Journal Pre-proof

Plant-plant interactions and resource dynamics of *Abies fabri and Picea brachytyla* as affected by phosphorus fertilization

Lei Yu, Mengya Song, Zhichao Xia, Helena Korpelainen, Chunyang Li

Please cite this article as: Yu L, Song M, Xia Z, Korpelainen H, Li C, Plant-plant interactions and resource dynamics of *Abies fabri and Picea brachytyla* as affected by phosphorus fertilization, *Environmental and Experimental Botany* (2019), doi: https://doi.org/10.1016/j.envexpbot.2019.103893

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The Revision of ***EEB-2019-1163***

Plant-plant interactions and resource dynamics of *Abies fabri and Picea brachytyla* as affected by phosphorus fertilization

Head title: P fertilization affects dynamics of plant-plant interactions and resources

Lei Yu [1], Mengya Song [2], Zhichao Xia [1],

Helena Korpelainen [3], Chunyang Li [1, *]

[1] College of Life and Environmental Sciences, Hangzhou Normal University,

Hangzhou 310036, China

[2] Key Laboratory of Geospatial Technology for the Middle and Lower Yellow River

Regions, College of Environment and Planning, Henan University, Kaifeng 475004,

China

[3] Department of Agricultural Sciences, Viikki Plant Science Centre, P.O. Box 27,

FI-00014 University of Helsinki, Finland

* Corresponding author: Chunyang Li, E-mail: licy@hznu.edu.cn

Highlights

- P fertilization affected the relative competition intensity.

- Plant-plant interactions caused temporal variation in C, N, P and NSC contents.

- *A. fabri* greatly benefited from the presence of *P. brachytyla* under P fertilization.

- The benefited species *A. fabri* showed higher biomass, C, N, P and NSC accumulations.

Abstract Although extensive research has been conducted on the temporal dynamics of plant-plant interactions, little is known about the effect of phosphorus (P) availability. In this study, *Abies fabri* and *Picea brachytyla* seedlings were collected from the late-stage Hailuogou glacier retreat area and grown under different P regimes (control and P fertilization) from year 2015 to 2016 in a common garden experiment to investigate whether plant-plant interactions are modulated by P availability. We found that P fertilization affected the relative competition intensity (RCI). Under control conditions in 2015, the growth of *A. fabri* was facilitated by the presence of *P. brachytyla*. Under P fertilization, the facilitative effect was more intensive: the leaf, stem and total biomass of *A. fabri* significantly increased under interspecific interaction compared with intraspecific interaction, but no effect was found in *P. brachytyla*. RCI showed similar tendencies both in 2015 and 2016. In addition, plant-plant interactions and P fertilization caused temporal variation in C, N, P and non-structural carbohydrate (NSC) contents. The growth of *A. fabri* greatly benefited from the presence of *P. brachytyla* when exposed to P fertilization and showed higher

biomass, and C, N, P and NSC accumulations. Our results demonstrated interactive effects between environmental conditions (i.e. P availability) and plant-plant interactions that are closely related to resource accumulation.

*Keywords***:** plant-plant interactions; temporal dynamics; resource accumulation; P fertilization; non-structural carbohydrates.

1. Introduction

The importance of plant-plant interactions in determining plant growth and performance, species abundance and distribution, and the composition and dynamics of plant communities is widely recognized (Tilman, 1988; Michalet, 2006; Raynaud et al., 2008; Pierik et al., 2013; Yamawo, 2015). Previous studies have showed that the relationships of plant-plant interactions can be altered by spatial and temporal environmental heterogeneity (Maestre et al., 2009; Soliveres et al., 2010; Liu et al., 2013; Biswas and Wagner, 2014; Song et al., 2019). Plants can discriminate conspecific and heterospecific individuals and this may lead to changes in plant-plant interactions over time (Weigelt and Jolliffe, 2003; Miller et al., 2007) confirming plant-plant interaction as a dynamic process (Andersen et al., 2007; Trinder et al., 2012, 2013; Crone, 2016).

Tilman's theory (1987, 1988) suggests that competition for soil nutrients is stronger

under low nutrient levels compared to high nutrient conditions. The stress gradient hypothesis proposes that plant-plant interactions vary along external environmental gradients and that negative interactions (competition) are more frequent in relatively productive environments, whereas positive interactions (facilitation) are more common in harsh conditions (Bertness and Callaway, 1994). Choler et al. (2001) and Kikvidze et al. (2006) have demonstrated that positive interactions (facilitation) mainly occur at high elevations and under high biotic and abiotic environmental stress conditions. In addition, Gamfeldt et al. (2013) has revealed that complementarity is the main mechanism behind the biodiversity-productivity relationship in forests, and complementarity is expected to be more frequent between species than between conspecific individuals (Díaz and Cabido, 2001).

In long-lived plants, storage is critical for survival under stress and disturbance conditions, such as shade, drought and herbivory (Adams et al., 2013; O'Brien et al., 2014; Dickman et al., 2015; Martínez-Vilalta et al., 2016; Wiley et al., 2017). Trinder et al. (2012) investigated competition between *Plantago lanceolate* and *Dactylis glomerata* for multiple harvests and found that nitrogen (N) accumulation by *P. lanceolate* exceeded that of *D. glomerata* during the first 62 days exhibiting a better competitiveness. However, thereafter, *D. glomerata* accumulated more N and showed a competitive advantage. Thus, the accumulation of N pools affects plant growth and competitive performance (Jordan et al., 2014). Non-structural carbohydrates (NSC), such as starch and soluble sugars, can be mobilize in plants playing key roles in

physiological processes, transcription, growth and respiration (Niinemets, 2010; Secchi and Zwieniecki, 2011; Sala et al., 2012; Wiley et al., 2013). NSC storage provides carbon for growth during the day and for respiration at night (Walter et al., 2005; Smith and Stitt, 2007). The maintenance during leafless seasons mainly relies on stored NSC (Barbaroux et al., 2003; Crone et al., 2009). Therefore, NSC pools are functionally important and may reflect competitive ability in plants.

Soil nutrient availability is often connected with plant-plant interactions and it plays an important role in plant growth and competitive ability (Boer et al., 2016; Guo et al., 2019; Xia et al., 2019; Yu et al., 2019a, 2019b). Soil nutrient availability not only alters the competitive outcomes but also affects the temporal dynamics of plant-plant interactions. For example, Guo et al. (2017) conducted an N fertilization experiment and found that the competition dynamics and phosphorus (P) accumulation of *Larix kaempferi* and *L. olgensis* varied differently when the plants were exposed to different N fertilization levels. Among soil nutrients, P often limits the reproductive and vegetative growth of plants and is considered to be one of the most limiting elements in terrestrial ecosystems (Elser et al., 2007; Vitousek et al., 2010). P availability affects plants' metabolic processes at biochemical and ecophysiological levels, alters plants' competitiveness and species diversity, and influences the structure and functioning of ecosystems (Sterner and Elser, 2002; Vance et al., 2003; Reich and Oleksyn, 2004; Lambers et al., 2011). For instance, Müller and Bartelheimer (2013) have shown that plant-plant interactions affect P absorption. In addition, atmospheric

5

N deposition from anthropogenic sources induces nutritional imbalances and accelerates the P demand of forest trees (Jonard et al., 2015). However, knowledge of the effects of P availability on the direction and magnitude of plant-plant interactions is still limited, especially concerning glacier retreat areas.

The Hailuogou glacier retreat area, located in the Gongga Mountain region (SW China), has developed a series of primary succession, including early successional deciduous shrubs of *Salix* and *Populus*, and *Abies fabri* appears during later stages of succession, followed by *Picea brachytyla* as a co-dominant species (Luo et al., 2012; Lei et al., 2015; Song et al., 2017). *A. fabri* and *P. brachytyla* occur mainly in the mountainous regions of Western Sichuan, China, and *A. fabri* exhibits higher shade-tolerance. They are endemic and dominant species of the dark subalpine coniferous forests (Liu, 2002; Guo et al., 2010). Plant primary production suffers from the initial N limitation to later P limitation during the primary succession (Vitousek et al., 2010; Lei et al., 2015; Song et al., 2017; Yu et al., 2017). Furthermore, a recent study has demonstrated that the leaf N:P ratio increased steadily in the Hailuogou glacier retreat area, and the limiting factor for plant growth shifted from nitrogen to phosphorus (Jiang et al., 2018). However, little is known whether P availability affects plant-plant interactions and alters the successional replacement of *A. fabri* by *P. brachytyla* in the Hailuogou glacier retreat area.

In the present study, we investigated plant-plant interactions of *A. fabri* and *P.*

6

brachytyla under different P regimes from 2015 to 2016. We also evaluated between-years variation in C, N, P and NSC contents. The following hypotheses were tested: (1) P fertilization affects plant-plant interactions, and *A. fabri* benefits from the presence of *P. brachytyla* under interspecific interaction and P fertilization. (2) The results of the interactions between *A. fabri* and *P. brachytyla* under P fertilization partly support Tilman's theory and the stress gradient hypothesis. (3) The accumulation of N, P and non-structural carbohydrates is affected by P availability and plant-plant interactions.

2. Materials and methods

2.1. Study site, plant material and experimental design

The study was conducted at the Gongga Mountain Alpine Ecosystem Observation and Experiment Station of the Chinese Academy of Sciences, located in the south-eastern fringe of the Tibetan Plateau (elevation: 3000 m a.s.l; 29°34' N, 101°59' E), where the mean annual temperature is 4.2 °C and mean annual precipitation 1947 mm (averages for 22 years). The Hailuogou glacier retreat area is 2 km away from the station and the climatic conditions are similar.

Healthy *A. fabri* and *P. brachytyla* seedlings with a height of about 25 cm were selected from a nursery near the station. Two seedlings (10 cm apart from each other)

7

were planted in each plastic pot (20 L; external diameter 32 cm, height 25 cm) with native soil in mid-September 2014. The planting soil was obtained from the natural habitat dominated by *A. fabri* and had a pH of 5.98 ± 0.07, soil organic matter content of 51.52 ± 3.61g kg^{-1}, total N content of 0.81 ± 0.02 g kg^{-1}, and total P content of 0.90 ± 0.02 g kg^{-1}.

The experimental layout was completely randomized with three factors (species, plant interactions and P fertilization). The plant interactions treatments were designed as follows: intraspecific interaction of *A. fabri* + *A. fabri* and *P. brachytyla* + *P. brachytyla* (monoculture planting, Mon), and interspecific interaction of *A. fabri* + *P. brachytyla* (mixed planting, Mix). Mon+P and Mix+P represented monoculture planting with P fertilization and mixed planting with P fertilization, respectively. Twenty replicates per treatment were included in the experiment. The experiment was performed in an open area under natural rain conditions. Plants were watered every other day. P fertilization was provided as NaH$_2$PO$_4$, 0.4 g P per pot in P-fertilized treatments (Siddique et al., 2010; Chen et al., 2015). We added P fertilization twice a year, in early May and July, during years 2015 and 2016.

2.2. *Harvesting and measurements*

The experiment continued through two harvests, which took place on 28 August 2015 and late August 2016. In 2015, five plants from each treatment were harvested

8

randomly and all individuals were divided into leaves, stems and roots. In 2016, five

plants from each treatment were selected randomly and all harvested plants were

divided into leaves, stems, fine roots (<2 mm in diameter) and coarse roots (>2 mm in

diameter). All harvested plants were dried at 70 °C to a constant mass. The root/shoot

ratio (R/S ratio) was calculated as the ratio between total root biomass and

aboveground biomass (the sum of leaf biomass and stem biomass). All dried samples

were measured for dry mass and ground into a power for further measurements.

C, N, P, soluble sugar and starch contents of organs (leaves, stems and roots) in

harvested plants were measured. C, N and P contents were determined by the rapid

dichromate oxidation technique (Nelson and Sommers, 1982), a semi-micro Kjeldahl

method (Mitchell, 1998), and induced plasma emission spectroscopy (Hötscher and

Hay, 1997), respectively. Soluble sugar and starch contents were determined as

glucose equivalents by the anthrone method according to Yemm and Willis (1954).

Methodological details have been described by Yu et al. (2017).

2.3. Data analysis

The relative competition intensity (RCI) of both conifers under different interaction

patterns and P fertilization in 2015 and 2016 was calculated as follows (Grace, 1995):

$RCI = (B_{inter} - B_{intra}) / B_{intra}$, where B_{inter} indicates the total biomass of an individual

from interspecific interaction and B_{intra} indicates the average total biomass of

9

corresponding plants from intraspecific interaction. For example, when calculating RCI of *A. fabri* under P fertilization, B_{inter} is that of *A. fabri* growing in interspecific interaction under P fertilization and B_{intra} should be the average biomass of *A. fabri* growing in intraspecific interaction under P fertilization (without considering unfertilized plants).

Concentration variations in C, N, P and NSC of each plant organ (leaves, stems and roots) were calculated from 2015 to 2016. NSC of each organ was the sum of starch and soluble sugars. The used formula was as follows:

Concentration variation = [Concentration$_{2016}$ − Concentration$_{2015}$] / Concentration$_{2015}$, where Concentration$_{2015}$ and Concentration$_{2016}$ represent the concentrations of C, N, P and NSC in 2015 and 2016, respectively. In 2016, the concentrations of roots were calculated as follows:

Concentration = [Biomass$_{coarse}$ × Concentration$_{coarse}$ + Biomass$_{fine}$ × Concentration$_{fine}$] / [Biomass$_{coarse}$ + Biomass$_{fine}$], where Biomass$_{coarse}$ and Biomass$_{fine}$ represent the biomasses of coarse and fine roots, respectively.

Content variations in C, N, P and NSC of each organ (leaves, stems and roots) were calculated as follows: Content accumulation = [Content$_{2016}$ − Content$_{2015}$] / Content$_{2015}$, where Content$_{2016}$ and Content$_{2015}$ represent the contents of C, N, P and NSC in 2016 and 2015, respectively. The content of each organ, for example, the leaf C content of an individual was calculated by multiplying the leaf C concentration by

leaf biomass.

Before statistical analyses, the data were checked for the normality and the homogeneity of variances and log-transformed to correct for deviations from these assumptions when needed. Tukey's HSD tests were carried out for each species to detect their RCI differences among treatments. Three-way analyses of variance (ANOVA) were performed to detect the effects of species, plant interactions and P fertilization, and their interactions. Tukey's HSD tests were conducted as well to discover significant differences among treatments. All statistical effects were considered significant at $P < 0.05$. The statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 18.0.

3. Results

3.1. Relative competition intensity

Under control conditions, the organ (leaves, stems, fine roots, and total roots) and total biomasses of *A. fabri* showed no differences between intra- and interspecific interaction, whereas those parameters of *P. brachytyla* decreased under interspecific interaction (Fig. 1). Under P fertilization, interspecific interaction led to higher organ and total biomasses in *A. fabri* compared to intraspecific interaction, while *P. brachytyla* showed no differences between the two interaction patterns. In addition, *A.*

11

fabri showed a higher relative total biomass accumulation than *P. brachytyla* under P fertilization from 2015 to 2016 (Fig. S1). Thus, the growth of *A. fabri* benefited from the presence of *P. brachytyla* under P fertilization (Fig. 1).

The relative competition intensity (RCI) of *A. fabri* and *P. brachytyla* was affected by the P fertilization regime. Both coniferous species showed little temporal variation in RCI from 2015 to 2016 (Fig. 2). Under control conditions in 2015, the growth of *A. fabri* was facilitated by *P. brachytyla*, and this facilitative effect was greater under P fertilization, as shown by significantly higher RCI in *A. fabri.* P fertilization increased RCI of *P. brachytyla*, which indicated that the competitive stress of *P. brachytyla* became ameliorated (Fig. 2).

3.2. Concentrations and contents of carbon and nutrients

Species, plant interactions and P fertilization as well as their interaction effects significantly influenced temporal variation in C, N and P concentrations (Table S1). Under control conditions, the C concentration variation of all organs in both conifers showed no differences between the two interaction patterns, except for the C concentration of shoots (Fig. 3a). Under P fertilization, the N concentration variation of *A. fabri* leaves was higher under interspecific interaction than under intraspecific interaction, while *P. brachytyla* showed a contrasting trend (Fig. 3b). In addition, the P concentration variation of *A. fabri* roots was higher under interspecific interaction

12

than under intraspecific interaction, while *P. brachytyla* showed no differences between the two interaction patterns under P fertilization. Furthermore, the P concentration variation of *A. fabri* leaves was significantly higher than that of *P. brachytyla* (Fig. 3c).

3.3. Concentrations and contents of non-structural carbohydrates

Species, plant interactions and P fertilization as well as their interaction effects significantly influenced the temporal variation in soluble sugar, starch and NSC concentrations (Table S1). Under P fertilization, the soluble sugar concentration variation of *P. brachytyla* leaves significantly decreased under interspecific interaction when compared to intraspecific interaction, while *A. fabri* showed no differences between the two interaction patterns (Fig. 3d). Starch and NSC concentrations had similar variation trends. P fertilization increased variation in the starch and NSC concentrations of roots in both conifers (Fig. 3e, f).

3.4. Between-years variation in element and non-structural carbohydrate pools

Under P fertilization, the C, N, and P contents of leaves and roots and of whole individuals were significantly higher under interspecific interaction than under intraspecific interaction in *A. fabri* (Tables S2, S3). In addition, under P fertilization, the C, N and P content accumulation ratios of leaves and whole individuals were

higher in *A. fabri* compared with *P. brachytyla* under both interaction patterns (Fig. 4 a-f). There were significant interaction effects of species and P fertilization on N and P content accumulation ratios of organs and whole individuals, except for the root N content accumulation ratio (Table S4).

The interaction effects of species, plant interactions and P fertilization significantly influenced soluble sugar, starch and NSC contents of plant organs, as well as those of whole individuals, except for the root starch content (Tables S3, S5). Under P fertilization, soluble sugar, starch, and NSC contents of organs and whole individuals of *A. fabri* were higher under interspecific interaction than under intraspecific interaction. However, *P. brachytyla* tended to show a contrasting trend (Tables S3, S5). In addition, P fertilization increased soluble sugar, starch and NSC accumulation ratios of organs and whole individuals of *A. fabri* more under interspecific interaction than under intraspecific interaction (Fig. 5a-f).

4. Discussion

4.1. The influence of P fertilization on relative competition intensity

In the present study, we investigated plant-plant interactions and resource accumulation in *A. fabri* and *P. brachytyla* under P fertilization from 2015 to 2016 and found that P fertilization significantly influenced plant-plant interactions and resource

14

accumulations in these two conifers. Under P fertilization, *A. fabri* had a higher total biomass under interspecific interaction than under intraspecific interaction, while *P. brachytyla* showed no differences between the two interaction patterns (Fig. 1d). In addition, from 2015 to 2016, the relative total biomass accumulation of *A. fabri* was higher than that of *P. brachytyla* under interspecific interaction and P fertilization (Fig. S1). These results demonstrated that *A. fabri* had a greater competitive capacity under interspecific interaction and P fertilization, which might be associated with its greater shade tolerance (Liu, 2002).

Previous studies have reported that complementarity is the main mechanism behind the biodiversity-productivity relationship in forests, and complementarity is expected to be more frequent between species than between conspecific individuals (Díaz and Cabido, 2001; Gamfeldt et al., 2013). In addition, there are beneficial effects of neighbours and species interactions in ecosystems, and the mechanism may be through the alteration of the microclimate by neighboring plants (Callaway and Walker, 1997; Maestre et al., 2005). In our study, the aboveground biomass of *P. brachytyla* was significantly higher than that of *A. fabri* under P fertilization (Fig. 1a, b), but the performance of *A. fabri* improved more strongly under interspecific interaction, as expected for a shade tolerant plant (Valladares and Niinemets, 2008; Valladares et al., 2016).

Species with minimum resource requirements and best tolerances are expected to be

superior competitors. Competition for soil nutrients has been demonstrated to be greater under low nutrient levels compared to high nutrient conditions (Tilman, 1987, 1988). Bertness and Callaway (1994) have showed that plant individuals may negatively compete for nutrients or space in a productive environment but they may positively compete (facilitation) in a severe or stress environment, as found in many other studies as well (Goldenheim et al., 2008; Maestre et al., 2009; Fajardo and McIntire, 2011; Liu et al., 2013). Under non-fertilization conditions, *A. fabri* showed a competitive advantage relative to *P. brachytyla*, as the latter species promoted the biomass accumulation of the former species (RCI of *A. fabri* was positive, but RCI of *P. brachytyla* was negative) (Figs. 1, 2). Under P fertilization (productive soil), RCI of *A. fabri* increased significantly and showed the presence of a strong interspecific interaction, which supported the stress gradient hypothesis (Bertness and Callaway, 1994). However, P fertilization increased the biomass of *P. brachytyla* while its RCI increased only little, which showed that P fertilization could ameliorate the inhibition of *P. brachytyla*. This result demonstrated that *P. brachytyla* suffered strong competition under non-fertilization (control) conditions, which supports Tilman's theory (Tilman, 1987, 1988). Thus, our results did not fully support either of the two contrasting competition theories, but showed that the relationships among plants are complex and closely related to the environmental conditions.

4.2. Between-years variation in plant-plant interactions and resources

16

In this study, although the RCI trends of the two coniferous were similar (Fig. 2), *A. fabri* had a higher relative total biomass accumulation than *P. brachytyla* under interspecific interaction and P fertilization (Fig. S1). Our results demonstrated that the competitive abilities of plants change with environmental factors. For instance, Song et al. (2017) found that nitrogen (N) fertilization can alter plant-plant interactions in glacier retreat areas. In the non-fertilized conditions, *Salix rehderiana* benefited from the presence of *Populus purdomii*, whereas under N fertilization, *P. purdomii* showed competitive advantage over *S. rehderiana*.

Guo et al. (2017) have found that *L. kaempferi* has a significantly higher leaf N content variation under intraspecific interaction than under interspecific interaction, and N fertilization significantly decreases the leaf N content variation of *L. kaempferi* under both interaction patterns. In the present study, under P fertilization, the N concentration variation of *A. fabri* leaves was higher under interspecific interaction than under intraspecific interaction, but *P. brachytyla* showed a contrasting trend (Fig. 3b). In addition, the P concentration variation of *A. fabri* roots was higher under interspecific interaction than under intraspecific interaction, but *P. brachytyla* showed no difference between the two interaction patterns under P fertilization (Fig. 3c). Previous studies have demonstrated that NSCs, being important photosynthetic products, may have greater or lower contents than required for plant functions (growth, respiration, etc.), resulting in temporal variations of NSCs in plants (Smith and Stitt, 2007; Crone et al., 2009; Martínez-Vilalta et al., 2016). For instance, Landhäusser and

Lieffers (2003) found that the NSC content of *Populus tremuloides* has seasonal variation and the NSC content of roots decreases with the growth. In this study, under P fertilization, the soluble sugar concentration variation of *A. fabri* shoots and roots was higher under interspecific interaction than under intraspecific interaction (Fig. 3d). Concentration variations in starch and NSC had similar trends, and P fertilization increased their variation in the roots of both conifers. We concluded that there is temporal variation in resource (C, N, P and NSC) concentrations in *A. fabri* and *P. brachytyla*, and that is affected by P fertilization.

4.3. Resource storage and plant-plant interactions

Storage carbon and nutrients can provide a buffer when respiratory growth or other physiological demands are not synchronized with photosynthesis, thus affecting plant growth and performance (Iwasa and kubo, 1997; Sakai et al., 1997). Pellicer et al. (2000) have reported that the N storage of *Larix × eurolepis* significantly affects root growth. Different studies have implied that stored resources are closely related to plant growth and performance (Uhde-Stone et al., 2003; Jordan et al., 2014). In line with these studies, we found that, at the whole plant level under P fertilization, C, N, P, soluble sugar, starch and NSC contents of *A. fabri* were significantly higher under interspecific interaction than under intraspecific interaction, but *P. brachytyla* showed no difference between the two interaction patterns, except for the N content (Table S3). On the other hand, neighbors can affect nutrient and NSC storages, thus influencing

plant-plant interactions under different environments (Liu et al., 2004; Duan et al., 2014; Guo et al., 2017). For example, Robinson et al. (1999) have reported that the greater localized root proliferation of *Lolium perenne* was related to its greater N capture compared to neighbouring *Poa pratensis*, providing the former species with a competitive advantage. Consistently with these results, we found that the C, N, P, starch and NSC accumulation ratios of *A. fabri* were significantly higher compared with *P. brachytyla* under interspecific interaction and P fertilization conditions (Figs. 4, 5). Thus, the benefiting species (*A. fabri*) showed higher element (C, N and P) and NSC accumulation ratios from 2015 to 2016, which resulted in a better competitiveness in *A. fabri* compared to *P. brachytyla* under interspecific interaction and P fertilization.

5. Conclusions

In the present study, we found that P fertilization altered plant-plant interactions in *A. fabri* and *P. brachytyla*, and increased the biomass accumulation of both coniferous species. *A. fabri* was the benefiting species and had a higher biomass and resource accumulation under interspecific interaction and P fertilization from 2015 to 2016. In addition, the between-years variation of C, N, P and NSC was affected by P fertilization in both species. Our results demonstrated that there are interactive effects between environmental conditions (e.g. P fertilization) and plant-plant interactions (among years), which are closely related to resource accumulation.

19

Author contributions Lei Yu had the main responsibility for data collection, analysis and writing, Mengya Song and Zhichao Xia had a significant contribution to data analysis, Helena Korpelainen had a significant contribution to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

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

References

Adams, H.D., Germino, M.J., Breshears, D.D., Barron-Gafford, G.A., Guardiola-Claramonte, M., Zou, C.B., Huxman, T.E., 2013. Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phytol. 197, 1142-1151.

Andersen, M.K., Hauggaard-Nielsen, H., Weinei, J., Jensen, E.S., 2007. Competitive dynamics in two- and three-component intercrops. J. Appl. Ecol. 44, 545-551.

Barbaroux, C., Bréda, N., Dufrêne, E., 2003. Distribution of above-ground and below-ground carbohydrate reserves in adult trees of two contrasting broad-leaved species (*Quercus petraea* and *Fagus sylvatica*). New Phytol. 157, 605-615.

Bertness, M.D., Callaway, R., 1994. Positive interactions in communities. Trends Ecol. Evol. 9, 191-193.

Biswas, S.R., Wagner, H.H., 2014. A temporal dimension to the stress gradient hypothesis for intraspecific interactions. Oikos 123, 1323-1330.

Boer, H.C., Deru, J.G.C., Hoekstra, N.J., Eekeren, N., 2016. Strategic timing of nitrogen fertilization to increase root biomass and nitrogen-use efficiency of *Lolium perenne* L. Plant Soil 407, 81-90.

Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78, 1958-1965.

Chen, F.S., Niklas, K.J., Liu, Y., Feng, X.M., Wan, S.Z., Wang, H., 2015. Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fir leaves and twigs differing in age. Tree Physiol. 35, 1106-1117.

Choler, P., Michalet, R., Callaway, R.M., 2001. Facilitation and competition on gradients in alpine plant communities. Ecology 82, 3295-3308.

Crone, E.E., 2016. Contrasting effects of spatial heterogeneity and environmental stochasticity on population dynamics of a perennial wildflower. J. Ecol. 104, 281-291.

Crone, E.E., Miller, E., Sala, A., 2009. How do plants know when other plants are flowering? Resource depletion, pollen limitation and mast-seeding in a perennial wildflower. Ecol. Lett. 12, 1119-1126.

Díaz, S., Cabido, M., 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16, 646-655.

Dickman, L.T., McDowell, N.G., Sevanto, S., Pangle, R.E., Pockman, W.T., 2015. Carbohydrate dynamics and mortality in a piñon-juniper woodland under three future precipitation scenarios. Plant Cell Environ. 38, 729-739.

Duan, B.L., Dong, T.F., Zhang, X.L., Zhang, Y.B., Chen, J., 2014. Ecophysiological responses of two dominant subalpine tree species *Betula albo-sinensis* and *Abies faxoniana* to intra- and interspecific competition under elevated temperature. For. Ecol. Manage. 323, 20-27.

Elser, J.J., Bracken, M., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H. et al., 2007. Global analysis of nitrogen and phosphorus limitation of primary

producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10, 1135-1142.

Fajardo, A., McIntire, E.J.B., 2011. Under strong niche overlap conspecifics do not compete but help each other to survive: Facilitation at the intraspecific level. J. Ecol. 99, 642-650.

Gamfeldt, L., Snäll, T., Bagchi, R., et al., 2013. Higher levels of multiple ecosystem services are found in forests with more tree species. Nat. Commun. 4, 1340.

Goldenheim, W.M., Irving, A.D., Bertness, M.D., 2008. Switching from negative to positive density-dependence among populations of a cobble beach plant. Oecologia 158, 473-483.

Grace, J.B., 1995. On the measurement of plant competition intensity. Ecology 76, 305-308.

Guo, J.Y., Yang, Y., Wang, G.X., Yang, L.D., Sun, X.Y., 2010. Ecophysiological responses of *Abies fabri* seedlings to drought stress and nitrogen supply. Physiol. Plant. 139, 335-347.

Guo, Q.X., Zhang, Y.B., Wang, D.L., Zhang, J.X., Korpelainen, H., Li, C.Y., 2017. Influence of soil qualities on intra- and interspecific competition dynamics of *Larix kaempferi* and *L. olgensis*. Environ. Exp. Bot. 135, 96-105.

Guo, Q.X., Yan, L.J., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2019. Plant-plant interactions and N fertilization shape soil bacterial and fungal communities. Soil Biol. Biochem. 128, 127-138.

Hötscher, M., Hay, M.J.M., 1997. Genotypic differences in physiological integration,

morphological plasticity and utilization of phosphorus induced by variation in phosphate supply in *Trifolium repens*. J. Ecol. 85, 341-350.

Iwasa, Y., Kubo, T., 1997. Optimal size of storage for recovery after unpredictable disturbances. Evol. Ecol. 11, 41-65.

Jiang, Y.L., Song, M.Y., Zhang, S., Cai, Z.Q., Lei, Y.B., 2018. Unravelling community assemblages through multi-element stoichiometry in plant leaves and roots across primary successional stages in a glacier retreat area. Plant Soil 428, 291-305.

Jonard, M., Furst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potocic, N., et al., 2015. Tree mineral nutrition is deteriorating in Europe. Global Change Biol. 21, 418-430.

Jordan, M.O., Vercambre, G., Gomez, L., Pagès, L., 2014. The early spring N uptake of young peach trees (*Prunus persica*) is affected by past and current fertilizations and levels of C and N stores. Tree Physiol. 34, 61-72.

Kikvidze, Z., Khetsuriani, L., Kikodze, D., Callaway, R.M., 2006. Seasonal shifts in competition and facilitation in subalpine plant communities of the central Caucasus. J. Veg. Sci. 17, 77-82.

Lambers, H., Brundrett, M.C., Raven, J.A., Hopper, S.D., 2011. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant Soil 348, 7-27.

Landhäusser, S.M., Lieffers, V.J., 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees 17, 471-476.

Lei, Y.B., Zhou, J., Xiao, H.F., Duan, B.L., Wu, Y.H., Korpelainen, H., Li, C.Y., 2015. Soil nematode assemblages as bioindicators of primary succession along a 120-year-old chronosequence on the Hailuogou Glacier forefield, SW China. Soil Biol. Biochem. 88, 362-371.

Liu, N., Ren, H., Yuan, S.F., Guo, Q.F., Yang, L., 2013. Testing the stress-gradient hypothesis during the restoration of tropical degraded land using the shrub *Rhodomyrtus tomentosa* as a nurse plant. Rest. Ecol. 21, 578-584.

Liu, Q., 2002. Ecological Research on Subalpine Coniferous Forests in China. Sichuan University Press, Chengdu. (in Chinese).

Liu, X.P., Kozovits, A.R., Grams, T.E.E., Blaschke, H., Rennenberg, H., Matyssek, R., 2004. Competition modifies effects of enhanced ozone/carbon dioxide concentrations on carbohydrate and biomass accumulation in juvenile Norway spruce and European beech. Tree Physiol. 24, 1045-1055.

Luo, J., Chen, Y.C., Wu, Y.H., Shi, P.L., She, J., Zhou, P., 2012. Temporal-spatial variation and controls of soil respiration in different primary succession stages on glacier forehead in Gongga Mountain, China. PloS One 7, e42354.

Maestre, F.T., Callaway, R.M., Valladares, F., Lortie, C.J., 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J. Ecol. 97, 199-205.

Maestre, F.T., Valladares, F., Reynolds, J.F., 2005. Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J. Ecol. 93, 748-757.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., Piper, F.I., Lloret, F., 2016. Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. Ecol. Monogr. 86, 495-516.

Michalet, R., 2006. Is facilitation in arid environments the result of direct or complex interaction? New Phytol. 169, 3-6.

Miller, A.E., Bowman, W.D., Suding, K.N., 2007. Plant uptake of inorganic and organic nitrogen: neighbor identity matters. Ecology 88, 1832-1840.

Mitchell, A.K., 1998. Acclimation of Pacific yew (*Taxus brevifolia*) foliage to sun and shade. Tree Physiol. 18, 749-757.

Müller, B., Bartelheimer, M., 2013. Interspecific competition in Arabidopsis thaliana: root hairs are important for competitive effect, but not for competitive response. Plant Soil 371, 167-177.

Nelson, D., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. Methods of soil analysis. Part 2. Chemical and microbiological properties pp. 539-579.

Niinemets, Ü., 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For. Ecol. Manage. 260, 1623-1639.

O'Brien, M.J., Leuzinger, S., Philipson, C.D., Tay, J., Hector, A., 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. Nat. Clim. Change 4, 710-714.

Pellicer, V., Guehl, J.M., Daudet, F.A., Cazet, M., Riviere, L.M., Maillard, P., 2000.

Carbon and nitrogen mobilization in *Larix* × *eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labeling: relationships with rooting. Tree Physiol. 20, 807-814.

Pierik, R., Mommer, L., Voesenek, L.A., 2013. Molecular mechanisms of plant competition: neighbour detection and response strategies. Funct. Ecol. 27, 841-853.

Raynaud, X., Jaillard, B., Leadley, P.W., 2008. Plants may alter competition by modifying nutrient bioavailability in rhizosphere: a modeling approach. Amer. Natural. 171, 44-58.

Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proc. Natl. Acad. Sci. USA. 101, 11001-11006.

Robinson, D., Hodge, A., Griffiths, B.S., Fitter, A.H., 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proc. Royal Soc. London B - Biol. Sci. 226, 431-435.

Sakai, A., Sakai, S., Akiyama, F., 1997. Do sprouting tree species on erosion-prone sites carry large reserves of resources? Ann. Bot. 79, 625-630.

Sala, A., Woodruff, D.R., Meinzer, F.C., 2012. Carbon dynamics in trees: feast or famine? Tree Physiol. 32, 764-775.

Secchi, F., Zwieniecki, M.A., 2011. Sensing embolism in xylem vessels: the role of sucrose as a trigger for refilling. Plant Cell Environ. 34, 514-524.

Siddique, I., Vieira, I.C.G., Schmidt, S., Lamb, D., Carvalho, C.J.R., Figueiredo, R.O., Blomberg, S., Davidson, E.A., 2010. Nitrogen and phosphorus additions

negatively affect tree species diversity in tropical forest regrowth trajectories. Ecology 91, 2121-2131.

Smith, A.M., Stitt, M., 2007. Coordination of carbon supply and plant growth. Plant Cell Environ. 30, 1126-1149.

Soliveres, S., DeSoto, L., Maestre, F.T., Olano, J.M., 2010. Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. Perspect. Plant Ecol. 12, 227-234.

Song, M.Y., Yu, L., Jiang, Y.L., Lei, Y.B., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2017. Nitrogen-controlled intra- and interspecific competition between *Populus purdomii* and *Salix rehderiana* drive primary succession in the Gongga Mountain glacier retreat area. Tree Physiol. 37, 799-814.

Song, M.Y., Yu, L., Jiang, Y.L., Korpelainen, H., Li, C.Y., 2019. Increasing soil age drives shifts in plant-plant interactions from positive to negative and affects primary succession dynamics in a subalpine glacier forefield. Geoderma 353, 435-448.

Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, USA.

Tilman, D., 1987. On the meaning of competition and the mechanisms of competitive superiority. Funct. Ecol. 1, 304-315.

Tilman, D., 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, USA.

Trinder, C.J., Brooker, R.W., Davidson, H., Robinson, D., 2012. Dynamic trajectories

of growth and nitrogen capture by competing plants. New Phytol. 193, 948-958.

Trinder, C.J., Brooker, R.W., Robinson, D., 2013. Plant ecology's guilty little secret: understanding the dynamics of plant competition. Funct. Ecol. 27, 918-929.

Uhde-Stone, C., Gilbert, G., Johnson, J.M.F., Litjens, R., Zinn, K.E., Temple, S.J., Vance, C.P., Allan, D.L., 2003. Acclimation of white lupin to phosphorus deficiency involves enhanced expression of genes related to organic acid metabolism. Plant Soil 248, 99-116.

Valladares, F., Laanisto, L., Niinemets, Ü., Zavala, M.A., 2016. Shedding light on the shade: ecological perspectives on understory plant life. Plant Ecol. Divers. 9, 237-251.

Valladares, F., Niinemets, Ü., 2008. Shade tolerance, a key plant feature of complex nature and consequences. Annu. Rev. Ecol. Evol. S39, 237-257.

Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157, 423-447.

Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol. Appl. 20, 5-15.

Walter, A., Christ, M.M., Barron-gafford, G.A., Grieve, K.A., Murthy, R., Rascher, U., 2005. The effect of elevated CO_2 on diel leaf growth cycle, leaf carbohydrate content and canopy growth performance of *Populus deltoides*. Global Change Biol. 11, 1207-1219.

Weigelt, A., Jolliffe, P., 2003. Indices of plant competition. J. Ecol. 91, 707-720.

Wiley, E., Hoch, G., Landhäusser, S.M., 2017. Dying piece by piece: carbohydrate dynamics in aspen (*Populus tremuloides*) seedlings under severe carbon stress. J. Exp. Bot. 68, 5221-5232.

Wiley, E., Huepenbecker, S., Casper, B.B., Helliker, B.R., 2013. The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favor of storage? Tree Physiol. 33, 1216-1228.

Xia, Z.C., He, Y., Yu, L., Lv, R.B., Korpelainen, H., Li, C.Y., 2019. Sex-specific strategies of phosphorus acquisition in *Populus cathayana* as affected by soil P availability and distribution. New Phytol. doi.org/10.1111/nph.16170 (online).

Yamawo, A., 2015. Relatedness of neighboring plants alters the expression of indirect defense traits in an extrafloral nectary-bearing plant. Evol. Biol. 42, 12-19.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508-514.

Yu, L., Song, M.Y., Lei, Y.B., Duan, B.L., Berninger, F., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2017. Effects of phosphorus availability on later stages of primary succession in Gongga Mountain glacier retreat area. Environ. Exp. Bot. 141, 103-112.

Yu, L., Song, M.Y., Lei, Y.B., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2019a. Effects of competition and phosphorus fertilization on leaf and root traits of late-successional conifers *Abies fabri* and *Picea brachytyla*. Environ. Exp. Bot. 162, 14-24.

Yu, L., Song, M.Y., Xia, Z.C., Korpelainen, H., Niinemets, Ü., Li, C.Y., 2019b. Elevated temperature differently affects growth, photosynthetic capacity, nutrient absorption and leaf ultrastructure of *Abies faxoniana* and *Picea purpurea* under intra- and interspecific competition. Tree Physiol. 39, 1342-1357.

Figure legends

Fig. 1. Biomass accumulation and allocation in *A. fabri* and *P. brachytyla* under different interaction patterns and P fertilization levels: (a) leaf dry matter accumulation, (b) stem dry matter accumulation, (c) root dry matter accumulation, (d) total dry matter accumulation, (e) fine root dry matter accumulation, (f) root/shoot ratio. Each value is the mean $\pm$ SE (n = 5). Different letters indicate significant treatment effects on *A. fabri* and *P. brachytyla* according to Tukey's HSD test at a significance level of $P < 0.05$. Three-way analyses of variance (ANOVA) were applied to evaluate the effects of different factors and their interactions. S, effect of

species; I, effect of plant interactions; P, effect of P fertilization; S × I, interaction effects of species × plant interactions; S × P, interaction effects of species × P fertilization; I × P, interaction effects of plant interactions × P fertilization; S × I × P, interaction effects of species × plant interactions × P fertilization. The black and white bars denote *A. fabri* and *P. brachytyla*, respectively. Mon, intraspecific interaction pattern; Mix, interspecific interaction pattern; Mon+P, intraspecific interaction under P fertilization; Mix+P, interspecific interaction under P fertilization.

Fig. 2. Relative competitive intensities (RCIs) of *A. fabri* and *P. brachytyla* in (a) 2015 and (b) 2016. The black and white bars denote *A. fabri* and *P. brachytyla*, respectively. Different lowercase letters indicate significant differences between treatments according to Tukey's HSD test at a significance level of $P < 0.05$.

Fig. 3. Concentration variation in C, N, P and non-structural carbohydrates (NSC) of *A. fabri* and *P. brachytyla* under different interaction patterns and P fertilization levels from 2015 to 2016. (a), (b) and (c) represent C, N and P concentration variation, respectively; (d), (e) and (f) represent soluble sugar, starch and NSC concentration variation, respectively. Each value is the mean ± SE ($n = 5$). The bars with and without oblique lines denote *P. brachytyla* and *A. fabri*, respectively. The white, grey and black portions denote root, stem and leaf, respectively. Mon, intraspecific interaction pattern; Mix, interspecific interaction pattern; Mon+P, intraspecific interaction under P fertilization; Mix+P, interspecific interaction under P fertilization.

Fig. 4. C, N and P content accumulation ratios in leaves, stems and roots, and in the whole plant of *A. fabri* and *P. brachytyla* under different interaction patterns and P fertilization levels from 2015 to 2016. (a), (b) and (c) represent C, N and P content accumulation ratios of leaves, stems and roots, respectively; (d), (e) and (f) represent C, N and P content accumulation ratios in the whole plant, respectively. Each value is the mean ± SE ($n = 5$). The bars with and without oblique lines denote *P. brachytyla* and *A. fabri*, respectively. The black and white bars indicate *A. fabri* and *P. brachytyla*, respectively. Mon, intraspecific interaction pattern; Mix, interspecific interaction pattern; Mon+P, intraspecific interaction under P fertilization; Mix+P, interspecific interaction under P fertilization.
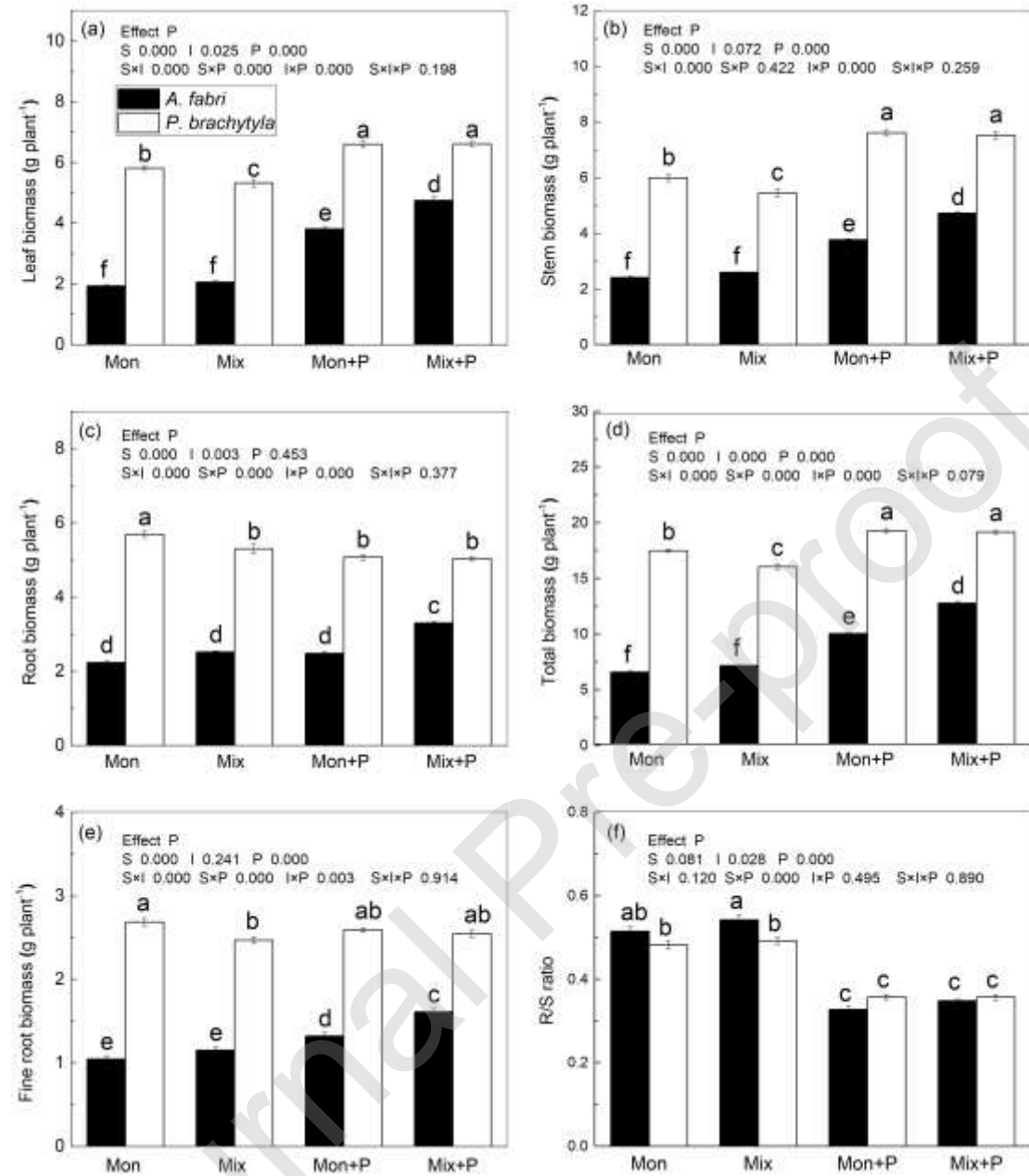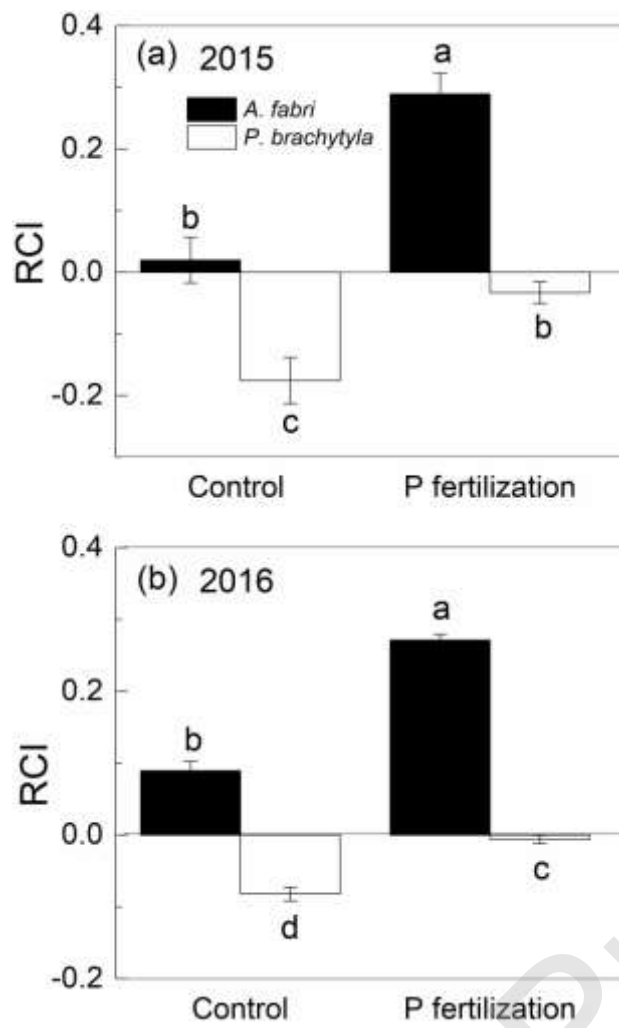
Fig. 5. Non-structural carbohydrate (NSC) content accumulation ratios in leaves, stems and roots, and in the whole plant of *A. fabri* and *P. brachytyla* under different interaction patterns and P fertilization levels from 2015 to 2016. (a), (b) and (c) represent soluble sugar, starch and NSC content accumulation ratios of leaves, stems and roots, respectively; (d), (e) and (f) represent soluble sugar, starch and NSC content accumulation ratios in the whole plant, respectively. Each value is the mean ± SE ($n = 5$). The bars with and without oblique lines denote *P. brachytyla* and *A. fabri*, respectively. The black and white bars indicate *A. fabri* and *P. brachytyla*, respectively. Mon, intraspecific interaction pattern; Mix, interspecific interaction pattern; Mon+P, intraspecific interaction under P fertilization; Mix+P, interspecific interaction under P
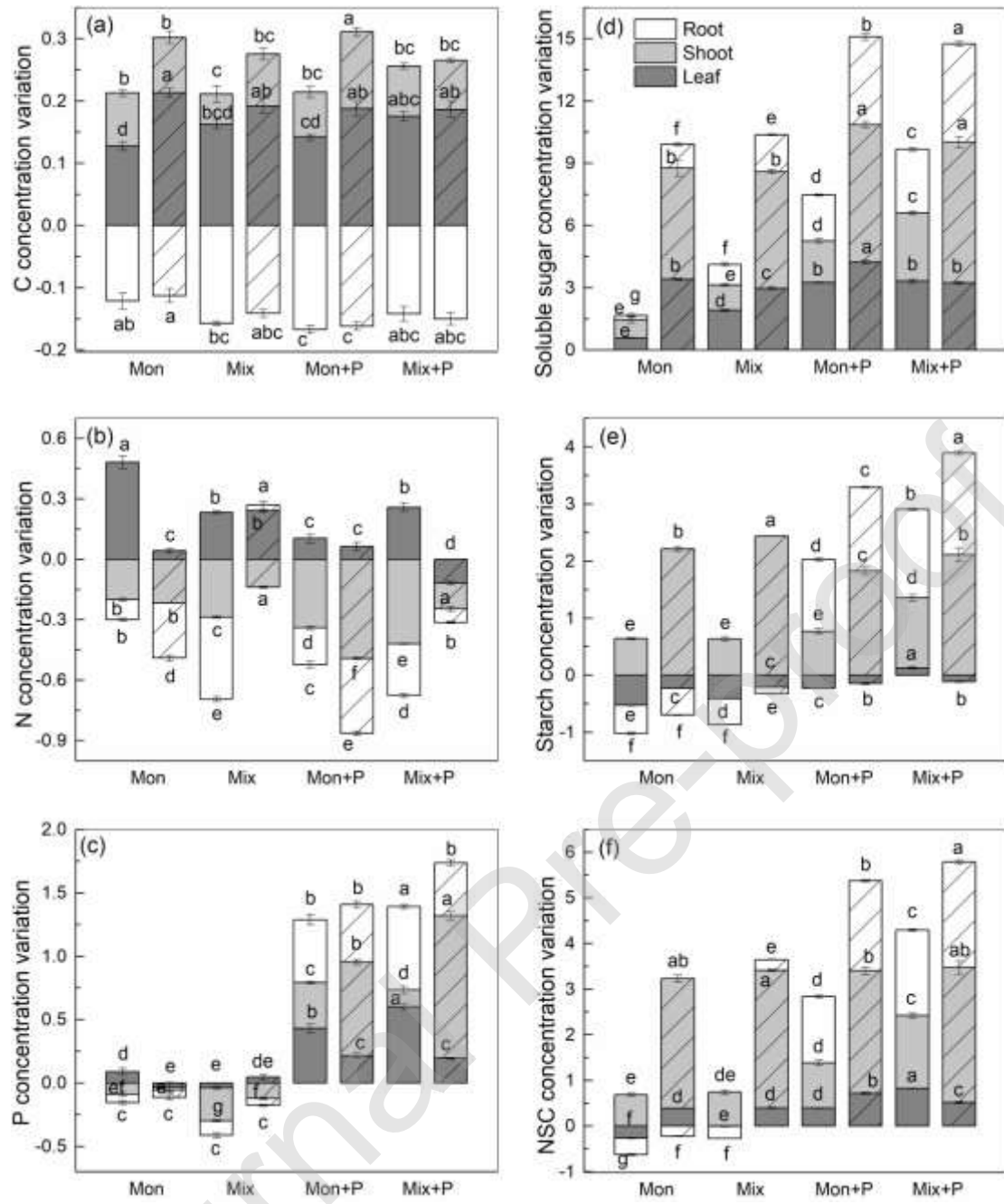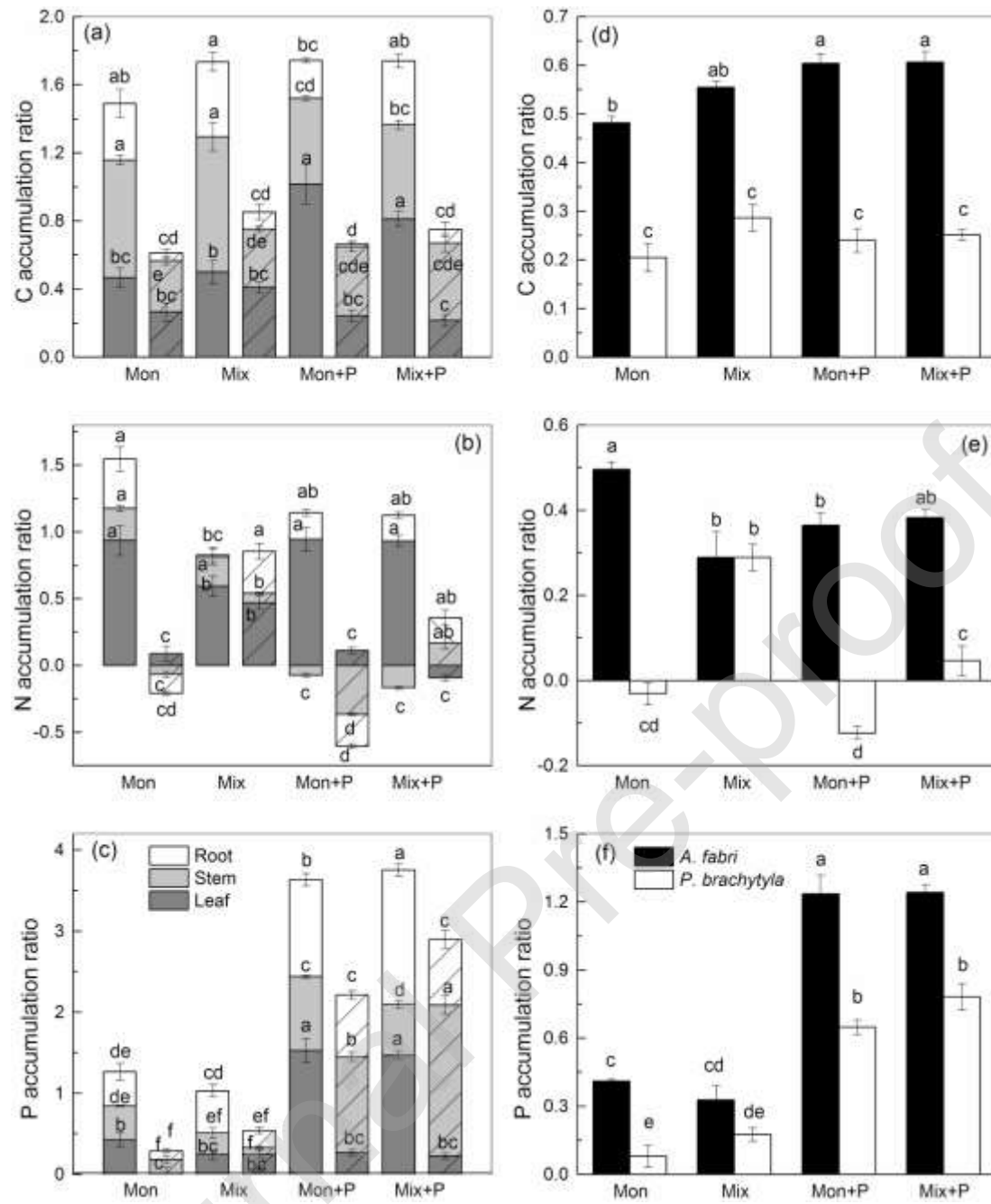
fertilization.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5