = 0.62$ and common slope = 0.5286) (Fig. 4, Fig. S4). Although significant positive relationships were observed between LDMC and LT in all samples ($r = 0.45$, $P < 0.0001$) (Table 1), no significant relationships were found for mature and old leaves (Fig. S5). For *F. mandshurica*, the slopes of all pairwise-trait relationships significantly varied with leaf phenology ($p < 0.05$) (Fig. 4, Fig. S5), except for the relationships between SLA and LT ($p = 0.065$ and common slope = −1.874) (Fig. S5) and between LT and N:P ($p = 0.078$ and common slope = −1.0148) (Fig. S5). For *A. mono*, the slopes of each pairwise-trait relationship significantly varied with leaf phenology ($p < 0.05$) (Fig. 4, Fig. S6). These results suggested that intraspecific trait-trait relationships were seriously modified by leaf phenology at the local scale.

In contrast, plant age affected the patterns of trait covariation less significantly than did leaf phenology for the three species (Fig. 5, Figs. S7–9). However, the slopes of most pairwise-trait relationships significantly varied with plant age for the three species, such as the slopes of the relationship between SLA and LDMC for the three species ($p < 0.05$) (Fig. 5) and the relationship between LDMC and LT for *F. mandshurica* ($p = 0.001$) and *A. mono* ($p = 0.001$) (Fig. 5, Figs. S8–9). Generally, the slopes of the relationships between leaf morphological traits were more easily affected by plant age than were the relationships between leaf chemical traits (Fig. 5, Figs. S7–9).

3.5. Coordination of LES traits along leaf phenology and plant age stages

We found the coordination of LES traits within the three species (Table 1, Fig. 6) and between the different species (Fig. 7), and this coordination was observed along both leaf phenology and plant age stages. The axis with the higher SLA, N and P indicated an acquisition strategy, while the axis with the higher LDMC and N:P ratio represented a conservation strategy (Figs. 6,7). However, LT could not be well integrated into the LES because of the varying correlations with N_{mass} and P_{mass} (Table 1, Figs. 6,7).

We found that the intraspecific trait variation among leaf development stages was driven by the different LES strategies, while the intraspecific trait variation between plant age stages was driven by the LT for both *F. mandshurica* and *A. mono* (Fig. 6). The young leaves showed acquisition strategies, while the mature and old leaves showed conservation strategies (Fig. 6b, c). The adult trees often had thicker leaves, while the sapling trees had thinner leaves (Fig. 6e, f). However, there was no apparent pattern of variation for *B. platyphylla* (Fig. 6a, d).

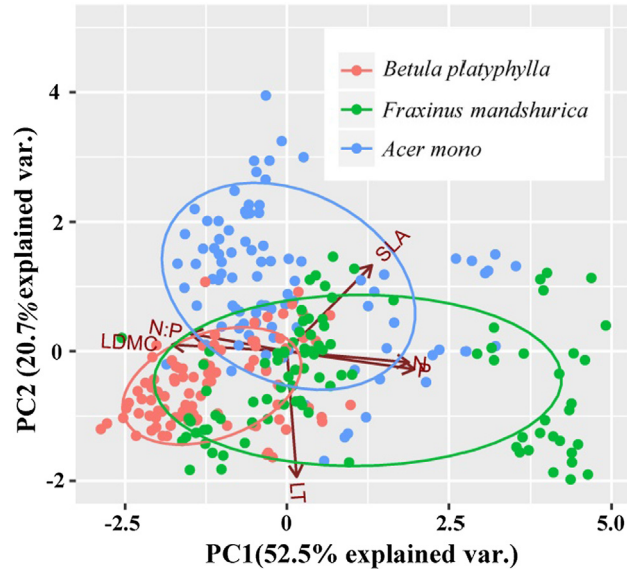

Fig. 7. Principal component analysis of six leaf traits for all species. Six leaf traits: SLA ($cm^2\ g^{-1}$), LDMC ($g\ g^{-1}$), LT (mm), N_{mass} ($mg\ g^{-1}$), P_{mass} ($mg\ g^{-1}$) and N:P.

4. Discussion

4.1. Leaf trait variations

Understanding differential variations in the suite of leaf traits responsible for the shade tolerance levels of co-occurring species is critical for predicting ecosystem responses to global change (Lavorel and Garnier, 2002; Valladares and Niinemets, 2008). Our results showed

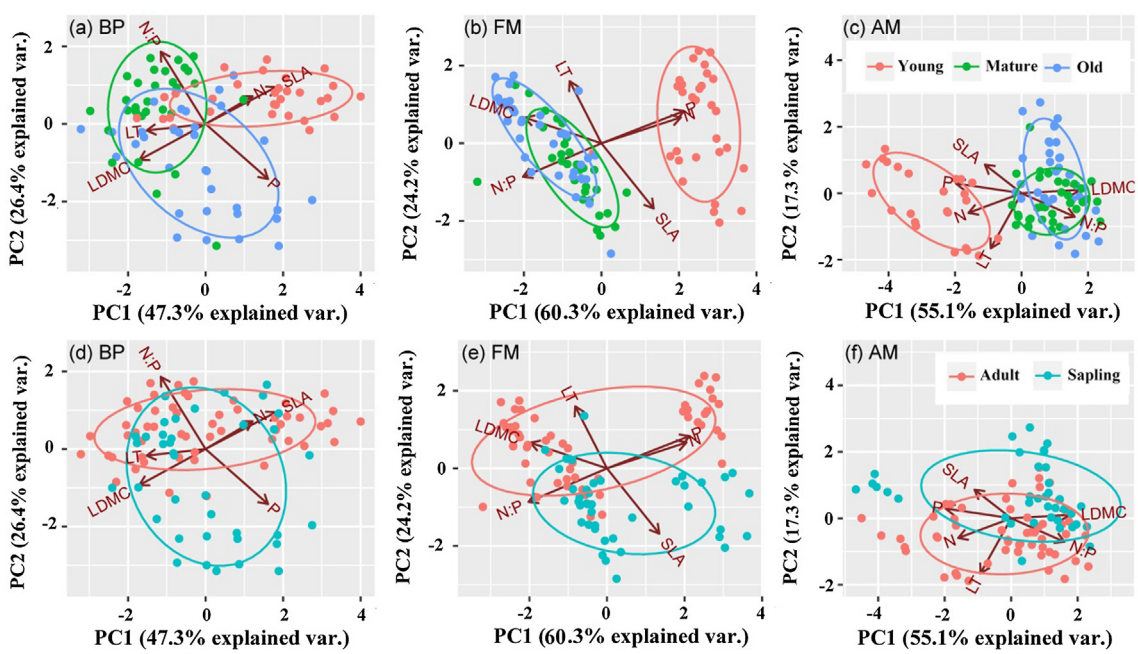

Fig. 6. Principal component analysis of six leaf traits within BP (*Betula platyphylla*; a and d), FM (*Fraxinus mandshurica*; b and e) and AM (*Acer mono*; c and f) across leaf phenology and plant age stages. Six leaf traits: SLA ($cm^2\ g^{-1}$), LDMC ($g\ g^{-1}$), LT (mm), N_{mass} ($mg\ g^{-1}$), P_{mass} ($mg\ g^{-1}$) and N:P.

that SLA was positively correlated with shade tolerance when the variations in leaf phenology and plant age were integrated for the three temperate deciduous broadleaf species at the local scale (Fig. 1). Thus, these results expand the applicability of the carbon gain hypothesis at the local scale (Givnish, 1988; Valladares and Niinemets, 2008), and suggest that later successional species are expected to have higher shade tolerance because only shade-tolerant species can survive and grow under the closed canopy of an old-growth forest. However, this pattern contradicts the ideas of global LES, which indicate that fast-growth species (i.e., shade-intolerant) usually have higher SLA (Wright et al., 2010) that could be resolved by changing leaf morphology with ontogeny. In contrast, the traits that were closely associated with the SLA that were expected by global LES did not exhibit the same variation patterns as those observed for the shade-tolerant species; for example, the LT, N_{mass} and P_{mass} values of the mid-shade-tolerant species (*F. mandshurica*) were all significantly larger than those for the other two species. At this point, the intraspecific trait-trait relationships may be decoupled from the global LES.

Moreover, the relative importance of intraspecific trait variation decreased with the increase in spatial scale (Siefert et al., 2015; Messier et al., 2017b); in this case, effectively accounting for intraspecific trait variation at relatively short environmental gradients (e.g., local scales) becomes especially relevant and urgent. We found that the mean intraspecific trait variation in the leaf morphological traits was lower than that in the chemical traits for the three species at the local scale (Fig. 1), indicating that leaf morphology tends to be more stable at the local scale; similar results have been reported by Rozendaal et al. (2006), Kazakou et al. (2014) and Siefert et al. (2015) at the community level. We also found that the mean value and CV of SLA of species generally increased with increasing shade tolerance (Fig. 1), which was supported by the fact that shade-tolerant species should have higher plasticity in their leaf morphological traits (e.g., SLA), which is important for light harvesting (Niinemets et al., 1998). In contrast, Chmura et al. (2017) found that seedlings of shade-intolerant species had higher CV of SLA than that of shade-tolerant species. Hence, the relationship between CV of SLA and species tolerance may differ in saplings and adult trees from that in seedlings (Lusk and Warton, 2007; Valladares and Niinemets, 2008).

Many previous studies have indicated that leaf plasticity in trees depends on leaf phenology and plant age (Reich and Oleksyn, 2004; Rozendaal et al., 2006; Poorter et al., 2009). The SLA values for the three deciduous broadleaf species were larger for young leaves than those for both mature and old leaves, which did not support our previous hypothesis (e.g., mature leaves had larger SLA than did young and old leaves for evergreen species) (Table S1). This result was partly caused by the differences in the leaf phenology processes between deciduous and evergreen species; for example, new leaves emerge in early May for most winter deciduous species, but most evergreen species were flushing new leaves in August and September (Wu et al., 2016). Similarly, the N_{mass} and P_{mass} values were high in young expanding leaves, but they decreased with leaf development in *F. mandshurica and A. mono*, likely because of the incorporation of N and P in structural materials; eventually, this results in the retranslocation of N and P during leaf senescence (Norby et al., 2000; Grassi et al., 2005). These patterns are also consistently seen in other broadleaf deciduous forests (Wilson et al., 2000; Simioni et al., 2004; Grassi et al., 2005; Mason et al., 2013). Additionally, N:P significantly varied with leaf phenology, and the value for young leaves was usually significantly lower than those for mature and old leaves for the three species (Table S1), likely because new leaves grow rapidly and reduce N and P, and P reductions occur much faster than do N reductions; thus, this leads to increased N:P ratios, and this case indicated that N:P was mainly determined by P, which was supported by our results (Figs. S4–6) and the results of others (Vanni et al., 2002; Elser et al., 2010). Thomas and Winner (2002) used meta-analysis and found that SLA was clearly lower in adults than in saplings for every reviewed species, and this finding was generally supported by our results (Fig. 2), Liu et al. (2010) and Martin and Thomas (2013); additionally, these results were expected, likely because low SLA helps larger trees effectively use light and water. Liu et al. (2010) reported that leaf P_{mass} in 47 species was clearly larger in small trees than in larger trees; however, we noted that P_{mass} was not clearly different between adults and saplings of *F. mandshurica* and *A. mono*, possibly because the allocation of P to the leaves was similar between adults and saplings of shade-tolerant deciduous species. Furthermore, we surprisingly found that leaf morphological traits were more sensitive to leaf phenology than were leaf chemical traits, which were more sensitive to plant age for the species *B. platyphylla*. As the shade tolerance of species increased, opposite results were observed, i.e., leaf morphological and chemical traits were sensitive to plant age and leaf phenology, respectively. These results modulated the widely recognized conclusion that leaf chemical traits are more highly labile than are morphological traits within species (Rozendaal et al., 2006; Kazakou et al., 2014; Siefert et al., 2015). These results are supported in Fig. 3.

Although many leaf traits (e.g., SLA, LDMC and N_{mass}) varied markedly with leaf phenology (Reich et al., 1991; Liu et al., 2010; Nouvellon et al., 2010; Sperlich et al., 2015; Chavana-Bryant et al., 2016) or plant age (Liu et al., 2010; Martin and Thomas, 2013; Kuusk et al., 2018), to date and to the best of our knowledge, the relative importance of leaf phenology and plant age in the determination of intraspecific trait variation at local scales had not yet been explored. On average, leaf phenology and plant age explained 38% and 28%, respectively, of the intraspecific trait variation in the six leaf traits of the three species, which supported our hypothesis (Fig. 3). These results indicated that both leaf phenology and plant age should be seriously considered in exploring intraspecific trait variation at local scales in the future.

4.2. Variations in trait-trait relationships along leaf phenology and plant age stages

The positive or negative correlations between most paired leaf traits for the three temperate deciduous broadleaf species were consistent with the results from previous studies conducted with different sites, tree species, functional groups and scales (Table 1) (Wright et al., 2004; Wright et al., 2005b). In other words, there was strong evidence that confirmed that the LES generally holds at the local scale, and this result provides a new dimension to the LES. For example, SLA was significantly and positively correlated with leaf N_{mass} and P_{mass} integrating sample leaves in different leaf development and plant age stages within species, consistent with the global LES prediction (Poorter and Evans, 1998; Reich et al., 1998; Wright et al., 2004; Grubb, 2016; Fajardo and Siefert, 2018). SLA was significantly and negatively correlated with LDMC and LT (Wilson et al., 1999; Shipley and Vu, 2002; Griffith et al., 2016); and N_{mass} and P_{mass} were significantly positively correlated (Wright et al., 2004; Osnas et al., 2013; Fajardo and Siefert, 2018). However, Liu et al. (2010) demonstrated that the patterns of some leaf trait-trait relationships could be modified by plant size in a study that used 47 species in a tropical montane rainforest. In this study, SLA was significantly correlated with N_{mass} (or P_{mass}) in adults, but no significant correlation was observed in saplings of *B. platyphylla* (Fig. 4). In contrast, for *F. mandshurica* and *A. mono*, there was a significant positive correlation between SLA and N_{mass} (or P_{mass}), but the slopes did not vary between the adults and saplings ($p = 0.09$ and $p = 0.18$), and the common slopes were 1.0086 [95%CI = (0.8371, 1.2136)] and 1.753 [95%CI = (1.486, 2.057)], respectively (Fig. 4). This was probably because of the variation in shade tolerance; specifically, the higher shade tolerance of *F. mandshurica* and *A. mono* enabled adults and saplings to better adapt to ambient light conditions and, thus, produce similar SLA at a given N cost. We also found that leaf N_{mass} and P_{mass} was significantly correlated in both adults and saplings, but the slopes did not significantly vary, with a common slope of 0.4685

[95%CI = (0.3898, 0.5637)], 0.3692 [95%CI = (0.3397, 0.4011)] and 0.448 [95%CI = (0.3845, 0.522)] for *B. platyphylla*, *F. mandshurica* and *A. mono*, respectively (Fig. 4). These results were inconsistent with the results reported by Liu et al. (2010), which stated that the slopes of the relationships between N_{mass} and P_{mass} significantly varied between adults and saplings. The differences in these conclusions could be explained by N:P, i.e., the mean value was larger for large trees (N:P with a value of 17) than for small trees (13), which indicated that the larger trees were more likely limited by P in the tropical montane rainforest; however, in cold-temperate forests (i.e., this study), there was no clear difference in N:P between adults and saplings, which had N:P values of 18 and 17 (Table S2), respectively; these results indicated that both adults and saplings were more likely to be limited by P, which was inconsistent with the traditional view of N limitation for plant growth in cold-temperate forests (Magnani et al., 2007; Lebauer and Treseder, 2008; Vitousek et al., 2010). The different conclusion was probably because we included all sampled leaves from the three leaf development stages when we calculated N:P for both adults and saplings.

In contrast, few studies have been designed to evaluate whether relationships between leaf traits varied with leaf phenology. Reich et al. (1991) and Bloomfield et al. (2018) demonstrated that leaf phenology might affect trait-trait relationships, but neither study found uniform leaf phenology patterns. In this study, we also found that most slopes of the relationships of the paired six key leaf traits significantly varied with leaf phenology for the three species. For instance, for *B. platyphylla* and *A. mono*, SLA was significantly correlated with N_{mass} and P_{mass} for data pooled across all leaf development stages (Table 1), but the only significant correlation was found for young leaves (Fig. 4); these results indicated that the production of SLA for young leaves (i.e., samples from June) of the two species was more sensitive to nutrient contents (i.e., N, P) than was the production of SLA for older leaves (i.e., mature and old leaves). In contrast, there was no significant correlation between SLA and P_{mass} for all pooled data of *F. mandshurica* (Table 1); however, a significant correlation was observed within a certain leaf development stage (e.g., young leaves; Fig. 4). There were different patterns in all relationships involving SLA, which suggested that the timing and magnitude of the N and P fluxes into and out of the leaf (versus other leaf constituents, such as carbon) differed (Reich et al., 1991). Although N_{mass} was significantly correlated with P_{mass} for data pooled across all leaf development stages for the three species ($P < 0.01$) (Table 1), no significant correlation was found within a certain leaf phenology stage, such as mature leaves of *B. platyphylla* and *F. mandshurica* (Fig. 4). This result was likely explained by clear leaf phenology variations in the N:P ratios. For the three species, the N:P ratios of young leaves (mean value of 13) were lower than 14, but they were larger than 16 for both mature (21) and old leaves (19) (Table S1), which indicated that the trees considered here were more likely to be limited by N when the leaves were young; however, the trees were more likely to be limited by P as leaf development progressed, which suggested that leaf phenology should be fully considered in the examination of nutrient limitations for plant growth in future studies. In general, our results suggest that the growth of plants in cold-temperate forests was co-limited by N and P (supported by Li et al., 2018), but was dependent on leaf phenology (supported by Townsend et al., 2007).

4.3. Coordination of intraspecific leaf economic traits along leaf phenology and plant age stages

We found evidence of within-species LES traits based on the three temperate species that were evaluated in northeastern China. Our study followed the recent focus of researchers, i.e., we downscaled the LES from the species level to the intraspecific level (Wright and Sutton-Grier, 2012; Niinemets, 2015; Martin et al., 2017; Fajardo and Siefert, 2018). Some recent studies have found coordination among LES traits within a single species across large study regions using static data (Hu et al., 2015; Niinemets, 2015; Martin et al., 2017). Consistent with previous studies, here, we found coordination among these traits at the local spatial scale (i.e., a 9-ha forest plot) but over a longer temporal scale (i.e., seasonal dynamics and the differences between plant age stages). However, Messier et al. (2017a) suggested that there might not be an LES at the local scale because they found different factors driving the variation in the LES traits. In our study, leaf phenology played a more important role in driving the variation in each LES trait than did plant age. Therefore, the strength of coordination among the LES traits decreased when we analyzed the traits of different plant age stages during each leaf phenology stage (Fig. 4), which may have been due to the shorter range of trait variation (Messier et al., 2017a). A broad covariation along the LES across the intraspecific level, species level and community level can help us understand how functional dimensionality influences the response to environmental gradients and climate change at scales ranging from individuals to ecosystems (Wright et al., 2004; Niinemets, 2015; Jiang et al., 2017).

We also found a differentiation in ecological strategies along leaf phenology and plant age (Fig. 6). First, the ecological strategies of leaves differed along the LES for the three leaf development stages, with acquisition ending for young leaves and conservation ending for mature and old leaves. This pattern might reflect the process of leaf development. In early spring, a leaf develops quickly to generate enough leaf area to intercept light. As time progresses into summer, however, the pressure posed by herbivores or pathogens increases relative to spring. Thus, more photosynthetic products were allocated to the construction of leaves to protect them from external environmental pressures and damage rather than to growth. This pattern was consistent for adult and sapling trees. Second, LT drove the intraspecific trait variation among plant age stages, with thicker leaves for adults and thinner leaves for saplings for both *F. mandshurica* and *A. mono* (Fig. 6). This pattern gave the adult trees a stronger advantage in protecting leaves from the complex environment. The pattern also implied that adult tress had a stronger acclimation ability to environmental variables. When analyzing the three species together, we found a larger intraspecific trait variation for *F. mandshurica* and *A. mono* than for *B. platyphylla*. *B. platyphylla* is an early-successional species; however, when living in a late-successional forest, lower intraspecific trait variation decreased the survival ability and further decreased the distribution (i.e., abundance) of *B. platyphylla* in this forest relative to the other two species (Umaña et al., 2015).

5. Conclusions

At the local scale, the key LES traits, including SLA, N_{mass} and P_{mass}, significantly varied with species, but changing patterns due to shade tolerance conflicted with the LES prediction and were sometimes even opposite. These surprising results suggested that variations in the LES traits were not always reliable for interpreting the differences in the resource-use strategies of plants (Anderegg et al., 2018). For shade-intolerant species (*B. platyphylla*), the variations in the leaf morphological traits were mainly driven by leaf phenology, while the variations in the leaf chemical traits were mainly driven by plant age; in contrast, the mid- and shade-tolerant species (*F. mandshurica* and *A. mono*) showed a reversal in their main drivers. These results suggested that intraspecific trait variation is not only caused by leaf phenology and plant age but also driven by shade tolerance of the species at the local scale. Generally, intraspecific trait variation was mainly caused by leaf phenology and plant age at the local scale; thus, leaf phenology and plant age should be seriously considered in future studies that use trait-based DGVMs to model species' responses to a changing environment. We found direct evidence for the coordination of global LES traits within species at the local scale, but the trait-trait relationships could be modulated by leaf phenology and plant age, which provides novel insights into understanding the LES at the intraspecific level.

Acknowledgement

The work was financially supported by the National Natural Science Foundation of China (No. 31730015, 31600587) and Yong Elite Scientists Sponsorship Program by CAST (2018QNRC001).

Author contributions

Guangze Jin and Zhili Liu planned and designed the research. Zhili Liu performed experiments and conducted fieldwork. Zhili Liu and Feng Jiang analysed data and wrote the manuscript. Guangze Jin and Fengri Li provided helpful comments in the draft.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2018.12.008.

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