

# **Regeneration patterns and intra- and inter-specific variation of the leaf traits of three temperate broadleaved species in mixed broad-leaved Korean pine forest**

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# Abstract

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Nowadays, under the background of climate change, people pay more and more attention to the protection and research of forest ecosystem. In this thesis, three broad-leaved tree species, *Betula platyphylla*, *Tilia amurensis*, and *Acer mono*, were selected in northeast China to study the regeneration patterns of seedlings and saplings and the variation of leaf functional traits. In the first study (Chapter 2), I explored the distribution characteristics of slope, aspect, topographic position, canopy openness and latitude on seedlings and saplings regeneration, I found that there were few seedlings and saplings of *B. platyphylla*, followed by *T. amurensis* in the middle of the succession, while there are many *A. mono* in the late succession, and they often occur on gentle slopes, middle and upper slopes, and sunny slopes. Canopy openness was strongly negatively correlated with the number of seedlings and saplings of *T. amurensis* and *A. mono*. In the second study (Chapter 3), I measured three economic traits, two anatomical traits and two hydraulic traits. I have found the leaf traits significantly varied across the different survey sites, there were correlations among leaf traits. Therefore, based on seedlings and saplings regeneration patterns and the intraspecific variation and coordination of different species along latitudinal gradients will help to further reveal the adaptation strategies and future development directions of plants to climate change.

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# Chapter 1: General introduction

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## 1.1 Coexistence theory

How similar species coexist, or how species richness in communities is maintained, are key questions in community ecology (Chesson 2000; Niu et al., 2009; Adler et al., 2010; Sedio and Ostling, 2013). Understanding the mechanisms of species coexistence in communities is crucial for understanding the process of community assembly and explaining community diversity (Bagchi et al., 2014). Coexistence theory is a framework for understanding how communities maintain species diversity and many models have been explored to examine species coexistence (Chesson, 2000). Mechanisms of species coexistence that have been explored explain how the variable competitive ability of different species (interspecific competition) are affected by equalizing processes that reduce competition and therefore exclusion of taxa, and how disturbance drives environmental heterogeneity in a landscape (Chesson, 1997, 2000). Other processes that support the maintenance of species diversity include intraspecific competition (stabilizing processes; Chesson, 2000). Studies on plant species coexistence suggest that species coexistence is determined by a combination of evolutionary, historical and ecological processes (Zhang et al., 2001; Hou & Ma, 2002). Recently, attention has been turned to how coexistence theory can provide a basis for the protection and restoration of plant communities in the context of changing climates and degradation of ecosystems (McGill et al., 2006).

Ecologists have proposed a range of mechanisms to understand species coexistence in plant communities. These mechanisms can be roughly divided into:

- 1) Equalising mechanisms – which affect inter-specific competition through processes that affect the fitness of a species relative to other co-occurring taxa..
- 2) Stabilising mechanisms – these are processes that promote coexistence through driving greater intra-specific competition than inter-specific competition including processes such as

resource partitioning.

3) Neutral theory—this is based on the assumption of ecological equivalence across species.

Equalizing mechanisms function on the basis that individual species have differences in fitness along environmental gradients; therefore, mechanisms that diversify habitat structure in a landscape, such as disturbance events like fire, will drive resource availability for a greater number of taxa. Equalizing mechanisms emphasize that the spatio-temporal changes of community environment and niche differentiation of coexisting species are the key to species coexistence (Tilman, 1982; Chesson, 2000; McGill et al., 2006). So at a broad scale, different species, with different life-histories and strategies, occupy different ecological niches and use space and resources differentially to achieve stable coexistence. However, equalising mechanisms alone do not always predict community structure: for example, many species can coexist stably even if niche differentiation is not obvious, take plants in tropical rain forest as an example (Bell, 2000; Hubbell, 2001). In tropical forest, many species occupy local communities without strong differentiation in niche space.

Stabilising mechanisms are those that examine the effect of intraspecific competition on coexistence. Interspecific competition should drive low species diversity due to differences across species' competitive ability; however, where intraspecific competition is greater than interspecific competition a stabilising effect is observed promoting species diversity (Bengtsson et al., 1994). Environmental factors can weaken the interspecific difference when species compete for different resources (resource partitioning), achieving stable coexistence (Bengtsson et al., 1994). Chesson (2000) expands on the ideas around stabilising mechanisms and suggests that there are several different types of stabilizing mechanism including:

- 1) Fluctuation-independent mechanisms – these are mechanisms that are highly localized in space and time, for example: resource partitioning;
- 2) Storage effects – species persisting due to differential effects of environmental variation (covariance between environment and competition) on competitive ability over space and time.

3) Predation – species specific predators will increase in locations where their prey is in higher abundance; any competitive benefit to an individual taxa due to environmental variation may be controlled by predation.

Evidence to support this theory has been observed in studies on forest species coexistence (Higgins et al., 2000; Kelly and Bowler, 2002; Gutiérrez et al., 2008; Norghauer and Newbery, 2014).

Unlike the theory of species coexistence described above, the neutral theory assumes that coexisting species (individuals) are ecologically equivalent (e.g., have the same mortality rates), that a community can be formed and maintained as a dynamic equilibrium between the stochastic migration / dispersal of species, the arising of new species, and the extinction of old species; species coexistence within a local community is determined by stochastic processes and dispersal limits (Hubbell, 2001). The introduction of the neutral theory has led to a heated discussion on the mechanisms of species diversity and community composition in tropical forests with high species richness. However, many studies have found that the neutral theory cannot always explain patterns of species diversity in a community (Niu et al., 2009). Further, studies have shown that species can have different ecological functions in the initial stage, which is not consistent with the view of neutral theory (all species supposedly being ecologically identical), and species are known to have different effects on population dynamics, community function, and ecological strategy (Zhou et al., 2006). Therefore, resource partitioning and species traits appear to play an important role in inter and intra-specific competition and the maintenance of species diversity.

## 1.2 Resource partitioning and the regeneration niche

The term regeneration niche was described by Grubb (1977); in the study of forest community species coexistence, the regeneration niche has been one of the hot spots. Seedlings of different species respond differently to the understory micro-environment during the early stages of tree growth, resulting in the differential survival rates of seedlings across the

environmental gradient, i.e., regeneration niches (Grubb, 1977; Harper, 1977). Thus, the tree regeneration niche includes a range of biotic and abiotic environments that are suitable for taxaregeneration. The seedling stage is the most vulnerable and environmentally sensitive stage of a tree's life history, therefore the regeneration niche is particularly important for population self-sustainability and forest cycling (Adler et al., 2010; Bagchi et al., 2014; Chu et al., 2016). Spatial and temporal differentiation of tree regeneration niche (niche differentiation), is particularly important to reduce competition—as an equalising mechanism *sensu* Chesson (2000)—in the seedling stage of populations and to promote coexistence of species. Therefore, in forest communities, resource differentiation of different species at the regeneration stage is considered an important mechanism to explain the coexistence of forest taxa (Grubb, 1977). Coexisting species with different life history traits in forest communities differ in their early life history stages (from seed germination to juvenile tree formation) in their response to the gap formation and the resulting spatial and temporal heterogeneity of the environment (regeneration niche differentiation) (Grubb, 1977). Nevertheless, Clark et al. (1992) did not find significant regeneration niche differentiation in coexisting species in tropical rain forests, and Sipe and Bazzaz (1995) obtained similar conclusions in temperate forests in the eastern United States. This suggests that the regeneration niche hypothesis needs further testing.

In temperate forests, species with different or even contrasting life history traits can often coexist over time to form stable communities (Lusk and Smith, 1998; Gutiérrez et al., 2008). Based on the regeneration niche hypothesis, many scholars have hypothesized that forest disturbance events are an important mechanism for the stable coexistence of these species with different life history traits (Grubb, 1977; Pearson et al., 2003; Gutiérrez et al., 2008). The regeneration of species in many temperate forest communities is driven by disturbance, which creates heterogeneity in the environment at different scales (e.g., gap formation, animal predation, litter cover, etc.), and differences in species' responses to environmental changes caused by disturbance due to the seed germination stage or seedling formation stage can be seen as resource

partitioning. Therefore, the heterogeneity of the understory environment caused by forest disturbance can provide regeneration opportunities for species with different regeneration requirements, effectively equalizing fitness differences across species and promoting coexistence.

### 1.3 Plant functional traits

Plant functional traits refer to a set of core plant attributes that are closely related to plant establishment, survival, growth and mortality, and which can significantly influence the function of an ecosystem and reflect the adaptation of plants to environmental changes (Liu and Ma, 2015). In contrast to many studies that focus on plant taxonomy and abundance, the study of plant functional traits has become an important and reliable way to address ecological questions at the population, community, and ecosystem scales (Reich et al., 2003). There are many relationships between functional traits of species, with many trading off against each other. Combinations of traits that result from natural selection also occur defining a species' "ecological strategy", and this can place a species in the most appropriate position on a competitive axis (Westoby et al., 2002; Díaz et al., 2004; Wright et al., 2007). Investigating the relationships among plant traits allows ecologists to understand the differences in plant ecological strategies within and among environments and to explore more deeply the mechanisms underlying ecological niche differentiation and species coexistence.

As plants differ in functional traits within small-scale communities, these differences lead to patterns of distribution of functional traits along latitudinal gradients or at large scales (Liu and Ma, 2015). Inter- and intra-community studies of differences in functional traits in woody plants have revealed that functional traits are not only distributed along soil moisture gradients, but also differ among species coexisting along a soil gradient (Ackerly and Cornwell, 2007). By examining and comparing the distribution of species functional traits, species coexistence mechanisms, and community dynamics within different local communities, these studies can help to identify the mechanisms underlying the distribution patterns of species at large scales (Wright

et al., 2004). Within small-scale communities, species coexistence occurs mainly because functional traits affect the arrangement of species on environmental gradients, competition among species, and the allocation of resources within the community by species, due to species with similar functional traits having similar resource requirements. Such species are likely subject to more intense inter-specific competition where equalizing and stabilizing mechanisms do not otherwise provide an escape from interspecific competition. Conversely, the greater the difference in functional traits of species, the likely greater difference in resource requirements of species, which facilitates interspecific coexistence (Sutherland et al., 2013).

In recent years, ecologists have done a lot of research on discrete patterns of functional traits in space and time as an entry point to investigate the role played by environmental filtering, species' ecological niche differentiation and neutral processes in community species coexistence (Liu et al., 2014). For the habitat filtering theory, ecologists focus on how functional traits of individual species are distributed along environmental gradients, and the competition across species due to the differences in functional traits. Scholars who subscribe to the niche differentiation theory usually focus on whether there are significant differences in functional traits among different communities, and whether traits are more divergent among co-existing species within communities, however, scholars who support the neutral theory have investigated the functional trait patterns of communities to examine whether these are consistent with the spatial aggregation patterns simulated by diffusion-limited causes (Lebrija-Trejos, 2010; Swenson and Enquist, 2009; Paine et al., 2011; Moles et al., 2009; Chave et al., 2010, Lake and Ostling, 2008;).

Findings of studies to date suggest that variation in species trait values within many communities is greater than expected, and that it is possible that differences in functional traits can promote species coexistence within communities. Nevertheless, many phenomenological-based approaches to discrete pattern analysis of traits have only identified variation in functional traits at the community level, but have not identified trait variation in response to environmental heterogeneity at the species level making it difficult to examine the

coexistence mechanisms and predict the effects of local environmental change on species diversity.

To understand how species co-exist within the equalizing (regeneration niche) and stabilizing (resource partitioning) mechanisms laid out in Chesson's (2000) coexistence framework, we must observe both patterns of regeneration, as well as examine functional traits of species along an environmental gradient. The aim of this thesis is to examine aspects of coexistence of three broadleaved tree taxa common to the mixed broadleaved Korean pine forest of north eastern China, and to achieve the following objectives:

1. Identify patterns in the regeneration niche, which can provide a mechanistic explanation for community species coexistence; and,
2. Identify functional trait variation along environmental gradients across these three taxa which will inform predictions of community diversity under different environments based on traits.

## 1.4 Study species

In this study, *Betula platyphylla*, *Tilia amurensis* and *Acer mono* were selected as the research objects, because they occupy different successional stages of dynamics within this forest. They generally occur in early, middle and late successional stages following disturbance respectively. All of them are broad-leaved species, and the shade-tolerance of their seedlings increases strongly along this same gradient (Zhang, Jin, & Liu, 2019).

### 1.4.1 *Betula platyphylla*

*Betula platyphylla* belongs to the deciduous trees of the Betulaceae (Fagales), and is one of the important broad-leaved timber trees in China. The bark is dark reddish brown and smooth when young, and white and peeling when adult. *Betula platyphylla* is a shade-intolerant tree species. It is fond of light and acidic soils, and often establishes on flat land and gentle slopes. It

grows best in moderately wet conditions (Ren, 1997). *Betula platyphylla* has rapid germination, rapid growth and regeneration.



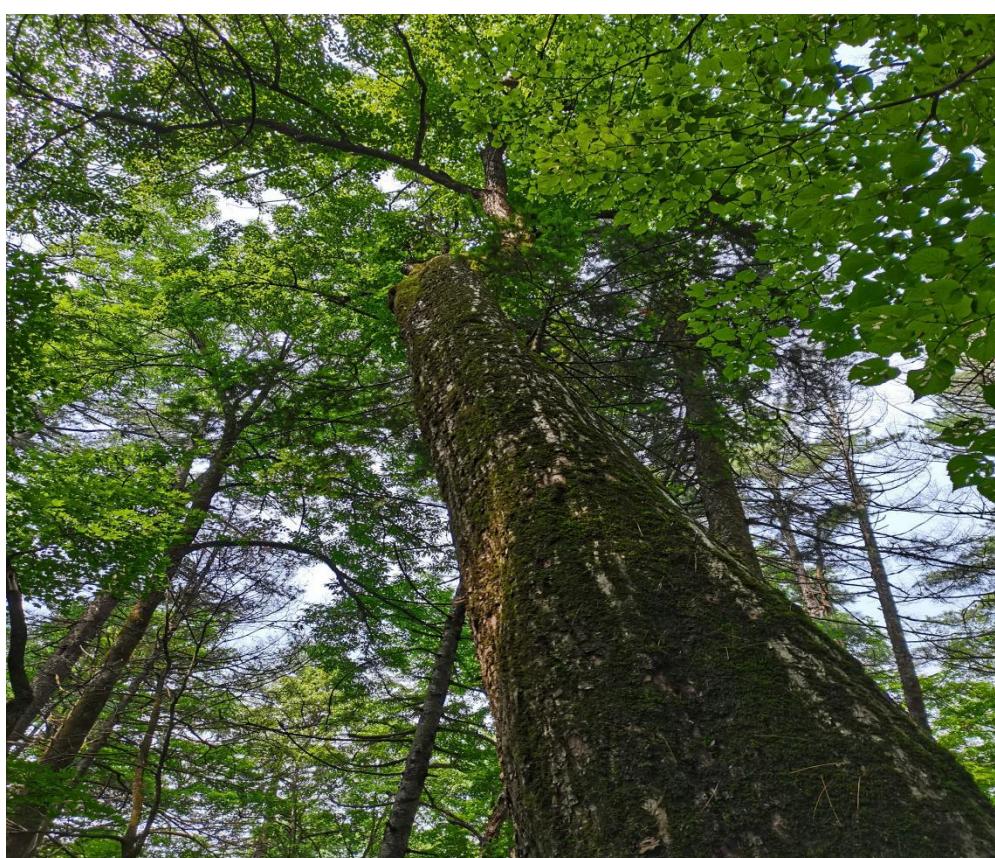
**Figure 1.1** *Betula platyphylla* in Northeast Forestry University  
[photo credit: Jiafa Tang].

*Betula platyphylla* is widely distributed across 14 provinces and regions in China, including northeast, north, northwest and southwest of China. It is distributed throughout nine main vegetation areas including the tropical monsoon rain forest and rain forest areas (Guan, 1998). According to the results of the ninth National Forestry Resources Survey in 2018, *Betula platyphylla* is the broad-leaved species with the largest stock volume, ranking second among all trees.

*Betula platyphylla* is a pioneer tree species that has an important role in forest secondary succession (Tung, 1981). *Betula platyphylla*, with its graceful trunk and unique ornamental value, is often cultivated as a garden tree, street tree and urban landscape tree in northern cities of China (Wang, 2021).

#### **1.4.2 *Tilia amurensis***

*Tilia amurensis* is a tall deciduous tree of the Malvaceae (Malvales), and a dominant tree species in broad-leaved Korean pine forest in northeast China (Zhang et al., 2007). *Tilia amurensis* grows in sandy loam of broad-leaved mixed forest, and is native to the Lesser Khingan Mountains and Changbai Mountains in China, whilst also being found in Korea, Russia and other regions (Wang, 2018). *Tilia amurensis* has a straight trunk, luxuriant foliage, is more shade tolerant than *Betula platyphylla*, and its cold tolerance increases as it ages (Zhou & Li, 1990). Its seedlings and saplings are shade-tolerant, and its trunks are branched when young (Zhou & Li, 1990). Its seedlings and saplings are shade-tolerant, and its trunks are branched when young (Zhou, 1994). *Tilia amurensis* has a high value as timber (Ma, 2009) and is used for afforestation, as well as providing medicinal resources and is an important nectar source (Li, 1990). Due to excessive logging, the stand volume and the quality of *Tilia amurensis* species has been reduced. At present, *Tilia amurensis* has become a national II-class key protected plant in China (Zhang et al., 2015).

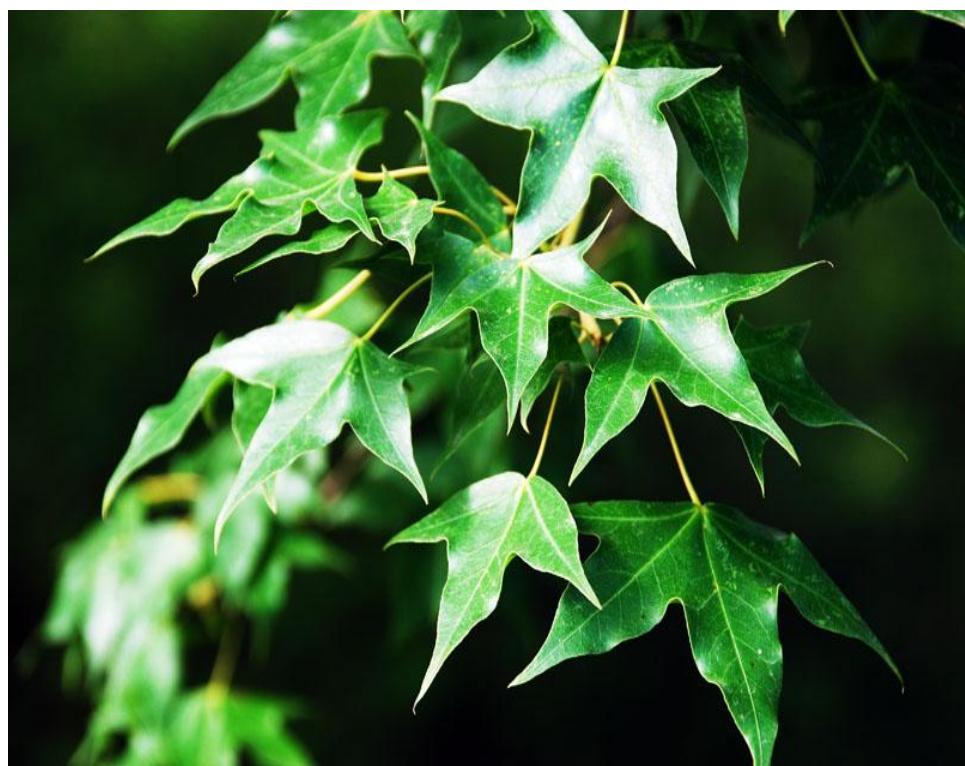


**Figure 1.2 *Tilia amurensis* in Liangshui national reserve.**

[photo credit: Ruizhe Wu].

### 1.4.3 *Acer mono*

*Acer mono* is a deciduous tree within the family Sapindaceae (Sapinales), and is one of the main components of broad-leaved Korean pine forest. There are many variations of *Acer mono* (Xu, 1992) as a result of its wide distribution across China (northeast and northern China, widely distributed in Songhua River area), in Japan and other locations (Zheng, 2004). *Acer mono* is a fast growing, shade-tolerant, drought, cold, and poor soil-tolerant tree, and occurs in a range of soil pH conditions. *Acer mono* is often used in furniture manufacturing, construction industry, agricultural tools, vehicles and other manufacturing because of its excellent wood qualities, dense and tough, finewood (Xu, 1988). *Acer mono* can also be used for the prevention and control of air pollutants and used for urban beautification (Nie et al., 2003).

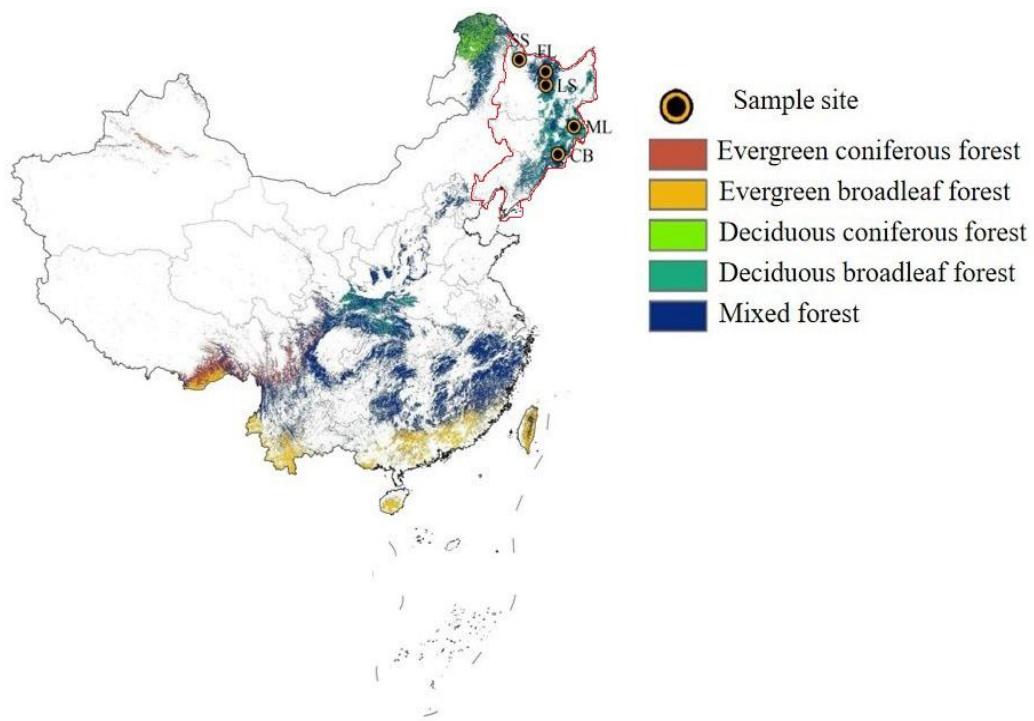


**Figure 1.3** *Acer mono*. [photo credit: Xinmao Li].

## 1.5 Study site overview

The elevation of my study sites are low and there are no obvious high mountains, with little

variation in topography. The soils are dark brown forest soils and dark brown loam, which is distributed in the mountainous area, accounting for 84.91% of the total area of the reserves, but the non-zonal soils are meadow soil, bog soil and peat soil, accounting for 15.09% of the total area of the reserves; meadow soils account for 1.2%, and are distributed in the forest clearing and terraces on both sides of the river; the bog soil accounts for 13.07% and the peat soil accounts for 0.82%, which are distributed in the low-lying areas on both sides of the river and the poorly drained areas in the valley between the mountains (Luan, 2010).



**Figure 1.4** Location of study sites in northeastern China (Circled in red). Each point represents a sampling point (Muling=ML, Liangshui=LS, Fenglin=FL, SS and CB represents two sites not included in field study locations for this chapter).

## 1.6 Outline of the Thesis structure

In this thesis, I discuss three separate but related studies that I carried out to gain a better understanding of the seedlings and saplings regeneration patterns and leaf functional traits across three species in Northeastern China.

In the first study (Chapter 2), I investigate the patterns relating to the species in terms of where they are regenerating and establishing in the landscape. I discuss the impact of

environmental factors on the regeneration of seedlings or saplings of three species and see if there is any effect of latitude.

In the second study (Chapter 3), I explore how each species differentially invests strategy through the variation of traits. I explore the intra-specific latitudinal pattern, the variation extent and causes of leaf economic, anatomical and hydraulic traits, I also explore the covariation of these three leaf traits and the effect of leaf anatomical and hydraulic traits on the intra-specific and inter-specific variation in the economic traits.

In Chapter 4, I synthesize the findings of the previous two chapters to analyze the relationship between environmental factors and traits, that is to examine whether the regeneration patterns of these three species responds to trait variation across the same latitudinal gradient. I compare inter-specific trait differences and discuss how these relate to the inter-specific differences in the regeneration niche. The respective chapters address the following questions:

#### Chapter 2:

- 1) What are the patterns relating to the species in terms of where they are regenerating and establishing in the landscape?
- 2) What are the important factors influencing the regeneration of seedlings or saplings of the three study species in Northeast China?

#### Chapter 3:

- 1) How does each species differentially invests strategy through the variation of traits?
- 2) Is there intra-specific latitudinal pattern across the three taxa, and what is the variation extent and causes of leaf functional traits?
- 3) Do leaf anatomical and hydraulic traits of the three taxa respond to the intra-specific and inter-specific variation in the economic traits?

#### Chapter 4:

- 1) Does the regeneration patterns of these three species respond to trait variation across the

same latitudinal gradient?

2) How inter-specific trait differences relate to the inter-specific differences in the regeneration niche?

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# Chapter 2: Regeneration patterns of seedlings and saplings of three species

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## 2.1 Abstract

Natural regeneration of the forest is important to the persistence of forest ecosystems. Therefore, it is of great significance to study the ecology, spatial distribution and growth of seedlings and saplings in forests. In this study, I examine how *Betula platyphylla*, *Tilia amurensis* and *Acer mono* partition resources along environmental gradients. I investigated the presence and absence of seedlings and saplings at 459 plots across Muling, Liangshui and Fenglin nature reserves in northeast China. I also recorded environmental parameters to explore the effects of slope, aspect, topographic position, canopy openness and latitude on the regeneration of seedlings and saplings of these species. The results are as follows:

- (1) I found a total of five seedlings and saplings of *B. platyphylla*, 77 *T. amurensis*, and 1317 *A. mono* in my survey. As a pioneer species, *B. platyphylla* appeared in the early stage of forest community succession only, and the sample sites I surveyed were in largely old-growth broad-leaved Korean pine forest, which is the top zonal vegetation in northeastern China. Therefore, there were few seedlings and saplings of *B. platyphylla*, followed by *T. amurensis* in the middle of the succession, while there are many *A. mono* in the late succession.
- (2) In this study, slope is the main environmental factor affecting natural regeneration. The greater the slope in a certain range, the more understory regeneration occurred; regeneration of seedlings and saplings was most frequent on grades with slopes of 6–25 degrees, mostly on gentle slopes. Regeneration is likely to occur on sunny slopes (S, SW). The middle and upper slopes are the slopes with the highest number of seedlings regenerating. The light environment is most suitable for the growth of seedlings in the interval of 20%–30% of canopy openness.
- (3) I found that the positive and negative correlations between the number of regenerating seedlings of *B. platyphylla* and environmental factors were less significant. The number of

regenerating seedlings and saplings of *T. amurensis* and *A. mono* were all positively correlated with slope. Canopy openness was strongly negatively correlated with the number of seedlings and saplings of *T. amurensis* and *A. mono*. While latitude was highly positively correlated with *A. mono*, latitude became negatively correlated with *T. amurensis*, and aspect and canopy openness were negatively correlated with all three species.

These species therefore avoid competition by targeting different niche space as seedlings and saplings.

**Keywords:** Seedlings and saplings, Regeneration, Distribution, Effects, Succession, Environmental factors

## 2.2 Introduction

Natural regeneration of forests is an important approach to community succession and ecosystem restoration. As Queenborough (2007) said, “the seedling stage is an important stage in the life cycle of plants and is the most sensitive period for individuals to environmental changes”. Seedling viability and its influence in different habitats affect the number and development of the population (Clark, 1998), and they are of great significance to the stability of the population and the composition of the community (Szwagrzyk, et al., 2001). A certain amount of seedling regeneration is the premise of good population development. Seedling regeneration is an important limiting factor in forest regeneration and plays a crucial role in the formation of the spatial distribution of the adult population.

Seedling regeneration is affected by many ecological factors, such as light, water and temperature (Fenner & Thompson, 2005). In small-scale geographical communities, topographic factors affect the spatial pattern of soil temperature, humidity and nutrients through redistribution of solar radiation and precipitation (Cantón, 2004), and then affect seedling renewal and subsequent tree growth (Dai, 2002). Topographic positions indirectly determines the distribution of forest vegetation (Kang, 2000).

In the forest community, seedlings with different life histories have different adaptability to topographic and environmental changes. For example, seedlings of late successional tree species need stable site conditions and can tolerate a shady environment, while seedlings of pioneer species can grow on unstable sites but need high light environment (Sagoff, 2017). Seedlings with different ecological habits can achieve niche differentiation by occupying various habitats and resources formed by terrain differences, thus promoting the coexistence of forest community species (Bell et al., 2000). Daws et al. (2002)'s study on the Barro Colorado Island sample site in Panama found that in tropical areas, topographic-induced changes in soil water content and litter layer thickness would affect tree emergence rate and mortality, providing effective conditions for species coexistence (Daws et al., 2002). Frey's study (2007) on the mixed deciduous forest of

New England in the southern United States showed that seedlings on the ridge were susceptible to water limitation, while seedlings in the valley were limited by light. Tsujin and Yumoto's study (2008) on Yakushima Island showed that the distribution of seedlings on the terrain could reflect the distribution characteristics of adult trees. Most of the existing domestic studies in China focus on the impact of topography on adult vegetation (e.g., Ou, Su, Li, & Lin, 2011), but there are few studies on the impact of topography on seedling regeneration.

Seedling regeneration is the basis for population sustainability, but only a certain number of seedlings and saplings are required to achieve sustainable population development. The heterogeneity of habitats makes population regeneration vary, therefore it is important to investigate and compare the number, adaptation strategies and growth patterns of seedlings and young trees in different habitats to promote population sustainability.

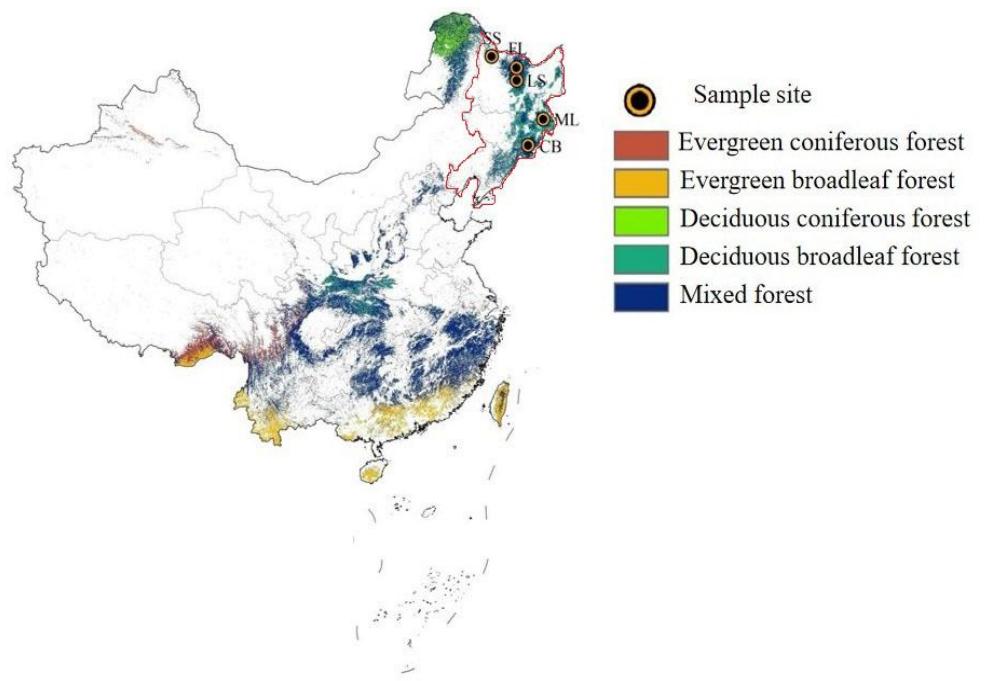
Broad-leaved Korean pine (*Pinus koraiensis*) forest is a old-growth vegetation in the mountains of northeastern China and is one of the forest type with the highest biodiversity in the north temperate zone (Lin et al., 2016). Due to long-term over-harvesting, the resources of broad-leaved Korean pine forests are now greatly reduced. Based on three national nature reserves of typical broad-leaved Korean pine forests in different latitudinal gradients in northeastern China, this study provides an in-depth and systematic study of the effects of different topographic factors on the composition and distribution of seedlings of the three species: *B. platyphylla*, *T. amurensis* and *A. mono*, as well as seedling regeneration and their mechanisms. The aim is to provide a scientific basis for understanding forest biodiversity maintenance mechanisms and the conservation and restoration of broad-leaved Korean pine forests.

## 2.3 Methods

### 2.3.1 Study Site overview

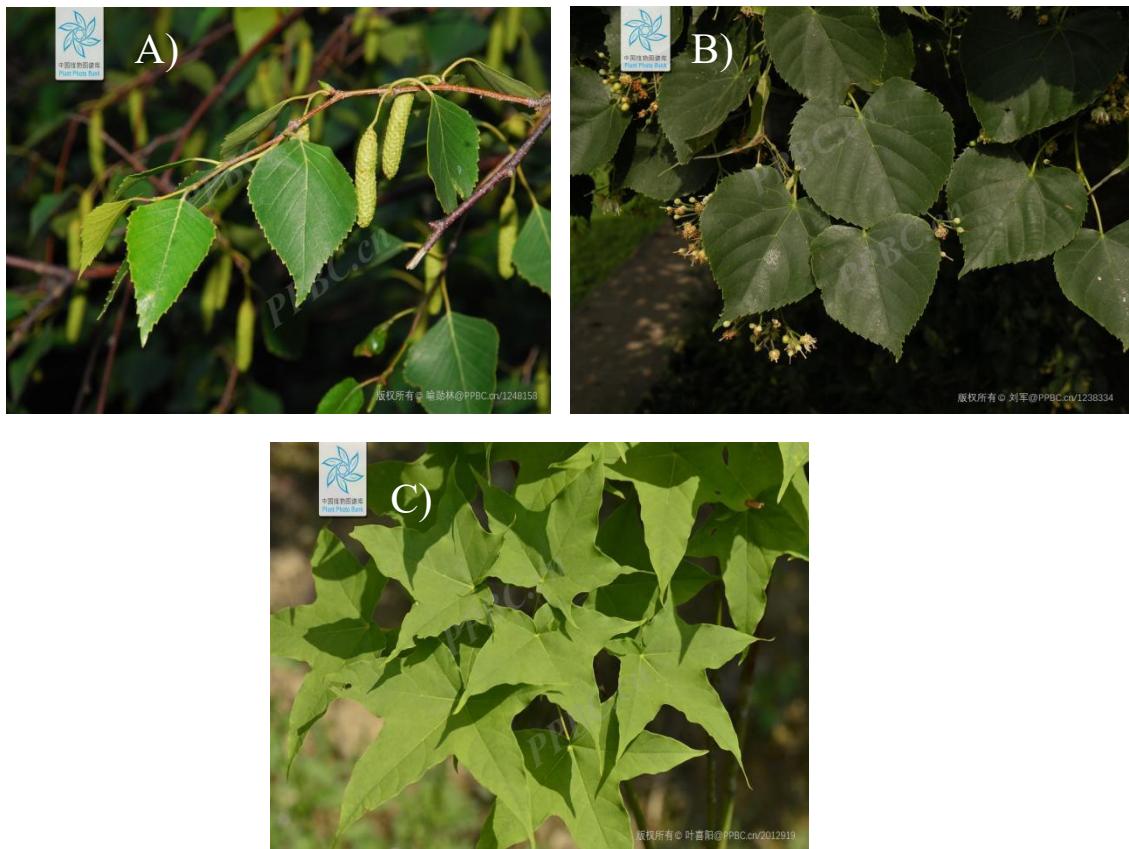
Data were collected in temperate mixed broad-leaved Korean pine Forest in northeastern China in 2021 at three typical mixed broad-leaved Korean pine forests across Muling, Liangshui and Fenglin nature reserves (Fig 2.1). The geographical locations of the study areas are Long.

128°47'–129°53' E, Lat. 44°20'–48°12' N, the altitude is 351–611 m asl, the mean annual precipitation is 514–676 mm, and the mean annual temperature is -0.5–3 °C (Table 2.1). The climate type of the study areas is a temperate continental monsoon climate, with sharp temperature changes in spring and autumn, high temperature and short duration in summer, common early frost in autumn, and a cold, dry and long winter. Typical broad-leaved tree species in the mixed broad-leaved Korean pine forest are *Populus davidiana*, *Betula platyphylla*, *B. costata*, *Tilia amurensis*, *Fraxinus mandshurica*, *Ulmus japonica*, *Ulmus laciniata*, and *Acer mono*.



**Figure 2.1** Location of study sites in northeastern China (Circled in red). Each point represents a sampling point (Muling=ML, Liangshui=LS, Fenglin=FL, SS and CB represents two sites not included in field study locations for this chapter).

In this study, *B. platyphylla*, *T. amurensis* and *A. mono* were selected as the research subjects (Fig 2.2), because they were in a very obvious succession stage, which were early, middle and late, respectively. All of them are broad-leaved species, and their shade tolerance gradually increases from *B. platyphylla* to *T. amurensis* to *A. mono* (Zhang, Jin, & Liu, 2019).



**Figure 2.2** Three species of broad-leaved trees with different leaves.

B. *platyphylla*, B) *T. amurensis*, and C) *A. mono*.

[Photo resources: <http://www.iplant.cn/>].

**Table 2.1** Information on the geographical locations and climates of three sampling sites for mixed broad-leaved Korean pine forests in Northeast China

Site	Longitude (E)	Latitude (N)	Elevation (m)	Mean annual precipitation (mm)	Mean annual temperature (°C)
Muling (ML)	129°40'~129°53'	44°20'~44°30'	611	514	3
Liangshui(LS)	128°47'~128°57'	47°06'~47°16'	400	676	-0.3
Fenglin (FL)	128°59'~129°15'	48°02'~48°12'	351	640	-0.5

### 2.3.2 Methods

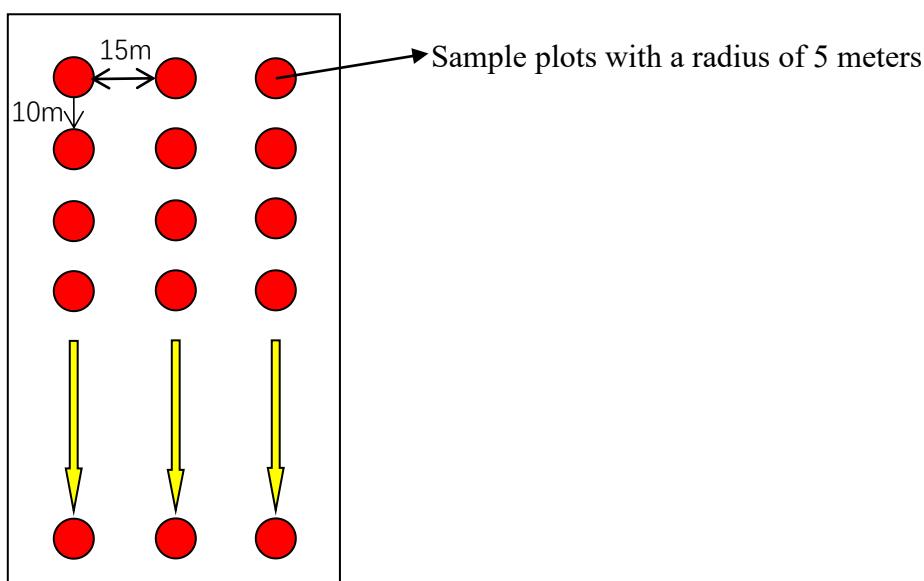
In July 2021 I selected representative conifer and broad-leaved mixed forest stands in three national nature reserves and established three transect zones at each site. I ran three transects across the landscape spaced ~15 m apart, and sampled 153 plots from each site giving 459

samples in total. Each plot was circular with a radius of 5 meters and all seedlings and saplings of my three study species were counted and recorded in each plot (Fig 2.3).

The definition of seedling was that it was less than 135 cm tall. Saplings were defined as greater than 135 cm tall but having a DBH of less than 5 cm.

I also recorded environmental data for each plot. The slope (0–90°) of the ground from horizontal was measured. I grouped the slopes into four groups: <6°, 6–15°, 16–25°, >25°. The direction the slope faces (aspect) as an angle in degrees was measured (0–360°). I divided this aspect into shady slope (337.5°–22.5° and 22.5°–67.5°, namely the NE, N), semi-shady slope (67.5°–112.5° and 292.5°–337.5°, namely E, NW), semi-sunny slope (112.5°–157.5° and 247.5°–292.5°, namely SE, W), and sunny slope (157.5°–247.5°, namely S, SW). Topographic position was divided into ridge, upper slope, mid-slope, lower slope, and gully.

I also recorded the species name, distance, DBH and height of the nearest tree with a DBH larger than 5 centimeters. Information on canopy cover for each plot was collected using digital hemispherical photography (DHP) (Macfarlane, 2011), and the images were collected at 1.2 meters above the ground. The collection time was set at 06:00–09:00 in the morning and 15:00–19:00 in the afternoon (Liu & Jin, 2013).



**Figure 2.3** Three transect maps of the study site, the red circles are the 78.5 m<sup>2</sup> sample plot

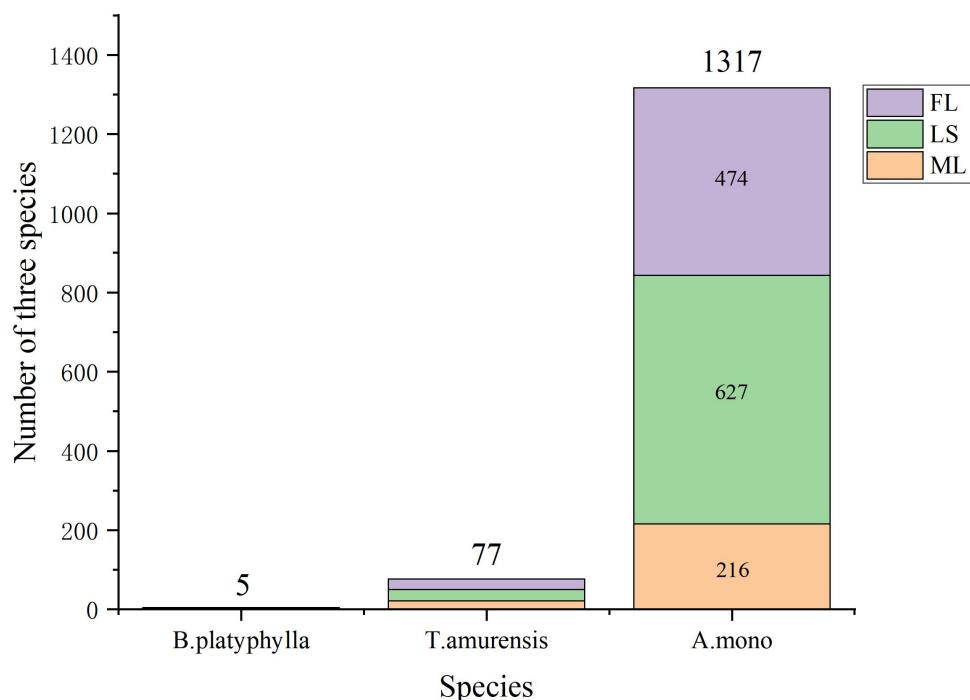
### 2.3.3 Statistical analysis

In this study, true-colour fisheye photographs were digitized using Gap Light Analyzer 2.0 (GLA) software (RUNKLE, & JR., 1982). SigmaPlot 10.0 was used to statistically analyze the distribution of seedlings and young trees under different environmental conditions. The relationship between the environmental factors and the number of regeneration was investigated using redundancy analysis (RDA) using R version 4.1.1 to identify the key environmental factors driving the number of seedlings and saplings recorded in each survey sample plot.

## 2.4 Results

### 2.4.1 Regeneration characteristics of seedlings and saplings of the three species

As shown in Figure 2.4, a total of five *B. platyphylla*, 77 *T. amurensis*, and 1,317 *A. mono* seedlings and saplings were encountered in the 459 sample plots. *B. platyphylla* occurred only in areas with higher light associated with disturbance events such as windthrow.



**Figure 2.4** The distribution map of the number of seedlings and saplings of the three species. Purple represents the number at Fenglin, green represents the number at Liangshui, and orange represents the number at Muling.

Among the 459 plots established and investigated in this research, the maximum seedling regeneration density was  $0.72 \text{ m}^{-2}$ , and a number of quadrats had no seedlings, (the coefficient of variation of seedling density was 2.04). In some plots, there was no sapling regeneration. However, in some plots, the sapling regeneration density was  $0.18 \text{ m}^{-2}$ , and the coefficient of variation of sapling regeneration was 1.68 (Table 2.2).

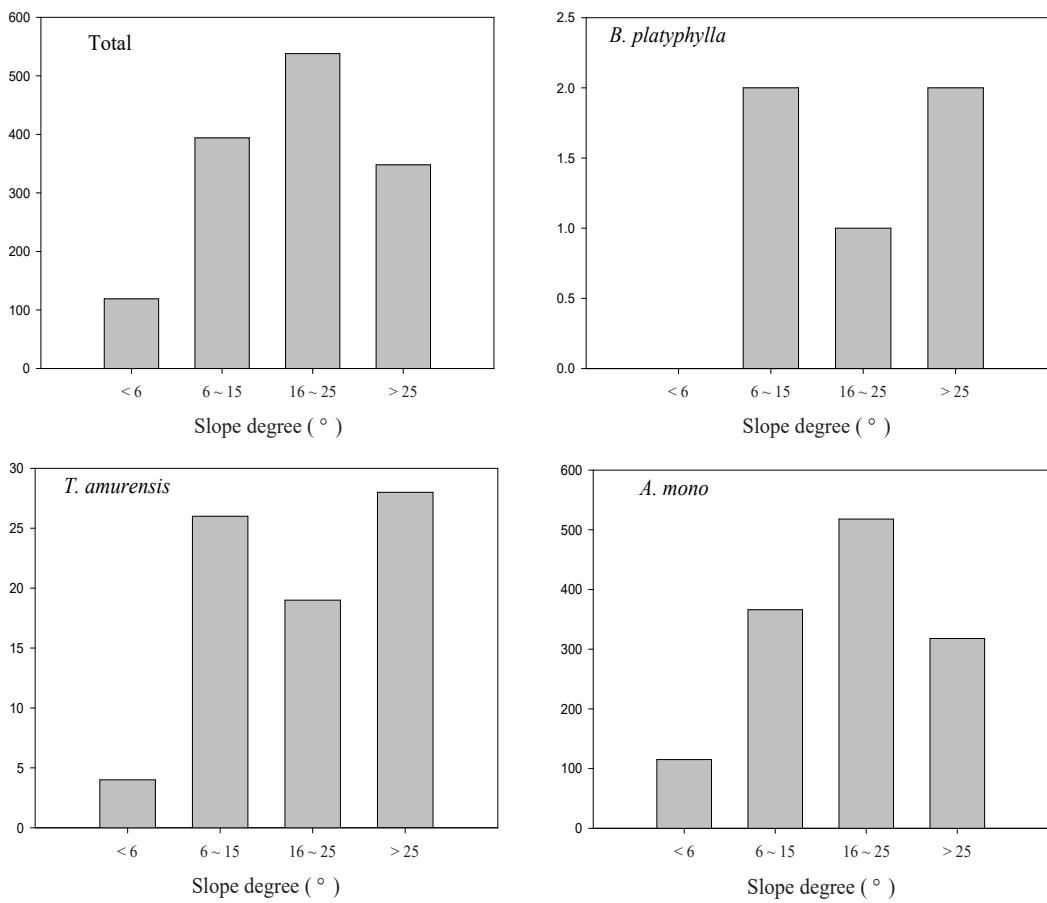
**Table 2.2** Difference statistics of seedlings and saplings density of three species in 459 plots

Project	Individual <sub>min</sub> /78.5m <sup>2</sup>	Individual <sub>max</sub> /78.5m <sup>2</sup> )	Variable Coefficient
Seedlings density	0	28	2.04
Saplings density	0	14	1.68

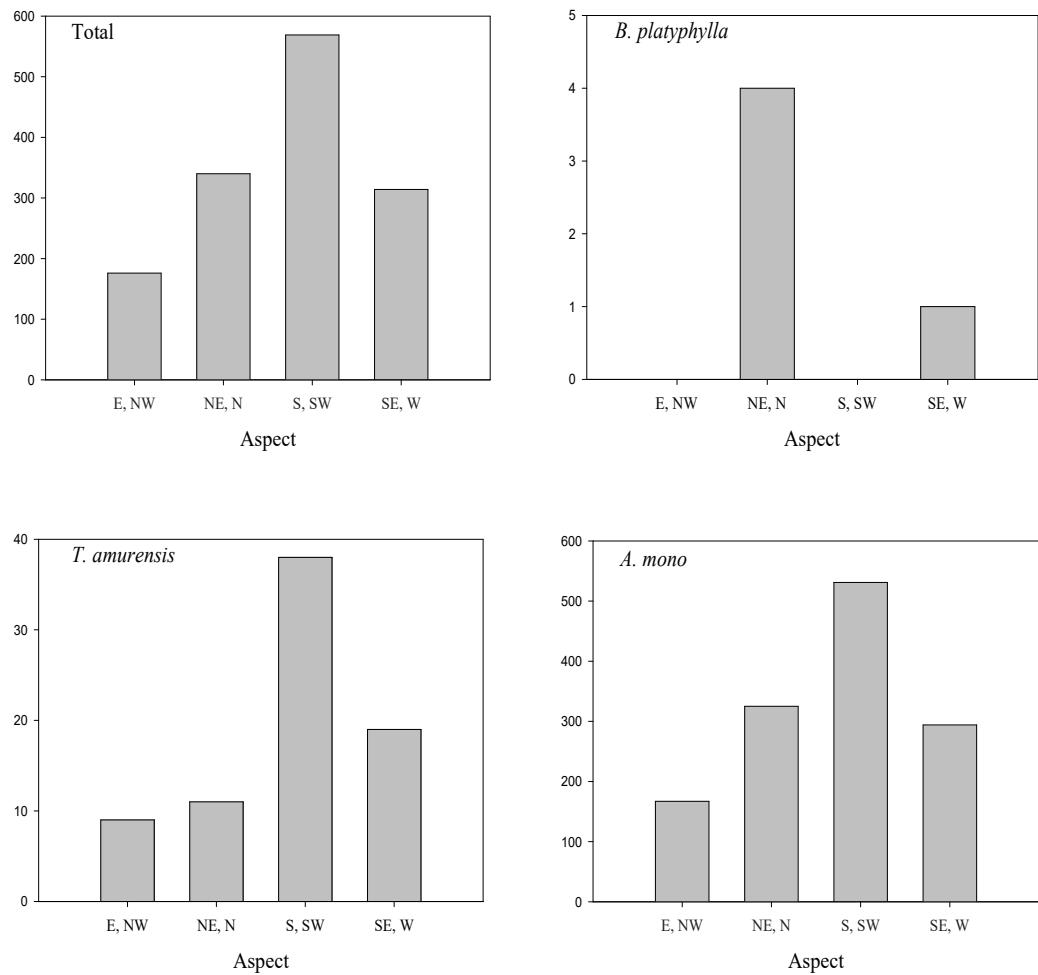
Looking at all three species combined, there are only 119 saplings of the three species in the area with a slope of less than 6 degrees, 393 saplings in the area with a slope of 6–15 degrees, 540 saplings in the area with a maximum of 16–5 degrees, and 348 saplings in the area above 25 degrees (Figure 2.5). In general, seedlings and saplings are mainly concentrated on gentle slopes between 6 and 25 degrees.

According to the aspect distribution in Figure 2.6, the total number of saplings of the three species was the least in the semi-shady slope (E, NW), with only 176 saplings, 340 saplings in the shady slope (NE, N), 569 saplings in the sunny slope (S, SW), and 314 saplings in the semi-sunny slope (SE, W). Saplings mainly appear on the sunny slopes.

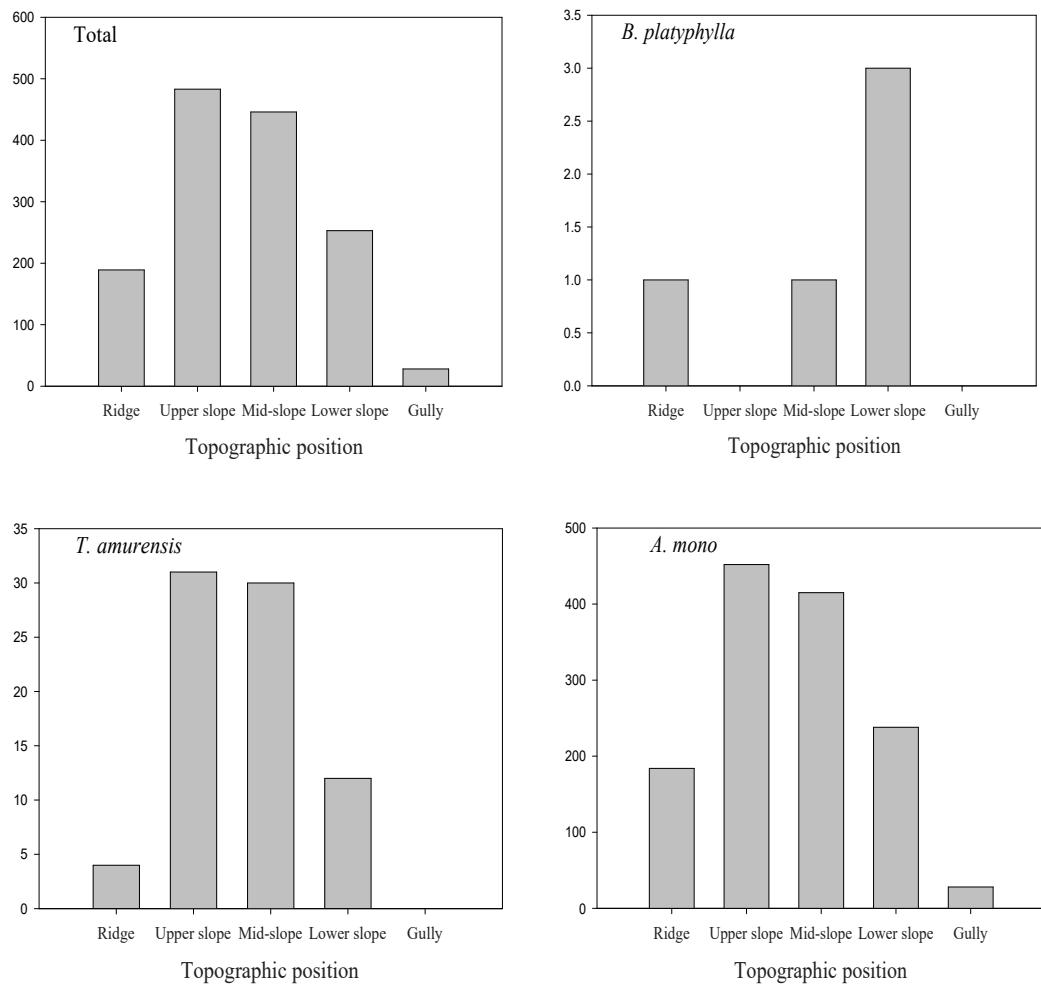
In Figure 2.7, from the topographic position, there are 189 saplings in the ridge section, 483 in the upper slope section, 446 in the mid-slope section, 253 in the lower slope section and only 28 in the gully, with the largest number in the upper slope and mid-slope. There are saplings in the gully occasionally, and the results are obviously different in different topographic positions.



**Figure 2.5** The distribution of seedlings and saplings in different slope degree.



**Figure 2.6** The difference of seedlings and saplings distribution in Semi-shady slope, Shady slope, Sunny slope and Semi-sunny Slope.



**Figure 2.7** The distribution of seedlings and saplings in different topographic positions.

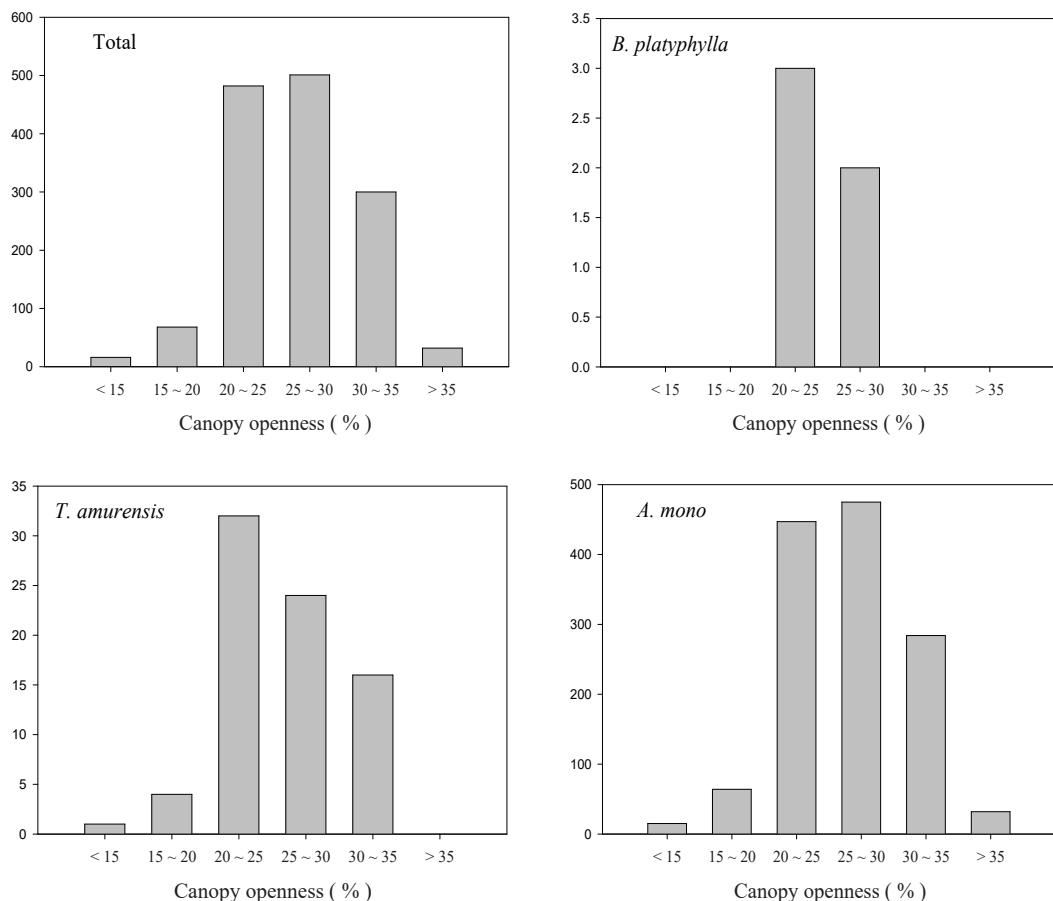
The three species seedlings and saplings are regenerating under different canopy openness.

My study found almost no natural regeneration of seedlings and saplings at a canopy openness of less than 10%, and only occasional regeneration of seedlings and saplings at a canopy openness of 10–15%. In these situations due to the high canopy density, seedlings and saplings would experience a serious lack of sunlight.

A canopy openness of 20–30% was the best regeneration situation for seedlings and saplings, which is sunny but not too strong (Fig 2.8). The number of *B. platyphylla* regenerated in these conditions were five, *T. amurensis* 56 and *A. mono* 922. In the 30%–35% section 0 seedlings and saplings of *B. platyphylla*, 16 *T. amurensis*, and 284 *A. mono* were recorded. However, sapling regeneration was also occasionally observed in segments larger than 35%. Most saplings were

recorded in plots with canopy openness values between 20%–30%.

However, saplings regeneration was occasionally observed in segments larger than 35%. Since canopy openness can affect the redistribution of light, heat, water and other conditions, and thus affect the growth and development of plants, but it varies from species to species, this study showed that the optimal regeneration canopy openness region of the three species was 20%–30%.



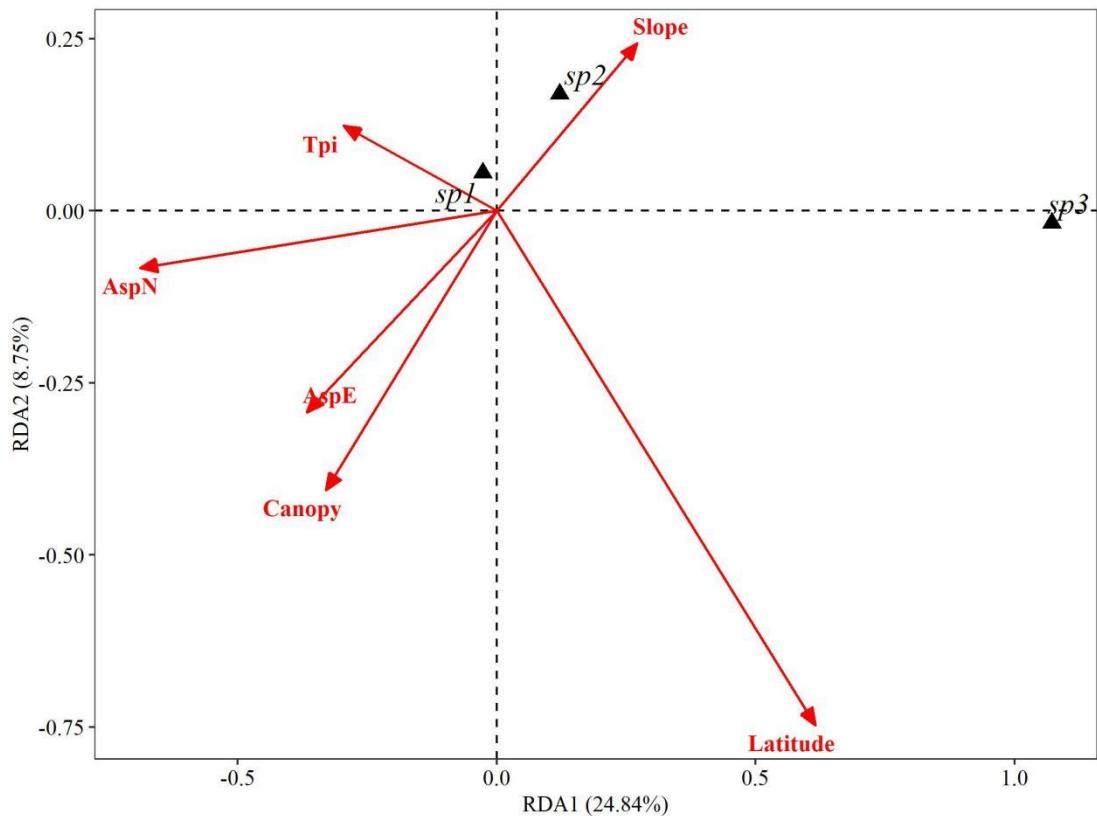
**Figure 2.8** The distribution of seedlings and saplings in different categories of canopy openness.

#### 2.4.2 Relationship between seedlings and saplings and environment of three species

The explanatory quantities of the RDA analysis show that the two axes represent 24.84% and 8.75% of the variation respectively, indicating that the relationship between regeneration density and environmental factors is complex (Fig 2.9). However, from the explanatory quantities of the first axis, environmental factors have a significant impact on regeneration.

*B. platyphylla* seedlings and sapling were not strongly correlated to environmental factors; I therefore report the results for *T. amurensis* and *A. mono* only. Both *T. amurensis* and *A. mono*

regeneration were correlated with slope. *T. amurensis* and *A. mono* regeneration also appears to be negatively correlated with canopy openness. Latitude was positively correlated with *A. mono* and negatively with *T. amurensis*. Aspect was negatively correlated across both *T. amurensis* and *A. mono*.



**Figure 2.9** Redundancy analysis (RDA) of seedlings and saplings regeneration and environmental factors in three species. *sp1* represents *B. platyphylla*, *sp2* represents *T. amurensis* and *sp3* represents *A. mono*. The environmental factors are represented by each arrow in the figure. The black font indicates the number of *B. platyphylla*, *T. amurensis* and *A. mono* seedlings and saplings. The length of the line indicates the relationship between regeneration and the environmental factors. In the quadrant where the arrow is located, the positive and negative correlation between environmental factors and the ranking axis is determined by the quadrant where the environmental factors are located, and the angle between environmental factors and the ranking axis represents the correlation.

## 2.5 Discussion

In the early stage of forest community succession, due to the lack of vegetation cover and buffer effect, plants grow in the environment with relatively full light. With the succession, although the total radiation above the community stays the same, the distribution of light, light intensity and light quality composition in the forest community will change. Due to the different adaptability of seed germination and seedling growth and configuration to the heterogeneity of light environment in early and late succession, the species of trees in early succession are gradually pushed out, while the species and number of trees in late succession became greater (Li & Ma, 2002). This is consistent with the results of this study that the regeneration of seedlings and saplings of different tree species increased with the advance of succession stage.

It can be inferred from the data and related studies that different topography has a significant effect on seedling and sapling regeneration of *B. platyphylla*, *T. amurensis* and *A. mono* populations in forest communities, among which slope, aspect and topographic position are the factors that significantly affect seedlings and saplings regeneration.

In this study, seedlings and saplings regeneration occurs mostly on the slope of 6–25 degrees, this might be in relation to differences in soil thickness along a slope gradient (Tokuchi et al., 1999). Places with gentle slopes have relatively thick soil layer and abundant water content, while places with steep slopes have relatively thin soil layer and relatively less water content. In addition, saplings are easy to regeneration on the sunny slope (S, SW), because the sunny slope has good light conditions. From the shady slope to the sunny slope, the light intensity gradually increases, the temperature gradually increases, and the water content decreases (Liu & Jin, 2009). In addition, the mid-slope and the upper slope are prone to the occurrence of saplings.

There are many reasons for the difference in the number of saplings among terrains. The combination of different slope position, aspect and slope can well reflect the level of multidimensional environmental factors, which will affect the distribution of saplings in various terrains (Oheimb et al., 2007).

This study found that three species seedling numbers were positively correlated with slope, which is consistent with Cai's findings (Wenhu et al., 2012). Through studying the Daxinanling forest region in Heilongjiang province, he found that the density of seedlings in broad-leaved species had the greatest correlation with slope.

In many studies, the light environment under the forest is considered to be an important abiotic environment affecting or even determining the survival of seedlings (Lin et al., 2016). In this study, it was found that the most suitable light environment was when the canopy openness was 20%–30%. Canopy had a negative correlation with the regeneration of the three species, indicating that too intense light under the forest was not suitable for the growth of saplings. From the species, because of different tree species tolerance and sensitivity of light, in the case of the smaller canopy openness, the shade of seedling compared with pioneer species with higher survival rate, it is also why the shadow resistance stronger, the most number of *A. mono* seedlings and saplings reflects the seedling plants live regeneration stage there are differences between species in their demand for light resources. Such differences allow plants to adapt to different intensities of light and thus survive and renew in complex community structures.

## 2.6 Conclusion

The regeneration of plant population is complicated by the environment. The juvenile stage of plants is the most sensitive stage in the whole life history of plants to the environment, which is easily eliminated by "environmental sieve". Therefore, seedlings and saplings are critical to population regeneration, and the study of seedlings and saplings is of great guiding significance to the sustainable development of population. In this paper, the three broad-leaved species, *B. platyphylla*, *T. amurensis* and *A. mono*, which are in different succession stages, were studied by statistical analysis, RDA sequencing and other methods. The relationship between the quantitative characteristics of seedlings and saplings of *B. platyphylla*, *T. amurensis* and *A. mono*, as well as morphological adaptation strategies and growth rules in heterogeneous habitats were explored.

The results showed that slope was the main topographic factor affecting natural regeneration, and the greater the slope was in a certain range, the greater the number of understory regeneration was, but the regeneration was the best in the 6–25 degree section. From the aspect of slope, regeneration is easy to occur on the sunny slope (S, SW). In addition, the middle slope and upper slope had the largest number of seedlings and saplings regenerating. Light conditions also vary greatly, with canopy openness between 20% and 30% being the most suitable light environment for the growth of saplings.

Of course, there are still some shortcomings in this study, the seedlings and saplings in the regeneration process are influenced by many environmental factors, including biological and abiotic environment. , This study selected the latitude, slope, slope direction, slope position, and canopy openness as abiotic environment factors to analysis of the impact of the existence of seedlings and saplings, and not to join the biological environment, such as biological neighbors, herbs, litter thickness, etc. In future studies, more biological environments should be considered, and seasonal dynamic surveys should be added, so as to be more comprehensive and convincing.

Moreover, studies on seedling functional traits can be increased to further explore the effects of environment on seedling regeneration.

## 2.7 References

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# **Chapter 3: Intraspecific variation and coordination of the leaf economic, anatomical and hydraulic traits of three temperate broad-leaved species along a latitudinal gradient**

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## **3.1 Abstract**

In the context of climate change, studies on intraspecific variation and coordination of key leaf functional traits along latitudinal gradient are helpful for further revealing the adaptation strategies and future development directions of plants to climate change. However, few studies have been systematically conducted on the intraspecific variation patterns and coordination of leaf economic, anatomical, and hydraulic traits for major species in the same forest type at a regional scale. In this study, I evaluated thirty-six trees belonging to three temperate broad-leaved species in four mixed broad-leaved Korean pine (*Pinus koraiensis*) forests along a latitudinal gradient in northeast China. I measured three economic traits, two anatomical traits and two hydraulic traits on leaves sampled from three tiers of the canopy and two zones (north and south) for each tier. I explored the intraspecific latitudinal pattern, the variation extent and causes of leaf economic, anatomical and hydraulic traits, and focused on exploring the coordination of these three suites of leaf traits and the effect of leaf anatomical and hydraulic traits on the intraspecific variation in the economic traits. The leaf traits significantly varied across the different survey sites. Position of leaf in the tree, individual tree, and survey site explained 22.06%, 17.36% and 16.43% of the intraspecific trait variation for the overall leaf traits. The epidermis thickness, leaf dry matter content, and mass-based leaf nitrogen content were more stable, whereas the leaf mass per area, palisade tissue thickness, and the length of the veins per unit area were more variable. There were correlations among leaf traits. Additionally, a high leaf mass per area resulted principally from a higher palisade tissue thickness, a high leaf dry matter content correlates with increased lengths of the minor veins per unit area. my results highlight the importance of intraspecific trait variation, supplement data of leaf economic,

anatomical and hydraulic traits at regional scales and effectively broaden current knowledge on the adaptation strategies of plants.

**Keywords:** intraspecific variation, coordination, leaf mass per area, palisade tissue thickness, lengths of the veins per unit area

## 3.2 Introduction

Plant functional traits are major predictors of ecosystem responses to global climate change (Fajardo & Siefert, 2016; Guittar et al., 2016; Zirbel et al., 2017). Leaf functional traits are important components of plant functional traits and comprise a series of strongly plastic traits. These leaf traits are critical to the function and adaptability of plants (Bonser et al., 2010; Poorter et al., 2018), and are closely related to the biomass of plants and their acquisition and utilization of resources (de la Riva et al., 2016; Zirbel et al., 2017). Among various leaf functional traits, those related to CO<sub>2</sub>, water exchange, and light capture have received the most attention, including leaf economic, anatomical, and hydraulic traits (Li et al., 2015; He et al., 2018). In the context of climate change, studies on intraspecific variation and the patterns and coordination of economic, anatomical, and hydraulic traits along latitudinal gradient are helpful for further revealing the adaptation strategies and future development directions of plants to climate change (An et al., 2021; Blonder et al., 2016; Cornelissen et al., 2003; Muir et al., 2016;).

Latitudinal patterns of leaf traits are frequently an important determinant of forest dynamics, helping us to better understand ecological strategies of plants in different environments (Fajardo & Siefert, 2016; Zhu et al., 2011). Ecological studies on latitudinal patterns of leaf traits have been focussed on plant functional groups and at a community levels (He et al., 2018). Studies to date have emphasized the likely importance of intraspecific leaf traits variation in species and population performance (Anderegg et al., 2018; Laughlin et al., 2017; Moran et al., 2016; Niinemets, 2015), and predicting forest community dynamics along an environment gradient (Fajardo & Siefert, 2018; Siefert et al., 2015). Although the likely importance of intraspecific variation has been highlighted, few studies have been conducted on the intraspecific latitudinal patterns of leaf traits for major coexisting species in the same forest type at a regional scale.

In recent years, researchers have become increasingly interested in the extent and causes of intraspecific variation in leaf traits (Albert et al., 2010; Li et al., 2015; Martin et al., 2017). There is evidence to show that leaf trait variation is constrained by light conditions, and that the extent of variation in leaf traits was correlated to plant habitat affiliation (Valladares et al., 2000). Most

attention has been paid to morphological, chemical, and physiological traits (Delagrange et al., 2004; Robakowski et al., 2003), and the information on the variation extent of leaf anatomical and hydraulic traits is limited. While it is evident that leaf traits are different for the same species growing in different regions (Hulshof & Swenson, 2010), leaf traits vary significantly within individuals, and across different individuals of the same species in the same area (Martin & Thomas, 2013; Martin et al., 2017). Further, significant canopy gradients of leaf traits have been identified as responding to variability in levels of irradiance and water availability (Azuma et al., 2015; Coble & Cavaleri, 2017; Hikosaka 2005). Thus, it is likely that intraspecific variation of leaf traits will be observed across the same individual, multiple individuals at the same site, and across an environmental gradient. However, few studies have quantified the extent to which position on an individual plant, variation across individuals within a site, and the effects of strong environmental gradients might explain intraspecific variation in leaf traits (Hulshof & Swenson, 2010).

Patterns of intraspecific correlations across leaf traits are being investigated (Martin et al., 2017; Muir et al., 2016; Niinemets 2015), and evidence suggests that hydraulic and economic leaf traits may decouple, or vary, in an un-coordinated manner (Blackman et al., 2016). Studies on correlations have not only been limited to the same suite of leaf traits (Niinemets et al., 2015; Anderegg et al., 2018), but have also considered the correlations among suites of leaf traits (Blackman et al., 2016; de la Riva et al., 2016; John et al., 2017; Onoda et al., 2017). Some studies have estimated the contributions of the given tissues to leaf mass per area (LMA) by fitting statistical relationships (Poorter et al., 2009; Villar et al., 2013). However, the effect of hydraulic traits on LMA, the anatomical and hydraulic traits that underlie leaf dry matter content (LDMC), and mass-based leaf nitrogen content ( $N_{mass}$ ) remains poorly understood. Therefore, the intraspecific correlations among various suites of leaf traits and the effect of leaf anatomical and hydraulic traits on leaf economic traits remain to be further studied.

In this study, I selected three major broad-leaved species of the mixed broad-leaved Korean pine (*Pinus koraiensis*) forests and sampled leaf traits of these species across four sites along a

latitudinal gradient in northeast China. The species selected for this study were *Betula platyphylla*, *Tilia amurensis*, and *Acer mono*. I measured a total of seven leaf traits, including three leaf economic traits (leaf mass per area (LMA), leaf dry matter content (LDMC) and mass-based leaf nitrogen content ( $N_{mass}$ )), two leaf anatomical traits (palisade tissue thickness (PT) and epidermis thickness (ET)) and two leaf hydraulic traits (total length of 1-2° veins per unit area (VLA<sub>1-2</sub>) and length of minor veins per unit area (VLA<sub>min</sub>)).

The objective of this study was to explore the intraspecific latitudinal pattern, the variation extent and causes of the leaf economic, anatomical and hydraulic traits of three major broad-leaved species in the same forest type at a regional scale, and focused on exploring the coordination of these three suites of leaf traits and the effect of leaf anatomical and hydraulic traits on leaf economic traits. I expected that (1) there were distinct latitudinal patterns and coordination of leaf traits among three species relating to species ecological habit; (2) the site, individual plant and the position of the leaf on a tree could explain the most intraspecific variation of leaf economic, anatomical and hydraulic traits; (3) leaf anatomical and hydraulic traits had a strong capacity to predict leaf economic traits.

### 3.3 Materials and methods

#### 3.3.1 Experimental material

Leaves of *B. platyphylla*, *T. amurensis* and *A. mono* were collected from four different locations supporting mixed broad-leaved Korean pine forests in northeast China (Table 3.1). The climatic region in which these sites occur is temperate continental monsoon: temperature changes rapidly occur in spring and autumn, the temperature is high and the duration is short in summer, early frost is common in autumn, and winter is cold, dry and long. In these sites, *B. platyphylla*, *T. amurensis*, and *A. mono* are common broad-leaved species and major components of the forest.

**Table 3.1** Information on the geographical locations and climates of four sampling sites for broad-leaved Korean pine forests in Northeast China.

Site	Longitude (E)	Latitude (N)	Elevation (m)	Mean annual	Mean annual
				precipitation (mm)	temperature (°C)
Changbai Mountain (CB)	127°42'~128°16'	41°41'~42°51'	852	700	3.6
Muling (ML)	129°40'~129°53'	44°20'~44°30'	611	514	3
Fenglin (FL)	128°59'~129°15'	48°02'~48°12'	351	640	0.2
Shengshan (SS)	126°27'~127°02'	49°25'~49°40'	510	520	-2

### 3.3.2 Experimental design

At each sampling site along a latitudinal gradient, three adult individuals of each species were randomly sampled. The diameter at breast height (DBH), height and height of the first living branch of the sample individuals were measured (Table 3.2). For each individual, the canopy was divided into three height levels (upper, middle and lower), and each canopy was further divided into south and north directions. That is, each individual had six sampling levels.

At each sampling level, mature leaves ( $40 < n < 60$ ) were collected. Five leaves were selected to measure leaf morphological traits and twenty leaves were fixed into a buffered formalin-acetic acid-alcohol fixation solution (70% ethanol: formalin: glacial acetic acid = 90: 5: 5) for measuring leaf anatomical and hydraulic traits. The remaining leaves were used to measure leaf chemical traits.

**Table 3.2** Means and standard deviations of the sizes of sampled trees of each species and site.

Species	Site	Height of first living branch (m)		
		DBH (cm)	Height (m)	Mean±SD
<i>Betula platyphylla</i>	CB	45.50±5.90	25.50±4.59	10.97±1.79
	ML	34.17±3.84	19.70±1.05	7.23±0.71
	FL	38.70±6.24	20.80±3.15	9.23±2.45
	SS	34.07±3.39	19.27±4.54	7.27±0.92
<i>Tilia amurensis</i>	CB	47.40±3.12	25.63±5.43	10.60±4.25
	ML	64.03±3.55	29.00±7.51	10.00±0.78
	FL	47.70±5.96	23.13±6.21	9.00±3.38
	SS	29.73±3.50	22.70±7.45	5.83±1.86
<i>Acer mono</i>	CB	38.20±6.55	19.03±2.05	6.00±1.87
	ML	43.40±0.70	19.57±1.01	6.57±0.95
	FL	28.80±7.55	12.27±1.46	5.03±1.14
	SS	27.70±4.03	12.03±2.90	3.93±1.37

CB, Changbai Mountain; ML, Muling; FL, Fenglin; SS, Shengshan

### 3.3.3 Leaf economic traits measurements

The area of each leaf was scanned using a portable scanner (Canon LiDE 120, Tokyo, Japan), and the leaf area was calculated by Motic Images Plus 2.0 software (with a precision of

$0.01 \text{ cm}^2$ ). The fresh mass was weighed (with a precision of 0.1 mg). I then placed the sampled leaves into distilled water for 12 hours after which the water was carefully wiped off each sample leaf with filter paper, and the water-saturated leaf was weighed (with a precision of 0.1 mg). The leaf samples were oven-dried at  $65^\circ\text{C}$  for 48 hours to a constant mass. The dry mass of the dried leaf samples was weighed (with a precision of 0.1 mg). The LMA ( $\text{g m}^{-2}$ ) was calculated as the ratio of the leaf dry mass to the leaf area. The LDMC ( $\text{g g}^{-1}$ ) was determined by dividing the leaf dry mass by the saturated fresh mass. All the sampled leaves used to measure the chemical traits were initially oven-dried at  $60^\circ\text{C}$  for 48 hours and were ground to a fine powder by a pulverizer. The  $\text{N}_{\text{mass}}$  ( $\text{mg g}^{-1}$ ) was measured using a Hanon K9840 auto-Kjeldahl analyzer (Jinan Hanon Instruments Co., Ltd., Jinan, China).

### 3.3.4 Leaf anatomical traits measurements

Based on the paraffin section technique (He et al., 2018), I obtained leaf transverse sections. The rectangular sections ( $1.0 \times 0.8 \text{ cm}^2$ ) were cut from the sample leaves. The leaf sections were progressively dehydrated in an ethanol series, infiltrated with warm paraffin, and then cut using a rotary microtome (KD-2258, Zhejiang, China). The mesophyll tissue were stained using safranin and fast green stain. After obtaining the anatomical sections of the leaves, the slides were observed under a light microscope (Olympus Electronics Inc., Tsukuba, Japan), photographed and measured using electronic image analysis equipment (cellSens Standard 1.11 software, Olympus Electronics Inc., Japan). The adaxial epidermis thickness (AD,  $\mu\text{m}$ ), abaxial epidermis thickness (AB,  $\mu\text{m}$ ) and PT ( $\mu\text{m}$ ) were measured.

### 3.3.5 Leaf hydraulic traits measurements

Before cutting the sample leaves, the leaves were scanned to obtain an electronic image for the further calculation of the leaf area and the measurement of  $1^\circ$  (primary) and  $2^\circ$  (secondary) vein lengths. The total lengths of the  $1^\circ$  and  $2^\circ$  veins in the scanning leaf area images were measured by using ImageJ software (NIH, Bethesda, MD, USA); the VLA<sub>1-2</sub> ( $\text{mm mm}^{-2}$ ) was

measured as the total length of the 1-2° veins per unit leaf area. For the minor veins, the remaining leaf portions that were used for measuring the anatomical traits were placed in 5% sodium hydroxide to soften the tissue and then the adaxial epidermis and palisade mesophyll were carefully brushed away using a banister brush to expose the minor veins. The sections were then placed in 5% sodium hypochlorite for bleaching for several hours to several days, until clear. After bleaching, the sections were carefully rinsed to remove excess bleach and then stained in 1% safranin (Sack et al., 2012). Finally, each section was observed using a light microscope, and 3 fields of view were photographed for each section using electronic image analysis equipment. the VLA<sub>min</sub> (mm mm<sup>-2</sup>) was measured as the total length of the minor veins per area.

### **3.3.6 Statistical analysis**

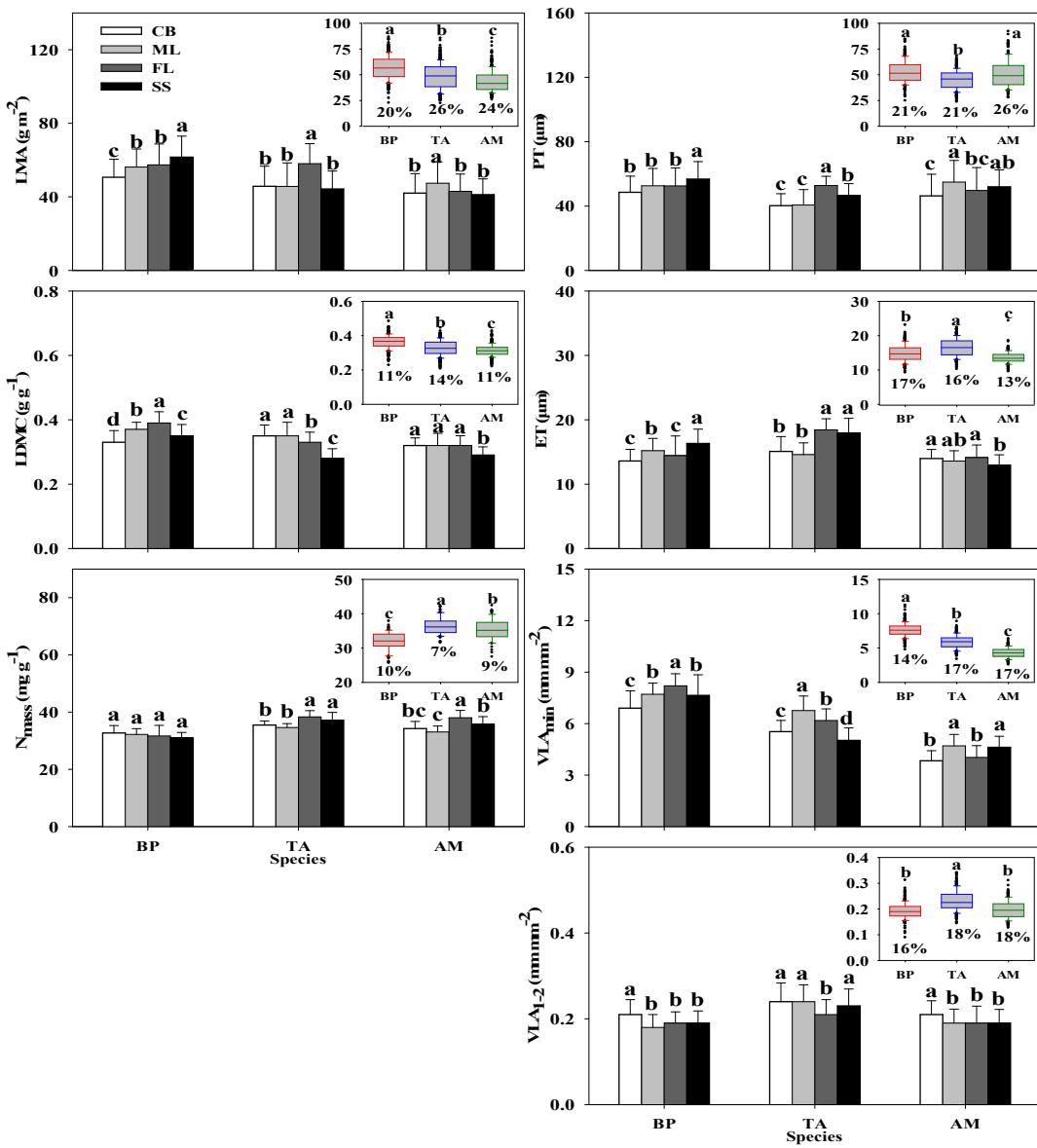
All the statistical analysis were performed using R-3.2.5 (Core Team 2016). The difference in the mean values of leaf traits among the three species were tested using one-way ANOVA, which was also used to assess the differences in the mean value of leaf traits among the four sites. In order to explore the variation extent of leaf traits for each species, I calculated the coefficient of variation (Valladares et al., 2000). I used nested analysis of variance coupled with variance partitioning techniques to evaluate the amount of variation in the leaf traits that was explained by the different ecological scales. A nested level (site > individual > canopy for each species) was employed.

I obtained the bivariate relationships between leaf anatomical and hydraulic traits against economic traits and then compared the slopes of bivariate relationships among the three species by standardized major axis (SMA) regression analysis using the ‘smatr’ (standardized major axis tests and routines) package (Warton et al., 2006). I then performed a principal components analysis (PCA) in the ‘vegan’ R package (Oksanen et al., 2013) to evaluate main axes of multivariate trait covariation for each species and all three species individually. Finally, I used redundancy analysis (RDA) to explore the main anatomical and hydraulic traits influencing leaf economic traits. All the analyses were based on log-transformed leaf trait values.

## 3.4 Results

### 3.4.1 The intraspecific latitudinal patterns of leaf economic, anatomical and hydraulic traits

For three study species, the VLA<sub>1-2</sub> in the higher latitude site was lower than that in the lower latitude site (Fig 3.1). For *T. amurensis* and *A. mono*, LDMC in the higher latitude site was higher than that in the lower latitude site, LMA and PT in middle latitude site were higher, N<sub>mass</sub> in higher latitude site was higher than that in lower latitude site (Fig 3.1). For *B. platyphylla*, LDMC in middle latitude site was higher, LMA and PT in the higher latitude site were higher than those in the lower latitude site, there was no significant difference of N<sub>mass</sub> among four sites (Fig 3.1). For *A. mono*, the ET was lowest and the VLA<sub>min</sub> was higher in the highest latitude site, for *B. platyphylla* and *T. amurensis*, the ET was highest and the VLA<sub>min</sub> was lower in the highest latitude site (Fig 3.1).



**Figure 3.1** Histogram and box plots of the LMA ( $\text{g m}^{-2}$ ), LDMC ( $\text{g g}^{-1}$ ), Nmass ( $\text{mg g}^{-1}$ ), PT ( $\mu\text{m}$ ), ET ( $\mu\text{m}$ ), VLA<sub>min</sub> ( $\text{mm mm}^{-2}$ ) and VLA<sub>1-2</sub> ( $\text{mm mm}^{-2}$ ) for the three temperate broad-leaved species. The histograms indicate the mean values and standard errors of the leaf traits across the different sites (i.e., CB: Changbai Mountain; ML: Muling; FL: Fenglin; and SS: Shengshan) for the three temperate broad-leaved species. Different lowercase letters for the four sites indicate that the leaf traits were significantly different among the sites for each species at the 0.05 level. The boxes indicate the difference in the leaf traits among the three species. The values in the insets are the coefficients of variation for each leaf trait. The boxes that share the same letter correspond to the species' mean trait values that were not significantly different at the 0.05 level. BP: *Betula platyphylla*; TA: *Tilia amurensis*; and AM: *Acer mono*. The same below.

### 3.4.2 The extent and causes of intraspecific variation in leaf economic, anatomical and hydraulic traits

The intraspecific variation extent of the three suites of leaf traits was consistent among three species. Within each species, the intraspecific variation extent of three suites of leaf traits was anatomical traits > hydraulic traits > economic traits (Fig 3.1). Specifically speaking, the LMA, PT, VLA<sub>1-2</sub> and VLA<sub>min</sub> were more variable, however, the ET, LDMC and N<sub>mass</sub> were more consistent (Fig 3.1).

Generally, the “canopy” (upper/middle/lower, north/south) level explained the largest proportion (mean value = 23.36%) of the intraspecific trait variation in the seven leaf traits for these three species, which was followed by the “individual” (mean value = 17.36%) and “site” level (mean value = 16.43%) (Table 3.3). Specifically, the amount of intraspecific variation in LMA and PT were more influenced by canopy (Table 3.3), the amount of intraspecific variation in LDMC and VLA<sub>min</sub> were more influenced by site and canopy (Table 3.3), the amount of intraspecific variation in N<sub>mass</sub> and VLA<sub>1-2</sub> were more influenced by individual (Table 3.3), the amount of intraspecific variation in ET was more influenced by site (Table 3.3).

**Table 3.3** Variance partitioning (%) of the seven leaf traits for the three temperate broad-leaved species across three nested levels: site, individual and canopy.

Species	Nested Levels	Economic traits			Anatomical traits		Hydraulic traits	
		LMA	LDMC	N <sub>mass</sub>	PT	ET	VLA <sub>min</sub>	VLA <sub>1-2</sub>
<i>Betula platyphylla</i>	Site	7.65	29.91	0.00	0.00	12.11	20.38	0.00
	Individual	8.60	5.06	53.16	22.03	22.04	10.24	26.00
	Canopy	30.62	21.88	18.72	14.35	2.44	27.16	6.36
	Unexplained	53.12	43.14	28.13	63.62	63.41	42.21	67.63
<i>Tilia</i>	Site	8.61	42.66	29.97	24.59	40.55	45.00	0.00

<i>amurensis</i>	Individual	28.01	11.64	17.69	29.25	18.29	11.41	29.89
	Canopy	40.90	28.42	33.68	18.30	2.38	22.68	16.67
	Unexplained	22.49	17.28	18.66	27.85	38.78	20.91	53.44
	Site	0.00	23.21	28.87	0.00	6.75	21.70	3.13
	Individual	2.05	0.00	32.93	12.92	0.00	17.68	5.61
<i>Acer mono</i>	Canopy	56.69	33.03	7.34	37.54	10.99	22.64	10.54
	Unexplained	41.26	43.76	30.86	49.53	82.26	37.98	80.73

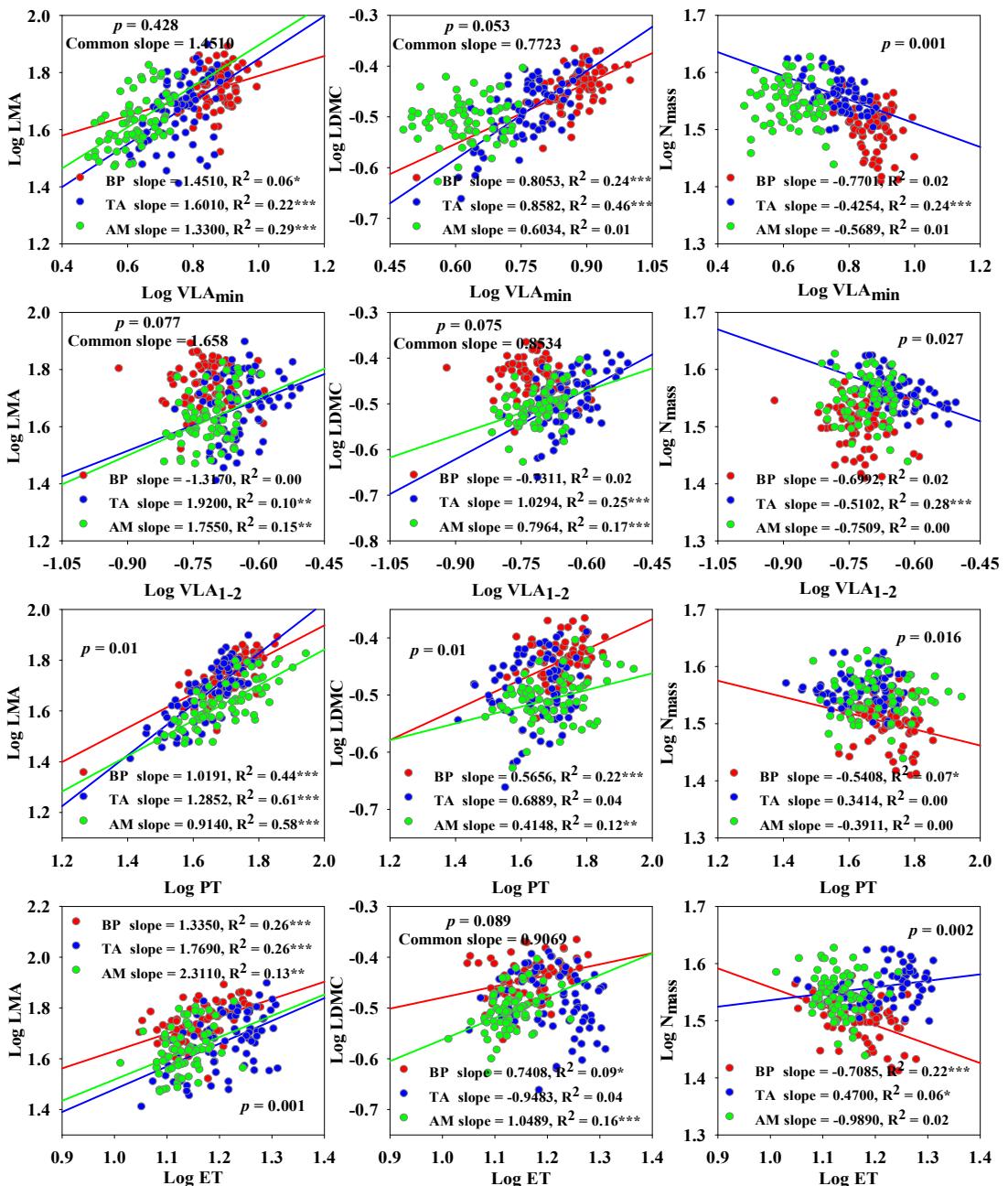
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LMA: leaf mass per area, LDMC: leaf dry matter content, N<sub>mass</sub>: mass-based leaf nitrogen content, PT: palisade tissue thickness, ET: epidermis thickness, VLA<sub>1-2</sub>: total length of the 1–2° veins per unit area and VLA<sub>min</sub>: the length of the minor veins per unit area. The same below.

### 3.4.3 The intraspecific bivariate relationships of the leaf traits

Although there were significant bivariate relationships between both anatomical / hydraulic traits and economic traits, some bivariate relationships were significantly affected by species ( $p < 0.05$ ) (Fig 3.2). The slope of bivariate relationship of LMA-ET of *B. platyphylla* was lowest, and that of *A. mono* was highest. The bivariate relationships of LMA-PT, LDMC-PT of *T. amurensis* were stronger than *B. platyphylla* and *A. mono* (Fig 3.2).

The bivariate relationships of anatomical and hydraulic traits against N<sub>mass</sub> were strongly affected by species (Fig 3.2). There were no bivariate relationships of anatomical and hydraulic traits against N<sub>mass</sub> for *A. mono* (Fig 3.2), there were negative bivariate relationships of hydraulic traits against N<sub>mass</sub> for *T. amurensis* and negative bivariate relationships of anatomical traits against N<sub>mass</sub> for *B. platyphylla* (Fig 3.2).



**Figure 3.2** Standardized major axis (SMA) bivariate relationships among the leaf economic, anatomical and hydraulic traits in the different temperate species. Different colours denote the different species. If there was no significant difference in the slopes of the relationships across the three species, the common slope and the 95% confidence intervals are shown. The line was not shown if there was no significant correlation between the leaf traits. All the analyses were based on log-transformed leaf trait values. The same below.

#### 3.4.4 The intraspecific coordination of the leaf economic, anatomical and hydraulic traits

The PCA results further confirmed that leaf anatomical traits were independent of leaf

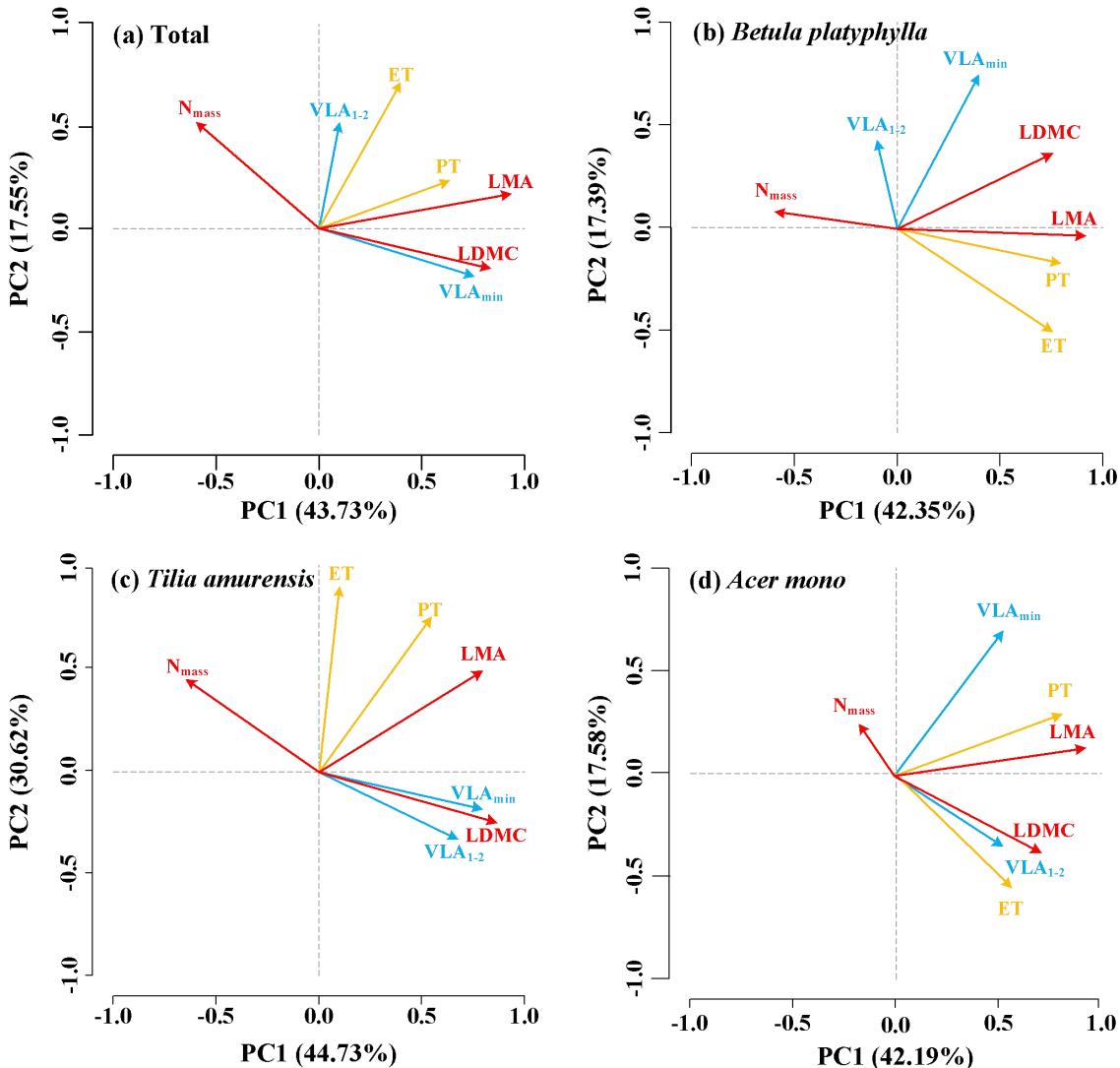
hydraulic traits for *B. platyphylla* and *T. amurensis* (Table 3.4, Fig 3.3). The economics and anatomical traits vary in a fully coordinated manner for *B. platyphylla*, whereas the economics and hydraulic traits vary in a full coordinated manner for *T. amurensis*. For *A. mono*, only VLA<sub>min</sub> and N<sub>mass</sub> were independent of the other five traits (Table 3.4, Fig 3.3).

With all species grouped, the first principal component was positively correlated with the LMA, LDMC, PT and VLA<sub>min</sub> and negatively correlated with the N<sub>mass</sub>. The second principal component was positively correlated with the ET and VLA<sub>1-2</sub> (Table 3.4, Fig 3.3).

**Table 3.4** Loading scores of seven leaf traits in the PCA within each species and among three species.

	<i>Betula platyphylla</i>		<i>Tilia amurensis</i>		<i>Acer mono</i>		Total	
Component	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
% of variance	42.35	17.39	44.73	30.62	42.19	17.58	43.73	17.50
Loadings of traits								
VLA <sub>min</sub>	0.38	<b>0.77</b>	<b>0.79</b>	-0.20	0.54	<b>0.72</b>	<b>0.76</b>	-0.23
VLA <sub>1-2</sub>	-0.08	<b>0.45</b>	<b>0.66</b>	-0.34	<b>0.54</b>	-0.35	0.10	<b>0.53</b>
PT	<b>0.78</b>	-0.15	0.52	<b>0.77</b>	<b>0.82</b>	0.31	<b>0.62</b>	0.24
ET	<b>0.72</b>	-0.49	0.08	<b>0.92</b>	<b>0.57</b>	-0.43	0.39	<b>0.73</b>
N <sub>mass</sub>	<b>-0.61</b>	0.08	<b>-0.64</b>	0.47	-0.17	0.26	<b>-0.60</b>	0.52
LMA	<b>0.89</b>	-0.01	<b>0.80</b>	0.51	<b>0.93</b>	0.13	<b>0.92</b>	0.18
LDMC	<b>0.74</b>	0.37	<b>0.85</b>	-0.25	<b>0.74</b>	-0.39	<b>0.84</b>	-0.19

The strong loadings traits of each principal component are indicated in bold. LMA: leaf mass per area, LDMC: leaf dry matter content, N<sub>mass</sub>: mass-based leaf nitrogen content, PT: palisade tissue thickness, ET: epidermis thickness, VLA<sub>1-2</sub>: total length of the 1–2° veins per unit area, VLA<sub>min</sub>: the length of the minor veins per unit area, the same below.



**Figure 3.3** Principal component analysis of the leaf economic, anatomical and hydraulic traits within each species and the “total” (all species grouped). The trait loading plot shows the leaf economic traits as red lines, the leaf anatomical traits as yellow lines and the leaf hydraulic traits as blue lines. The same below.

### 3.4.5 The effect of the anatomical and hydraulic traits on intraspecific variation in the economic traits

For three species, the anatomical and hydraulic traits best explain the intraspecific variation of leaf economics traits (46.56–69.63% for LMA; 24.36–51.72% for the LDMC; 0.00–34.22% for N<sub>mass</sub>) (Table 3.5, Fig 3.4). The trait that best explains LMA was PT for all three species. The trait that best explains LDMC was VLA<sub>min</sub> for *B. platyphylla* and *T. amurensis* and ET for *A.*

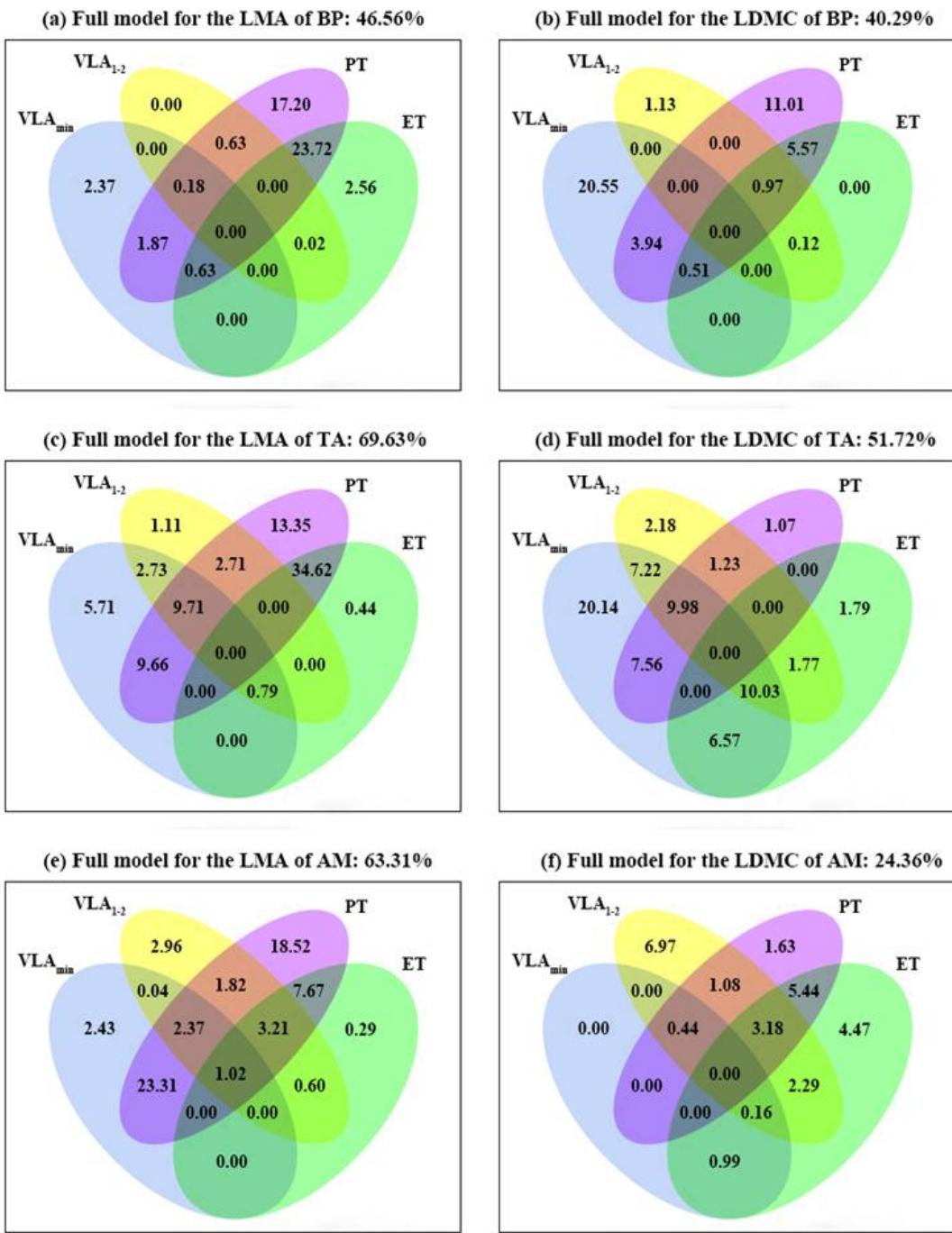
*mono*. The trait that best explains N<sub>mass</sub> was ET for *B. platyphylla* and VLA<sub>1-2</sub> for *T. amurensis* (Table 3.5, Fig 3.4).

There were trait-trait joint effects on the intraspecific variation of LMA and LDMC for three species (Fig 3.4). The traits that best explained variation in LMA were joint effect of PT and ET for *B. platyphylla* and *T. amurensis*, and between PT and VLA<sub>min</sub> for *A. mono* (Fig 3.4). The trait-trait effect that best explained LDMC between PT and ET for *B. platyphylla* and *A. mono*, and between PT and VLA<sub>min</sub> for *T. amurensis* (Fig 3.4).

**Table 3.5** Variation partitioning (%) of anatomical and hydraulic traits in accounting for leaf economic traits.

Explanatory variable	<i>Betula platyphylla</i>			<i>Tilia amurensis</i>			<i>Acer mono</i>		
	LMA	LDMC	N <sub>mass</sub>	LMA	LDMC	N <sub>mass</sub>	LMA	LDMC	N <sub>mass</sub>
VLA <sub>min</sub>	4.44	23.85	0.79	20.70	46.70	22.69	28.25	0.00	0.00
VLA <sub>1-2</sub>	0.00	0.00	0.24	8.69	22.85	26.24	11.77	13.87	0.00
PT	42.77	20.42	5.53	60.26	2.02	0.00	57.84	10.42	0.00
ET	25.06	5.84	20.76	24.65	2.34	4.45	11.87	15.28	0.53
ALL	46.56	40.29	24.98	69.63	51.72	34.22	63.31	24.36	0.00

Leaf economic traits were used as dependent variables, leaf anatomical and hydraulic traits as explanatory variables. The table shows the independent effects of each explanatory variable when not controlling for the other three explanatory variables, respectively.



**Figure 3.4** Variation partitioning (%) of the leaf anatomical and hydraulic traits that account for leaf economic traits. The leaf economic traits were used as dependent variables, and the leaf anatomical and hydraulic traits were used as explanatory variables. (a) LMA of BP. (b) LDMC of BP. (c) LMA of TA. (d) LDMC of TA. (e) LMA of AM. (f) LDMC of AM. Different colours denote the different traits. Places that do not intersect denote the independent effects of each explanatory variable when controlling for the other three explanatory variables; the intersection is their joint effect. Values < 0 are shown as 0.00. The same below.

## 3.5 Discussion

### 3.5.1 The intraspecific latitudinal patterns of leaf economic, anatomical and hydraulic traits

Differences in environmental factors such as temperature, light, and water conditions at four sites along a latitudinal gradient may result in associated patterns of leaf economic, anatomical and hydraulic traits (He et al., 2018), which would suggest that leaf traits have strong potential for acclimation to local environmental conditions (Poorter et al., 2018). Of my three study species, *B. platyphylla* is a light-demanding species, whereas *T. amurensis* and *A. mono* are more shade-tolerant species (Zhang, et al., 2019). The interspecific differences of the latitudinal patterns might often be dominated by plants adaptations to the site climate, which implied that species with different shade tolerances might have specific adaptive strategy in faced of the latitudinal environment changes (Bayeur et al., 2018; Niinemets 2015).

The LMA and PT first increased and then decreased with increasing latitude for *T. amurensis* and *A. mono* (Fig 3.1), which were conformed with the results of Zhu et al. (2012) and He et al. (2018). However, the PT and LMA of *B. platyphylla* increased with increasing latitude (Fig 3.1), a higher LMA and a thicker palisade tissue could improve photosynthetic capacity and the accumulation of photosynthetic products (Krober et al., 2015), which are beneficial for shade-intolerant species living at higher latitudes (Kitajima and Poorter 2010; Kitajima et al., 2012).

My results also showed that VLA<sub>1-2</sub> was lower at higher latitudes (Fig 3.1). This is showing a similar patterns to that in *Quercus variabilis* (Zhu et al. 2012). Low temperatures may particularly decrease cell division, reduce the transpiration demand and decrease the flow rate in the venation system, and then resulting in a reduced requirement for VLA<sub>1-2</sub> (Blonder et al., 2016). The thicker epidermis might decrease leaf transpiration and improve the ability of leaves to resist cold damage at higher latitudes (Krober et al., 2015), whereas the thinner epidermis might benefit gas exchange and improve CO<sub>2</sub> distribution from stomata throughout leaves (He et al., 2018). The different intraspecific latitude pattern of ET among three species depends on their survival strategy in high latitudes. *A. mono* does not have much of a pattern of ET along a

latitudinal gradient, whereas both *T. amurensis* and *B. platyphylla* do (they increase). These are also interesting inter-specific differences.

### 3.5.2 The extent and causes of intraspecific leaf trait variation

My results showed that ET, LDMC, and N<sub>mass</sub> were more stable, whereas the LMA, PT, VLA<sub>1-2</sub>, and VLA<sub>min</sub> were more variable (Fig 3.1) as Martin et al. (2017) and Li et al. (2015). One possible explanation for this result is that there are differences in the response, adaptation time, and sensitivity of leaf traits to environmental changes (Bonser et al., 2010). VLA<sub>1-2</sub> and VLA<sub>min</sub> are size-related traits, LMA and PT are light capture-related traits (Poorter et al., 2018), PT can maximize light absorption (Coble et al., 2017; He et al., 2018). Further, VLA<sub>1-2</sub> and VLA<sub>min</sub> can determine the mechanical support capacity and the transport capacity of water and material in the leaves (Kawai et al., 2016; Li et al., 2013), they are the main factors for optimizing photosynthesis and influencing plant performance. Plants require these traits to respond rapidly in faced of the environment changes (Brodrribb et al., 2013; Martin et al., 2017). Epidermal tissue traits were more stable, because the epidermis is formed by a single layer of closely packed cells, closely contacted with other cells and has a greater cell wall thickness (Onoda et al., 2008). The way to increase epidermis thickness is to increase the cell size or decrease the cell number (Villar et al., 2013). Leaf dry matter content is related to tissue quality and toughness, my results showed that LDMC is less sensitive to changes in environmental conditions (Fig 3.1), which implies that LDMC is phylogenetically more conserved and may makes corresponding adjustments with a change in other traits (Oguchi et al., 2005). Previous studies found that the leaf nutrient concentrations tended to have a higher intraspecific variation than LMA (Siefert et al., 2015), my results suggest that the variation of N<sub>mass</sub> may be influenced by study species.

I found that the ecological scale that contributes the most to intraspecific trait variation is the canopy, followed by the individual and lastly the site (Table 3.3). Previous studies provide appropriate explanations for my results: Light, water pressure, gas temperature, and wind speed

change noticeably with the canopy, resulting in the high plasticity of leaf traits at the canopy scale (Poorter et al., 2009). Leaves growing in high light conditions with a thicker palisade layer enhance light penetration to the deeper layers of the mesophyll (He et al., 2018; Oguchi et al., 2005). Further, gravitational potential gradient appears to limit leaf development at greater heights and may restrict leaf expansion, resulting in leaves with a greater LMA (Coble et al., 2017). A greater investment in vascular tissue for leaves growing at high light intensities may enhance the water supply to leaves (Brodribb & Field, 2010; Oguchi et al., 2005), which may compensate for a high evaporative demand and reduce the gradients in the leaf water potential (Brodribb & Field, 2010). Martin and Thomas (2013) showed that individuals explained little (< 10%) of the intraspecific trait variation in coffee (Martin and Thomas 2013), whereas my results showed that individuals explained 17.60% of the intraspecific trait variation (Table 3.3). The study presented by Martin and Thomas (2013) was designed to limit the influence of plant size and there was a limitation on the size range of the trees in My study, confirmed an important role of plant ontogeny in driving intraspecific trait variation (Delagrange et al., 2004; Martin et al., 2017). A portion of the intraspecific trait variation remained unexplained by the nested characteristics, which indicates that the leaf economic, anatomical and hydraulic traits maybe change significantly with the microclimatic factors (Martin et al., 2017). The intraspecific trait variation of the leaf traits may be determined by the environment in which a plant or leaf is situated (Martin et al., 2017) and may also be related to leaf size and leaf age (Brodribb et al., 2013; Kitajima et al., 2012; Schneider et al., 2017).

### **3.5.3 Correlations of the leaf economic, anatomical and hydraulic traits**

The “whole-plant economic spectrum” hypothesis makes a key prediction: traits that are related to resource acquisition and transport will be correlated across all vascular plant species and will span a single dimension of variation (Reich 2014). However, my results showed that not all the leaf traits related to ecophysiology will span a single dimension of variation, which was reflected in our findings that not all traits were captured by the first PCA axis (Fig 3.3; Table 3.4).

Further, not all the species had the same trait combinations, which also was reflected in the loadings of the traits in the first PCA axis which were different among these three study species (Fig 3.3; Table 3.4). One of the most important abilities of vascular tissue is mechanical support (Kawai et al., 2016), for leaves, the vascular tissue mainly support mesophyll and epidermis tissue, and these three tissues together enhance leaf strength and optimize photosynthesis. Thus, it is expected that leaf anatomical traits and hydraulic traits co-vary due to the functional association; however, our findings showed that these two suites of traits were independent for *B. platyphylla* and *T. amurensis* (Fig 3.3; Table 3.4). This result suggested that coordination among these two suites of traits may not be as simple as previously thought. The independence of these two suites of leaf traits may be related to the contrasting evolutionary trajectories and physical separation of leaf structures that correspond with leaf anatomical and hydraulic traits (Li et al., 2015). A webbing of photosynthetic mesophyll tissues may have appeared long after the vascular tissue in the leaf (Beerling 2005). In angiosperms, the epidermis surrounds the mesophyll, and the mesophyll can be divided into an upper subsystem and a lower subsystem by palisade and spongy tissues. Vascular tissues are mainly located in the middle and lower compartment of the leaf (Brodribb & Field, 2010), whereas the palisade tissue is located in the upper compartment of the leaf and different combinations of leaf lower and upper layers will likely give a certain leaf much more freedom to adjust its functioning to a local environment.

I found that leaf traits were coordinated with different economic traits across the three study species (Fig 3.3; Table 3.4). Plants can optimize their performance and form unique ecological strategies by adjusting the combination of their traits (Costa et al., 2020). Although previous research found that there were no correlations between leaf hydraulic traits and economic traits (Blackman et al., 2016; Li et al., 2015; Sack et al., 2014), my results showed that, except for the independence of leaf economic and hydraulic traits of *B. platyphylla*, these two suites of leaf traits of the other two species vary in a coordinated manner to some extent (Fig 3.3; Table 3.4). It can be seen that these two suites of leaf traits cannot be simply regarded as independent or coordinated, but their coordination is influenced by species. In the future, identifying the

coordination among various suites of leaf traits for species with different shade tolerances should lead to better understanding of species ecology strategy in diverse ecosystems.

My study also revealed the effect of leaf anatomical and hydraulic traits on leaf economic leaf traits. Leaf mass per area and LDMC were best explained by leaf anatomical and hydraulic traits, whereas the  $N_{mass}$  was only poorly explained by leaf anatomical and hydraulic traits (Fig 3.4; Table 3.5). More specifically, LMA was mainly correlated to PT, which is consistent with previous studies that found that palisade tissue thickness can drive LMA variation (Coble and Cavaleri 2017; de la Riva et al., 2016; John et al., 2017). Epidermis tissue has a very low density, mesophyll has an intermediate density, and vascular tissue has thick cell walls and a high density (John et al., 2017; Poorter et al., 2009), which likely explains the observed patterns between changes in PT and  $VLA_{min}$  and the change in LMA (Villar et al., 2013). I also found that the LDMC was mainly influenced by the  $VLA_{min}$ , which is an effective supplement for related research. A greater proportional representation of veins indicates increases in cell wall volume fraction (Poorter et al., 2009), fibre contents per unit volume and fracture toughness (Kitajima & Poorter, 2010; Lucas, 2000), which leading to increases in LDMC. It can be seen that variation in economic traits is not random but rather occurs in a coordinated way with the anatomical and hydraulic traits (Villar et al., 2013). Leaf anatomical and hydraulic traits had certain predictive effects on leaf economic traits.

### 3.6 Conclusions

One of the key frontiers in the field of plant ecology is to better understand intraspecific variation patterns of key leaf functional traits for the most common plants. In this study, we found that leaf economic, anatomical and hydraulic traits showed significant intraspecific latitudinal patterns for three coexisting species in the same forest type at regional scales, and the amount of intraspecific variation in leaf traits could be explained by site, individual and canopy, which could make important contributions to our further understanding the intraspecific variation of key leaf functional traits. The coordination among various suites of leaf traits leads to the

formation of unique ecological strategies in plants and enables them to better adapt to their environments, we found that there were the intraspecific coordination among leaf economic, anatomical and hydraulic traits within three coexisting species; however, coordination varied by tree species. Furthermore, leaf anatomical and hydraulic traits were important to determine leaf economic traits, which provides new evidence for the idea that intraspecific variation in leaf economic traits is not random but rather occurs in a coordinated way with leaf anatomical and hydraulic traits.

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# Chapter 4: Synthesis

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## 4.1 Summary of Findings

This chapter reexamines the overall objectives outlined in the introduction to this thesis and discusses them in the light of the results outlined in Chapters 2 and 3. This thesis focuses on three typical broad-leaved species, *B. platyphylla*, *T. amurensis*, and *A. mono*, in a broad-leaved Korean pine forest in northeast China. One-way ANOVA, nested analysis of variance, standardized major axis (SMA) regression analysis, PCA, and RDA investigated the regeneration patterns of seedlings and saplings and leaf functional traits of adult trees under different latitudinal gradients, and analyzed the differences in regeneration niche of coexisting species. The relationship between the quantitative characteristics of *B. platyphylla*, *T. amurensis*, and *A. mono* seedlings and saplings and the morphological adaptation strategies and growth rules in heterogeneous habitats were studied. Based on the different leaf functional traits, I also systematically studied the intraspecific variation patterns and coordination of leaf economic, anatomical, and hydraulic traits for major species in the same forest type at a regional scale. The main conclusions are as follows:

- (1) I found a total of five seedlings and saplings of *B. platyphylla*, 77 *T. amurensis*, and 1317 *A. mono* in our survey. As a pioneer species, *B. platyphylla* appeared in the early stage of forest community succession, and the sample sites I surveyed were in the original broad-leaved Korean pine forest, which is the top zonal vegetation in northeastern China. Therefore, there are very few seedlings and saplings of *B. platyphylla*, followed by *T. amurensis* in the middle of the succession, while there are many *A. mono* in the late succession.
- (2) The results showed that slope is the main environmental factor affecting regeneration patterns. The greater the slope in a certain range, the more understory plants appear to regenerate; regeneration of seedlings and saplings is most frequent on grades with slopes of 6–25 degrees, mostly on gentle slopes. Regeneration is likely to occur on sunny slopes (S, SW). The middle

and upper slopes are the slopes with the highest number of seedlings regeneration. The light environment is most suitable for the growth of seedlings in the interval of 20%–30% of canopy openness.

(3) Any correlations between the *B. platyphylla* and environmental factors were less significant, due to low numbers of individuals recorded. The number of regeneration of seedlings and saplings of *T. amurensis* and *A. mono* were all positively correlated with slope. Canopy openness was strongly negatively correlated with the number of regeneration of *T. amurensis* and *A. mono*. While latitude was highly positively correlated with *A. mono*, latitude became negatively correlated with *T. amurensis*, and aspect and canopy openness were negatively correlated with all three species.

(4) I found that the leaf traits significantly varied across the different survey sites. Position of leaf in the tree, individual tree, and survey site explained 22.06%, 17.36%, and 16.43% of the intraspecific trait variation for the overall leaf traits.

(5) The epidermis thickness, leaf dry matter content, and mass-based leaf nitrogen content were more stable, whereas the leaf mass per area, palisade tissue thickness, and the length of the veins per unit area were more variable. There were correlations among leaf traits.

(6) My study showed that a high leaf mass per area resulted principally from a higher palisade tissue thickness, a high leaf dry matter content correlates with increased lengths of the minor veins per unit area. Therefore, my results highlight the importance of intraspecific trait variation, supplement data of leaf economic, anatomical and hydraulic traits at regional scales and effectively broaden current knowledge on the adaptation strategies of plants.

## 4.2 Regeneration ecology

In the early stage of forest community succession, due to the lack of vegetation coverage and buffer effect, plants grow in the environment with relatively full light. With the succession, although the total radiation above the community has more difference in different stages of succession, the distribution of light, light intensity and light quality composition in the forest

community will change. The species of trees in early and mid-succession were gradually pushed out, likely due to species requirements for germination and seedling growth in response to the heterogeneity of the light environment between early and late succession, while the species and number of trees in late succession were more abundant (Li & Ma, 2002).

It can be inferred from the data and related studies that terrain change has a significant effect on the regeneration of *T. amurensis* and *A. mono* populations in forest communities, among which slope, aspect and topographic position are the factors that significantly affect seedlings and saplings regeneration.

In this study, seedlings and saplings regeneration the most on the slope of 6–25 degrees, this might be in relation to differences in soil thickness along a slope gradient (Tokuchi et al., 1999). Places with gentle slopes have relatively thick soil layer and abundant water content, while places with steep slopes have relatively thin soil layer and relatively less water content. In addition, saplings are easy to regeneration on the sunny slope (S, SW), because the sunny slope has good light conditions. From the shady slope to the sunny slope, the light intensity gradually increases, the temperature gradually increases, and the water content decreases (Liu & Jin, 2009). In addition, the mid-slope and the upper slope are prone to the occurrence of saplings.

There are many reasons for the difference in the number of saplings among terrains. The combination of different slope position, aspect and slope can well reflect the level of multidimensional environmental factors, which will affect the distribution of saplings in various terrains (Oheimb et al., 2007).

My study found that three species seedlings on the number of regeneration are positively correlation with slope. Cai (Wenhu et al., 2012) found that the density of seedlings in broad-leaved species had the greatest correlation with slope through studying the Daxinanling forest region in Heilongjiang province fire to be forest regeneration of three factors (vegetation, fire before the fire interference characteristics and site conditions).

In many studies, the light environment under the forest is considered to be an important abiotic environment affecting or even determining the survival of seedlings (Lin et al., 2016). In

this study, it was found that the most suitable light environment was when the canopy openness was 20%–30%. Canopy had a negative correlation with the regeneration of the three species, indicating that too intense light under the forest was not suitable for the growth of saplings. From the species, because of different tree species tolerance and sensitivity of light, in the case of the smaller the canopy openness, the shade of seedling compared with pioneer species with higher survival rate, it is also why the shadow resistance stronger, the most number of *A. mono* seedlings of saplings reflects the seedling plants live regeneration stage there are differences between the demand for light resources, Such differences allow plants to adapt to different intensities of light and thus survive and renew in complex community structures.

## 4.3 Leaf traits and ecology

### 4.3.1 The intraspecific latitudinal patterns of leaf economic, anatomical and hydraulic traits

Differences in environmental factors such as temperature, light, and water conditions at four sites along a latitudinal gradient may result in associated patterns of leaf economic, anatomical and hydraulic traits (He et al., 2018), which would suggest that leaf traits have strong potential for acclimation to local environmental conditions (Poorter et al., 2018). Of my three study species, *B. platyphylla* is a light-demanding species, whereas *T. amurensis* and *A. mono* are more shade-tolerant species (Zhang, X. et al., 2019). The interspecific differences of the latitudinal patterns might often be dominated by plants adaptations to the site climate, which implied that species with different shade tolerances might have specific adaptive strategy in faced of the latitudinal environment changes (Bayeur et al., 2018; Niinemets 2015).

The LMA and PT first increased and then decreased with increasing latitude for *T. amurensis* and *A. mono*, which were conformed with the results of Zhu et al. (2012) and He et al. (2018). However, the PT and LMA of *B. platyphylla* increased with increasing latitude, a higher LMA and a thicker palisade tissue could improve photosynthetic capacity and the accumulation of photosynthetic products (Krober et al., 2015), which are beneficial for shade-intolerant species living at higher latitudes (Kitajima and Poorter 2010; Kitajima et al., 2012).

My results also showed that VLA<sub>1-2</sub> was lower at higher latitudes. This is showing a similar patterns to that in *Quercus variabilis* (Zhu et al. 2012). Low temperatures may particularly decrease cell division, reduce the transpiration demand and decrease the flow rate in the venation system, and then resulting in a reduced requirement for VLA<sub>1-2</sub> (Blonder et al., 2016). The thicker epidermis might decrease leaf transpiration and improve the ability of leaves to resist cold damage at higher latitudes (Krober et al., 2015), whereas the thinner epidermis might benefit gas exchange and improve CO<sub>2</sub> distribution from stomata throughout leaves (He et al., 2018). The different intraspecific latitude pattern of ET among three species depends on their survival strategy in high latitudes. *A. mono* does not have much of a pattern of ET along a latitudinal gradient, whereas both *T. amurensis* and *B. platyphylla* do (they increase). These are also interesting inter-specific differences.

#### **4.3.2 The extent and causes of intraspecific leaf trait variation**

My results showed that ET, LDMC, and N<sub>mass</sub> were more stable, whereas the LMA, PT, VLA<sub>1-2</sub>, and VLA<sub>min</sub> were more variable as Martin et al. (2017) and Li et al. (2015). One possible explanation for this result is that there are differences in the response, adaptation time, and sensitivity of leaf traits to environmental changes (Bonser et al., 2010). VLA<sub>1-2</sub> and VLA<sub>min</sub> are size-related traits, LMA and PT are light capture-related traits (Poorter et al., 2018), PT can maximize light absorption (Coble et al., 2017; He et al., 2018). Further, VLA<sub>1-2</sub> and VLA<sub>min</sub> can determine the mechanical support capacity and the transport capacity of water and material in the leaves (Kawai et al., 2016; Li et al., 2013), they are the main factors for optimizing photosynthesis and influencing plant performance. Plants require these traits to respond rapidly in faced of the environment changes (Brodribb et al., 2013; Martin et al., 2017). Epidermal tissue traits were more stable, because the epidermis is formed by a single layer of closely packed cells, closely contacted with other cells and has a greater cell wall thickness (Onoda et al., 2008). The way to increase epidermis thickness is to increase the cell size or decrease the cell number (Villar et al., 2013). Leaf dry matter content is related to tissue quality and toughness, my results showed that LDMC is less sensitive to changes in environmental conditions, which implies that

LDMC is phylogenetically more conserved and may makes corresponding adjustments with a change in other traits (Oguchi et al., 2005). Previous studies found that the leaf nutrient concentrations tended to have a higher intraspecific variation than LMA (Siefert et al., 2015), my results suggest that the variation of N<sub>mass</sub> may be influenced by study species.

I found that the ecological scale that contributes the most to intraspecific trait variation is the canopy, followed by the individual and lastly the site. Previous studies provide appropriate explanations for my results: Light, water pressure, gas temperature, and wind speed change noticeably with the canopy, resulting in the high plasticity of leaf traits at the canopy scale (Poorter et al., 2009). Leaves growing in high light conditions with a thicker palisade layer enhance light penetration to the deeper layers of the mesophyll (He et al., 2018; Oguchi et al., 2005). Further, gravitational potential gradient appears to limit leaf development at greater heights and may restrict leaf expansion, resulting in leaves with a greater LMA (Coble et al., 2017). A greater investment in vascular tissue for leaves growing at high light intensities may enhance the water supply to leaves (Brodribb & Field, 2010; Oguchi et al., 2005), which may compensate for a high evaporative demand and reduce the gradients in the leaf water potential (Brodribb & Field, 2010). Martin and Thomas (2013) showed that individuals explained little (< 10%) of the intraspecific trait variation in coffee (Martin and Thomas 2013), whereas my results showed that individuals explained 17.60% of the intraspecific trait variation. The study presented by Martin and Thomas (2013) was designed to limit the influence of plant size and there was a limitation on the size range of the trees in My study, confirmed an important role of plant ontogeny in driving intraspecific trait variation (Delagrange et al., 2004; Martin et al., 2017). A portion of the intraspecific trait variation remained unexplained by the nested characteristics, which indicates that the leaf economic, anatomical and hydraulic traits maybe change significantly with the microclimatic factors (Martin et al., 2017). The intraspecific trait variation of the leaf traits may be determined by the environment in which a plant or leaf is situated (Martin et al., 2017) and may also be related to leaf size and leaf age (Brodribb et al., 2013; Kitajima et al., 2012; Schneider et al., 2017).

#### **4.3.3 Correlations of the leaf economic, anatomical and hydraulic traits**

The “whole-plant economic spectrum” hypothesis makes a key prediction: traits that are related to resource acquisition and transport will be correlated across all vascular plant species and will span a single dimension of variation (Reich 2014). However, my results showed that not all the leaf traits related to ecophysiology will span a single dimension of variation, which was reflected in my findings that not all traits were captured by the first PCA axis. Further, not all the species had the same trait combinations, which also was reflected in the loadings of the traits in the first PCA axis which were different among these three study species. One of the most important abilities of vascular tissue is mechanical support (Kawai et al., 2016), for leaves, the vascular tissue mainly support mesophyll and epidermis tissue, and these three tissues together enhance leaf strength and optimize photosynthesis. Thus, it is expected that leaf anatomical traits and hydraulic traits co-vary due to the functional association; however, our findings showed that these two suites of traits were independent for *B. platyphylla* and *T. amurensis*. This result suggested that coordination among these two suites of traits may not be as simple as previously thought. The independence of these two suites of leaf traits may be related to the contrasting evolutionary trajectories and physical separation of leaf structures that correspond with leaf anatomical and hydraulic traits (Li et al., 2015). A webbing of photosynthetic mesophyll tissues may have appeared long after the vascular tissue in the leaf (Beerling 2005). In angiosperms, the epidermis surrounds the mesophyll, and the mesophyll can be divided into an upper subsystem and a lower subsystem by palisade and spongy tissues. Vascular tissues are mainly located in the middle and lower compartment of the leaf (Brodribb & Field, 2010), whereas the palisade tissue is located in the upper compartment of the leaf and different combinations of leaf lower and upper layers will likely give a certain leaf much more freedom to adjust its functioning to a local environment.

I found that leaf traits were coordinated with different economic traits across the three study species. Plants can optimize their performance and form unique ecological strategies by adjusting the combination of their traits (Costa et al., 2020). Although previous research found that there

were no correlations between leaf hydraulic traits and economic traits (Blackman et al., 2016; Li et al., 2015; Sack et al., 2014), my results showed that, except for the independence of leaf economic and hydraulic traits of *B. platyphylla*, these two suites of leaf traits of the other two species vary in a coordinated manner to some extent. It can be seen that these two suites of leaf traits cannot be simply regarded as independent or coordinated, but their coordination is influenced by species. In the future, identifying the coordination among various suites of leaf traits for species with different shade tolerances should lead to better understanding of species ecology strategy in diverse ecosystems.

My study also revealed the effect of leaf anatomical and hydraulic traits on leaf economic leaf traits. Leaf mass per area and LDMC were best explained by leaf anatomical and hydraulic traits, whereas the  $N_{mass}$  was only poorly explained by leaf anatomical and hydraulic traits. More specifically, LMA was mainly correlated to PT, which is consistent with previous studies that found that palisade tissue thickness can drive LMA variation (Coble and Cavaleri 2017; de la Riva et al., 2016; John et al., 2017). Epidermis tissue has a very low density, mesophyll has an intermediate density, and vascular tissue has thick cell walls and a high density (John et al., 2017; Poorter et al., 2009), which likely explains the observed patterns between changes in PT and  $VLA_{min}$  and the change in LMA (Villar et al., 2013). I also found that the LDMC was mainly influenced by the  $VLA_{min}$ , which is an effective supplement for related research. A greater proportional representation of veins indicates increases in cell wall volume fraction (Poorter et al., 2009), fibre contents per unit volume and fracture toughness (Kitajima & Poorter, 2010; Lucas, 2000), which leading to increases in LDMC. It can be seen that variation in economic traits is not random but rather occurs in a coordinated way with the anatomical and hydraulic traits (Villar et al., 2013). Leaf anatomical and hydraulic traits had certain predictive effects on leaf economic traits.

#### 4.4 Limitations

Limited by the COVID-19 lockdown, this thesis only selected a few environmental factors for research and analysis from the complex factors affecting population regeneration. In fact, seedlings and saplings are affected by many factors in the process of regeneration, including biotic environment and abiotic environment. In this study, canopy openness and topographic factors were selected as abiotic environments to analyze the effects on seedling richness and survival, without adding too many abiotic environment factors, such as soil nutrient content and soil water content. In future studies, more abiotic environments should be considered. In order to further explore the impact of environment on seedling regeneration, studies on the functional traits of seedlings and saplings should be increased, which should be linked to underground soil nutrients. In addition, the seedling stage is the most vulnerable to external environment, but later growth stage testing, such as sapling stage, is also very necessary. In future studies, the effects of environmental factors on seedling, sapling and big tree stages should be comprehensively analyzed.

Secondly, the selection of indicators for the response of *B. platyphylla*, *T. amurensis* and *A. mono* sapling populations to the environment is too thin, and many new ideas and related scientific problems generated in the writing process of the thesis have not been studied and verified. The regeneration of plant populations is extremely complex under the influence of the environment. The juvenile stage of plants is the most sensitive stage in the whole life history of plants, which is easily eliminated by "environmental sieve". Therefore, the dynamic monitoring of seedlings and saplings is an extremely critical link in population regeneration, and the study on the dynamics of seedlings and saplings has important guiding significance for the sustainable development of the population. I hope to follow up the research on the regeneration of seedlings and saplings in the future scientific research.

## 4.5 Recommendations

Since there are still some limitations in this thesis, I would like to further explore the

following issues:

- (1) Effects of microhabitats on seed germination of *B. platyphylla*, *T. amurensis* and *A. mono* during natural regeneration. Seeds are the starting point of plant life history, and paying attention to the response of their "source" to the environment is a key entry point to explore population regeneration.
- (2) Plant configuration is the result of the interaction between plants and the environment. With the development of component population theory, the indicators describing plant configuration are constantly improved and systematized. In this thesis, only a few indexes, there is a lack of more detailed and systematic configuration index parameters, which need to be further improved and complemented.
- (3) The underground part of plants is also sensitive to the environment. The responses of physiological and morphological indexes of the underground roots of *B. platyphylla*, *T. amurensis* and *A. mono* to the environment were also included in the study of population regeneration.
- (4) The dynamic monitoring of plant growth is an important means to reflect objective facts. In the future long-term regeneration research, the dynamic monitoring of research indicators of *B. platyphylla*, *T. amurensis* and *A. mono* should become its main content. In the study of population regeneration, the core idea of "community as background, population as unit, and individual as basis" is adhered to, and the concept of "scale" runs through the whole process. Exploring population regeneration from all angles is the basic principle of scientific, objective and comprehensive reflection of population regeneration.

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# Appendices

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## Appendix A

**Table A.** Means and standard deviations of the sizes of sampled trees of each species and site.

Species	Site	DBH (cm) Mean±SD	Height of first living branch (m)	
			Height (m) Mean±SD	Mean±SD
<i>Betula</i> <i>platyphylla</i>	CB	45.50±5.90	25.50±4.59	10.97±1.79
	ML	34.17±3.84	19.70±1.05	7.23±0.71
	FL	38.70±6.24	20.80±3.15	9.23±2.45
	SS	34.07±3.39	19.27±4.54	7.27±0.92
<i>Tilia amurensis</i>	CB	47.40±3.12	25.63±5.43	10.60±4.25
	ML	64.03±3.55	29.00±7.51	10.00±0.78
	FL	47.70±5.96	23.13±6.21	9.00±3.38
	SS	29.73±3.50	22.70±7.45	5.83±1.86
<i>Acer mono</i>	CB	38.20±6.55	19.03±2.05	6.00±1.87
	ML	43.40±0.70	19.57±1.01	6.57±0.95
	FL	28.80±7.55	12.27±1.46	5.03±1.14
	SS	27.70±4.03	12.03±2.90	3.93±1.37

CB, Changbai Mountain; ML, Muling; FL, Fenglin; SS, Shengshan

## Appendix B

**Table B.** Loading scores of seven leaf traits in the PCA within each species and among three species.

	<i>Betula platyphylla</i>		<i>Tilia amurensis</i>		<i>Acer mono</i>		Total	
Component	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
% of variance	42.35	17.39	44.73	30.62	42.19	17.58	43.73	17.50
Loadings of traits								
VLA <sub>min</sub>	0.38	<b>0.77</b>	<b>0.79</b>	-0.20	0.54	<b>0.72</b>	<b>0.76</b>	-0.23
VLA <sub>1-2</sub>	-0.08	<b>0.45</b>	<b>0.66</b>	-0.34	<b>0.54</b>	-0.35	0.10	<b>0.53</b>
PT	<b>0.78</b>	-0.15	0.52	<b>0.77</b>	<b>0.82</b>	0.31	<b>0.62</b>	0.24
ET	<b>0.72</b>	-0.49	0.08	<b>0.92</b>	<b>0.57</b>	-0.43	0.39	<b>0.73</b>
N <sub>mass</sub>	<b>-0.61</b>	0.08	<b>-0.64</b>	0.47	-0.17	0.26	<b>-0.60</b>	0.52
LMA	<b>0.89</b>	-0.01	<b>0.80</b>	0.51	<b>0.93</b>	0.13	<b>0.92</b>	0.18
LDMC	<b>0.74</b>	0.37	<b>0.85</b>	-0.25	<b>0.74</b>	-0.39	<b>0.84</b>	-0.19

The strong loadings traits of each principal component are indicated in bold. LMA: leaf mass per area, LDMC: leaf dry matter content, N<sub>mass</sub>: mass-based leaf nitrogen content, PT: palisade tissue thickness, ET: epidermis thickness, VLA<sub>1-2</sub>: total length of the 1-2° veins per unit area, VLA<sub>min</sub>: the length of the minor veins per unit area, the same below.

## Appendix C

**Table C.** Variation partitioning (%) of anatomical and hydraulic traits in accounting for leaf economic traits.

Explanatory variable	<i>Betula platyphylla</i>			<i>Tilia amurensis</i>			<i>Acer mono</i>		
	LMA	LDMC	N <sub>mass</sub>	LMA	LDMC	N <sub>mass</sub>	LMA	LDMC	N <sub>mass</sub>
VLA <sub>min</sub>	4.44	23.85	0.79	20.70	46.70	22.69	28.25	0.00	0.00
VLA <sub>1-2</sub>	0.00	0.00	0.24	8.69	22.85	26.24	11.77	13.87	0.00
PT	42.77	20.42	5.53	60.26	2.02	0.00	57.84	10.42	0.00
ET	25.06	5.84	20.76	24.65	2.34	4.45	11.87	15.28	0.53
ALL	46.56	40.29	24.98	69.63	51.72	34.22	63.31	24.36	0.00

Leaf economic traits were used as dependent variables, leaf anatomical and hydraulic traits as explanatory variables. The table showed the independent effects of each explanatory variable when not controlling for the other three explanatory variables, respectively.