



Effects of defoliation modalities on plant growth, leaf traits, and carbohydrate allocation in *Amorpha fruticosa* L. and *Robinia pseudoacacia* L. seedlings

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Abstract

• **Key message** Defoliation modality significantly affected leaf traits and carbon allocation especially in the early recovery period of two Leguminosae woody species. *Robinia pseudoacacia* L. recovered more quickly following defoliation than *Amorpha fruticosa* L.

• **Context** Defoliation resulting from anthropogenic disturbance, insects, and grazing animals affects plant growth and vegetation dynamics. *Robinia pseudoacacia* L. and *Amorpha fruticosa* L. with different susceptibility to biotic attack are commonly used for vegetation restoration in North China. However, plant responses to defoliation were not fully understood in the two Leguminosae woody species.

• **Aims** We selected *R. pseudoacacia* and *A. fruticosa* to determine the effects of defoliation on individual plant growth, leaf traits, and carbohydrate allocation and compared the two species in terms of recovery strategies following defoliation for vegetation restoration.

• **Methods** In a greenhouse experiment, *A. fruticosa* and *R. pseudoacacia* seedlings were subjected to three defoliation treatments: defoliation of the top down (TD) or bottom up (BU) 50% of the crown, and a control without defoliation (CK). The physiological and growth traits of seedlings were determined at 1, 3, 14, 30, and 60 days following defoliation.

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- **Results** Plant growth in both species recovered after 2 months whatever defoliation modalities. Carbon allocation in both species was greatly affected by defoliation modalities during the early recovery period but had been fully recovered after 60 days of treatment. In the end of the experiment, defoliation modalities still had obvious impacts on leaf characteristics, especially in chlorophyll concentration and specific leaf area. *R. pseudoacacia* exhibited more evident compensatory mechanisms than *A. fruticosa* following defoliation.
- **Conclusion** Defoliation modality treatments significantly affect most leaf traits and carbon allocation, especially in the early recovery period of both species. Considering the faster recovery ability of *R. pseudoacacia* than *A. fruticosa* after defoliation, *R. pseudoacacia* seedlings may be more suitable for vegetation restoration in North China.

Keywords Leaf damage · Leaf morphology · Photosynthetic rate · Recovery stage

1 Introduction

Plants often suffer from leaf damage due to anthropogenic disturbance, insects, and grazing animals, especially in the context of increasing anthropogenic activities and changing climate conditions (Eyles et al. 2013a, b). As the climate changes, warmer temperatures and drier conditions may become more widespread and occur more frequently. These changes would increase the fitness and abundance of some forest pests (Dale and Frank 2017; Root et al. 2003), affecting plant growth and vegetation dynamics (Kuusmanen et al. 2018; Leather et al. 2010; Yang et al. 2005). Studying the influence of biological disturbance on young trees and the response of individual plants may provide a theoretical basis for selecting and managing species for vegetation restoration. Defoliation experiments are commonly used to simulate biological disturbance on plant species (Wiley et al. 2017; Wyka et al. 2017). Leaf removal decreases leaf photosynthetic area and affects root vigor, plant nutrient concentrations, secondary metabolite, and non-structural carbohydrate (NSC) content (Eyles et al. 2013a, b; Karolewski et al. 2010; Wiley et al. 2013). Thus, the recovery process and the allocation and partitioning of carbohydrates in individual plants following defoliation are important research topics in the study of plant ecology for developing improved management strategies (Barry et al. 2012; Jacquet et al. 2014; Quentin et al. 2010; Wiley et al. 2017).

Leaves are the main organ used for production of organic matter and are the main source of carbohydrates for a plant (Liu et al. 2017). Stems and roots are long-term storage organs and represent important carbon sinks (Lourens et al. 2010). The accumulation of carbohydrates in the leaves depends on the balance between carbon sources and sinks and the transport of carbohydrates in the phloem (Asao and Ryan 2015; Galiano et al. 2011). Sources provide assimilates through phloem transport to sinks that compete with each other (Dewar 1993). Carbohydrates are the main product of plant photosynthesis and are divided into structural carbohydrates and NSCs (Asao and Ryan 2015; Piper et al. 2017). NSCs are commonly used to assess the balance between carbon sources

and sinks in plants (Bansal and Germino 2009). Carbohydrate concentration and its transport are indicators of the ecological strategies of trees, and they are important parameters in the development of tree growth and forest carbon cycle models (Chuste et al. 2019; Hinman and Fridley 2018; Richardson et al. 2013).

Generally, species, even in the same genus, respond differently after defoliation, and the time and extent of recovery vary. The responses of *Eucalyptus globulus* and *Eucalyptus nitens* to defoliation were similar, and growth (height and stem diameter) was found to have significantly reduced after defoliation (Barry and Pinkard 2013). However, the responses of white oak (*Quercus alba*) and black oak (*Quercus velutina*) to defoliation were different. White oak significantly changed in above-ground biomass, while black oak had a more pronounced chemical change in foliar chemistry after defoliation (Rieske and Dillaway 2008). After full defoliation, *Quercus velutina* seedlings had lower starch levels than those in the control in the short term but could recover to control levels in the longer term (Wiley et al. 2013). In another evergreen oak species, *Quercus ilex*, carbohydrate reserves were also found to decrease in the short term after defoliation (Schmid et al. 2017). However, in the deciduous *Quercus petraea*, NSC tissue concentrations were not found to decrease either in the short term or in the longer time after defoliation (Schmid et al. 2017). Deciduous larches and evergreen pines were also found to respond differently to defoliation. Defoliation reduced *Larix decidua* height growth but had no effect on radial growth, while *Pinus resinosa* stem radial growth decreased immediately, but height did not decrease until the following year (Krause and Raffa 1996). Consequently, these findings suggest that after leaf area is severely decreased, the recovery ability is species specific.

The extent and timing of recovery of individual plants after leaf removal may also be related to leaf defoliation modalities. Leaf position within canopy influences photosynthesis (Nautiyal et al. 1999). Defoliation pattern had no effect on the incident light reaching the upper or mid-crown. Thus, defoliation of the upper crown is likely to increase the penetration of light throughout the crown (Quentin et al. 2011),

which may increase the productivity of the lower crown. In addition, defoliation of the upper crown would decrease branch senescence in the lower crown and increase the rate of leaf area production in the mid-crown as well as mean specific leaf area (SLA) (Quentin et al. 2011). The upper leaves are functionally different from the lower leaves, which have higher photosynthetic activities than the upper leaves during the vigorous growth period (Wilson and Cooper 1969). Leaves will transform from carbon sinks to carbon sources with growth development. However, the effect of lower crown defoliation on plant growth and physiological traits has rarely been reported. Therefore, irrespective of leaf senescence, the lower leaves are regarded as a mature carbon source at the seedling stage, while the upper leaves are regarded as a partially underdeveloped carbon pool. The defoliation of the lower leaves should have a greater impact on carbohydrate production; therefore, we suspect that the recovery pattern will be different based on leaf defoliation modalities. The photosynthetic ability of the remaining leaves will increase (Qiu et al. 2016) and plants rapidly produce new leaves (Korpita and Orians 2014) after defoliation. However, how to select the most economical strategy for rapid recovery under different defoliation modalities remains unclear.

In the present study, two leguminous woody species, *Robinia pseudoacacia* L. and *Amorpha fruticosa* L., were selected to study the effects of different defoliation modalities on individual plant growth, carbohydrate allocation, and leaf traits. *R. pseudoacacia* is the dominant species in the tree layer of forests in the warm temperate zone, while *A. fruticosa* is normally found in the shrub layer as an important companion species (Wang and Zhou 2000). Both species are commonly used for vegetation restoration in warm temperate regions, owing to their rapid growth rates (Dehaan et al. 2006; Guo et al. 2018). Although *R. pseudoacacia* is listed as an invasive species, it has not caused much harm and is widely used in the afforestation and vegetation restoration in warm temperate zones of China for several years (Cierjacks et al. 2013; Petrović et al. 2016; Weber et al. 2008). *R. pseudoacacia* has been planted since the 1980s in China for the purpose of vegetation restoration with approximately 8000 ha planted (Zhang and Xing 2009). However, the numbers of insect pests associated with *R. pseudoacacia* have greatly increased, such as *Cyclopetta siccifolia* and *Bagrada cruciferarum* (Sharma et al. 2008). These pests feed on new shoots and leaves of *R. pseudoacacia*. For *R. pseudoacacia*, *Phyllactinia moricola* is a fungus mainly harmful to leaves, mainly occurring on the back of mature leaves but also in the early stage of seedlings; the symptom starts from the lower leaves. However, *A. fruticosa* is not susceptible to diseases and insect pests. To our knowledge, no previous studies have reported the effects of defoliation modalities on *R. pseudoacacia* and *A. fruticosa*. Therefore, in the present study, three different defoliation treatments were set up in a greenhouse experiment

to measure plant growth, leaf traits, and carbohydrate allocation. We hypothesized that (i) defoliation reduces plant growth in terms of height and biomass at the end of the experiment, (ii) physiological compensatory mechanisms differ between the defoliation of top and bottom leaves, and (iii) *R. pseudoacacia* and *A. fruticosa* have different recovery strategies after defoliation.

2 Material and methods

2.1 Site

The study was conducted at Fanggan Research Station of Shandong University in Jinan, Shandong province, China (36° 26'N, 117° 27'E), which is in the warm temperate zone with mean annual precipitation of 700 ± 100 mm and an average temperature of 13 ± 1 °C. Seedlings were irrigated every second day, each time to saturation. The experiment was carried out in a greenhouse with a steel pipe frame covered by a plastic film. The greenhouse had a light transmittance of ~66%. During the growth period, the microclimate in the greenhouse was monitored with HOBO data loggers (U12-012, Onset, Bourne, MA, USA): mean air temperature of 29.6 °C (18.7–36.7 °C) in the daytime and 20.8 °C (10.2–27.5 °C) at night, mean relative humidity of 59.3% (28.2–97.8%) in the daytime and 93.6% (56.3–100%) at night.

2.2 Plant materials

Seeds of *R. pseudoacacia* and *A. fruticosa* were purchased from Qiluyuanyi Seed Company (Linyi, China) and were collected from their own garden in the early winter of 2016. The seeds were stored at 0–4 °C through the winter. In late April of 2017, after soaking in distilled water for 24 h, the seeds were allowed to germinate in a growth chamber. When most radicles reached 2 cm, healthy and uniform germinants were sown in plastic pots (25 × 21 cm, height × diameter; one plant per pot) with 7-kg mixed sandy loam and humus soil and allowed to grow for 3 months.

2.3 Experimental design

For each species, 3-month-old vigorous seedlings of similar size were selected and randomly assigned to treatments. The initial plant height of *A. fruticosa* was about 0.34 ± 0.01 m, and the initial plant height of *R. pseudoacacia* was about 0.75 ± 0.01 m. The three defoliation treatments were as follows: defoliation of top down 50% of the crown height (TD), defoliation of the bottom up 50% of the crown height (BU); and a control group without leaf removal (CK). The treatments were conducted from July 6 to September 4. There were 20 replicates in each treatment for each species. During the

experiment, seedlings were harvested at days 1 (July 7), 3 (July 9), 14 (July 20), 30 (August 5), and 60 (September 4). Four individuals of each species and treatment were randomly selected to measure in each sampling.

2.4 Growth measurements

Seedling height (H) and total biomass were recorded at each harvest. Four seedlings in each treatment were harvested at around noon (12:00–3:00 pm) and separated into roots, stems, and leaves. Then, the samples were oven-dried (30 min at 105 °C, followed by 72 h at 75 °C) and weighed. Total biomass (TB) was calculated as follows:

$$TB = RB + SB + LB$$

where TB is the total biomass, RB is the root biomass, SB is the stem biomass, and LB is the leaf biomass.

2.5 Leaf morphological and physiological traits

After 60 days of treatment, leaf morphological and physiological traits were measured at the beginning of September. For each species, 50 fully expanded and healthy reflusing leaves from the upper part of each seedling were scanned, and images were analyzed with the WinFOLIA Pro 2009a software (Regent Instruments, Inc., Quebec, QC, Canada) to determine leaf area. Leaf thickness was measured with an electronic digital micrometer and was averaged from 20 fresh leaves per seedling. After scanning, these leaves were oven-dried for 72 h at 75 °C and weighed. SLA was calculated as leaf area/leaf dry mass.

Three fully expanded and healthy leaves from the upper shoots (one leaf per seedling) in each treatment in both species were sampled to determine leaf chlorophyll concentration. After extraction by 95% ethanol (v/v), the concentration of leaf chlorophyll a and b and total chlorophyll concentration were determined using the spectrophotometric method according to Lichtenthaler and Wellburn (1983). Net photosynthetic rate (A) of seedlings was measured before each harvest at days 1 (July 7), 3 (July 9), 14 (July 20), 30 (August 5), and 60 (September 4). Net photosynthetic rate (A) was measured in situ with an infrared gas analysis system (Li-6800, Li-Cor, Lincoln, NE, USA). The measurements were conducted at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR supplied by an external LED light, which was high enough to obtain the maximum photosynthetic rate according to a pre-experiment. Gas-exchange characteristics were measured between 9:00 and 11:30 on sunny days. During the measurement, temperature, relative humidity, and CO₂ concentration inside the chamber were controlled at 28 °C, 50%, and 400 ppm, respectively. The measurements were taken both for remaining leaves and reflusing leaves.

Since the lower leaves of the TD and CK plants naturally fell after 30 days of experimental treatment, the net photosynthetic rate of the remaining leaves was not measured at day 60. Gas exchange in the remaining leaves was measured at days 1, 3, 14, and 30 for the lower crown (TD), upper crown (BU), and both upper and lower crowns (CK). The reflusing leaves were fully expanded at day 14 after defoliation, and the gas exchange of reflusing leaves was measured at days 14, 30, and 60 for the top of the upper crown in all treatments with four replicates for each measurement. For each species, four or eight fully expanded and healthy leaves from the top of the upper crown in BU (one leaf per seedling), top of the lower crown in TD (one leaf per seedling), and both top of the upper and lower crowns in CK (two leaves per seedling) were selected from each treatment.

2.6 Non-structural carbohydrate concentration and mass

The leaf samples were collected at midday (12:00–15:00 h). After biomass determination, dried samples were grounded with a ball mill to quantify NSC (defined as the sum of starch and soluble sugars) concentration in the leaves, stems, and roots. Soluble sugars were extracted twice from 20 mg of dried tissue in 5 ml of 80% (v/v) ethanol at 60 °C. The total soluble sugar was determined by using the anthrone colorimetric method at 620 nm by spectrophotometry (UV-9000S, Metash, Shanghai, China). Then, starch was measured after the solid residue of each sample had been extracted twice with boiling water and 9.2-mol L⁻¹ perchloric acid. The absorbance of samples was measured at 620 nm by spectrophotometry (UV-9000S, Metash, Shanghai, China) after an anthracenone-sulfuric acid reaction as previously described (Cao et al. 2017), and the concentrations of sugars and starch (measured as glucose equivalents) were calculated for dry mass (mg g⁻¹). NSC mass for a given organ was calculated as the product of an individual organ's biomass and NSC concentration (mg). The soluble sugar/starch ratio (SS/ST), ratio of leaf NSC mass increment (R_{LN}), ratio of stem NSC mass increment (R_{SN}), ratio of root NSC mass increment (R_{RN}), and the NSC source/sink ratio were calculated as follows:

$$\frac{SS}{ST} = \frac{\text{Soluble sugar concentration}}{\text{Starch concentration}}$$

$$R_{LN} = \frac{\Delta \text{Leaf NSC mass of TD (BU)}}{\Delta \text{Leaf NSC mass of CK}}$$

$$R_{SN} = \frac{\Delta \text{Stem NSC mass of TD (BU)}}{\Delta \text{Stem NSC mass of CK}}$$

$$R_{RN} = \frac{\Delta \text{Root NSC mass of TD (BU)}}{\Delta \text{Root NSC mass of CK}}$$

$$\text{NSC}_{\text{Sink}}^{\text{Source}} = \frac{\text{Leaf NSC mass}}{\text{Stem NSC mass} + \text{Root NSC mass}}$$

where Δ is the difference between NSC mass of TD (BU) at day 60 and that at day 1. Percentage mass represents the proportion of day 1 to day 60 NSC mass.

2.7 Statistical analysis

Two-way analysis of variance (ANOVA) was applied to detect differences between species and defoliation treatments for every parameter. Three-way ANOVA was applied to detect differences among species, time, and defoliation treatments for NSC allocation. All ANOVAs were followed by Duncan's multiple comparison tests at $\alpha = 0.05$ when significant differences were found. Before ANOVAs, data were checked for normality and homogeneity of variance. All of the statistical analyses were performed using the SPSS 23.0 software package (SPSS Inc., Chicago, IL, USA), and all figures were drawn using OriginPro 2016 (Originlab Co., Northampton, MA, USA).

3 Results

3.1 Plant growth

Generally, seedling height was not significantly different between defoliation treatments and CK in both species (Fig. 1). Considering seedlings biomass, the seedling total biomass in TD was significantly decreased at days 3–14, and the seedling total biomass in BU significantly decreased at day 14 in

A. fruticosa (Fig. 2a). The seedling leaf biomass, stem biomass, and root biomass in TD and BU significantly decreased at day 14 in *A. fruticosa* (Table 1). For *R. pseudoacacia*, the seedling total biomass and leaf biomass in TD significantly decreased at days 1–3, and the seedling total biomass in BU significantly decreased at day 3 (Table 1, Fig. 2b). In addition, the seedling stem biomass in TD and BU significantly decreased at day 3 compared with the CK treatment (Table 1). Especially at day 60, the root biomass increased significantly in the BU treatment compared with the CK treatment (Tables 1 and 2).

3.2 Leaf traits

There was no significant difference among the three defoliation treatments in *A. fruticosa* of the remaining leaves (Fig. 3a and b). In addition, the net photosynthetic rate in reflusing leaves was significantly higher in TD than in BU in *A. fruticosa* at day 30 (Fig. 4a). For *R. pseudoacacia*, TD and BU significantly increased the net photosynthetic rate of the remaining leaves at day 14 (Fig. 3c and d). Defoliation significantly increased the net photosynthesis of the reflusing leaves in TD at day 14 (Fig. 4b). After 30 days, defoliation treatments did not affect the net photosynthesis of the new leaves in *R. pseudoacacia*.

The total chlorophyll concentration and chlorophyll a/b of BU increased significantly compared with that of CK in *R. pseudoacacia* (Fig. 5b and d). The SLA of BU decreased significantly compared with that of CK both in *A. fruticosa* and *R. pseudoacacia* (Fig. 5e and f). However, different defoliation treatments did not significantly affect leaf thickness in both species (Fig. 5g and h).

Fig. 1 Seedling height of *Amorpha fruticosa* (a) and *Robinia pseudoacacia* (b) under different defoliation treatments. The values shown are mean \pm SE ($n = 4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

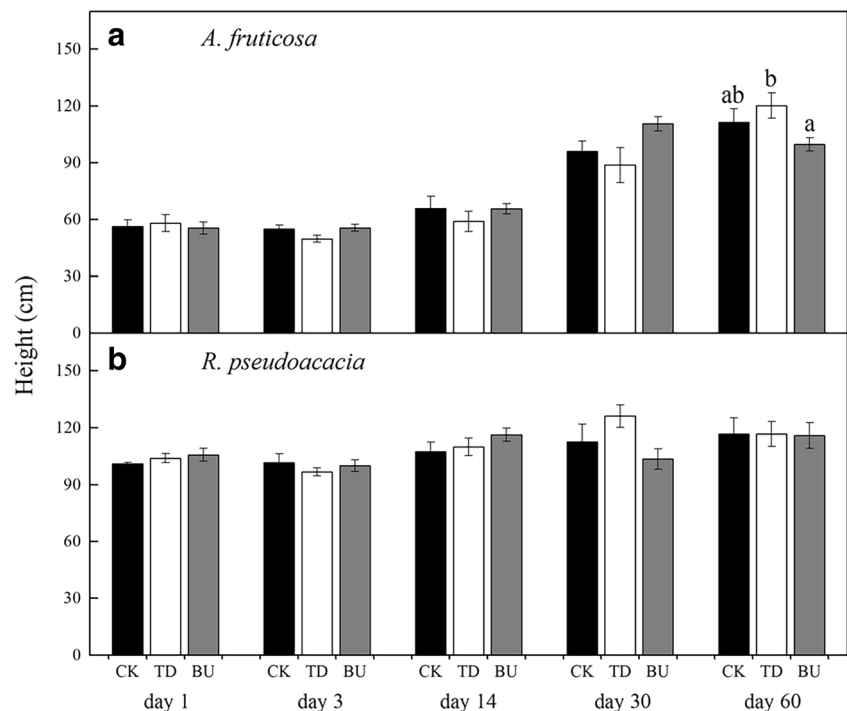
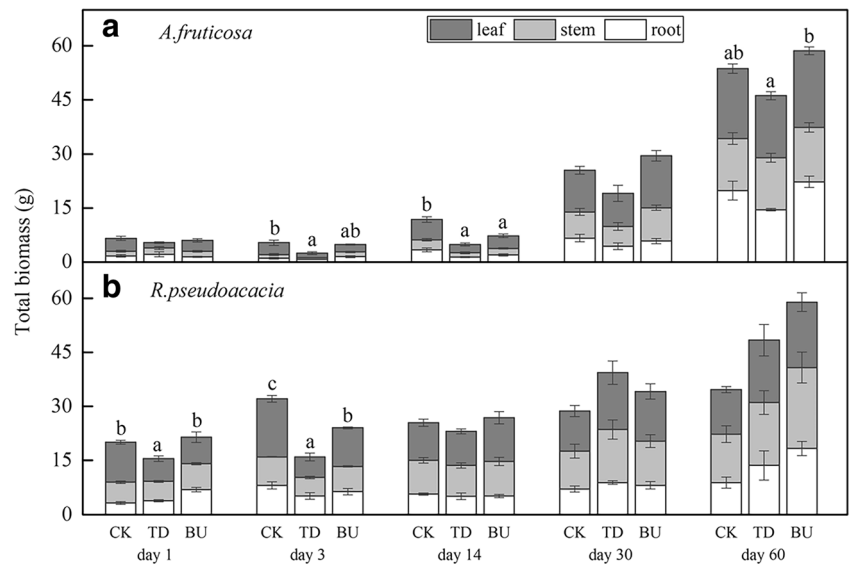


Fig. 2 Seedling total biomass of *Amorpha fruticosa* (a) and *Robinia pseudoacacia* (b) under different defoliation treatments. The values shown are mean ± SE (n = 4). Different letters indicate significant differences among different defoliation treatments (p < 0.05) with Duncan’s test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown



3.3 Soluble sugar/starch ratio and NSC concentration in different defoliation treatments

Seedling carbon allocation patterns were affected by species, time, and defoliation treatments (Table 3). The SS/ST reflects the NSC allocation pattern and can be helpful for understanding the carbohydrate-utilization strategies of plants. In this

experiment, temporal changes in SS/ST ratio and NSC concentration varied by organ (Figs. 6 and 7).

For *A. fruticosa*, the stem SS/ST ratio in the TD treatment significantly decreased from days 1 to 3, and the stem SS/ST ratio in the BU treatment significantly decreased at day 3 (Fig. 7c). The stem NSC concentration and soluble sugar concentration in TD and BU treatments significantly decreased at day

Table 1 Biomass (mean ± SE) of *Amorpha fruticosa* and *Robinia pseudoacacia* seedlings harvested at days 1 (July 7), 3 (July 9), 14 (July 20), 30 (August 5), and 60 (September 4). Different lowercase

letters indicate significant differences among different defoliation treatments (p < 0.05) with Duncan’s test

Organ	Date	Biomass by treatment (g)					
		<i>Amorpha fruticosa</i>			<i>Robinia pseudoacacia</i>		
		CK	TD	BU	CK	TD	BU
Leaf	Day 1	3.56 ± 0.56 ^b	1.50 ± 0.24 ^a	3.00 ± 0.44 ^b	11.02 ± 0.54 ^b	6.20 ± 0.77 ^a	7.35 ± 1.49 ^a
	Day 3	3.26 ± 0.73 ^{ab}	1.22 ± 0.40 ^a	2.01 ± 0.16 ^b	16.17 ± 0.93 ^c	5.63 ± 1.09 ^a	10.70 ± 0.24 ^b
	Day 14	5.60 ± 0.76 ^b	2.28 ± 0.41 ^a	3.48 ± 0.52 ^a	10.43 ± 0.97 ^a	9.48 ± 0.69 ^a	12.11 ± 1.69 ^a
	Day 30	11.57 ± 1.10 ^a	9.20 ± 2.23 ^a	14.45 ± 1.48 ^a	11.19 ± 1.55 ^a	15.81 ± 3.28 ^a	13.82 ± 2.15 ^a
	Day 60	19.33 ± 1.35 ^{ab}	17.16 ± 1.13 ^a	21.17 ± 1.10 ^b	12.36 ± 0.88 ^a	17.33 ± 4.38 ^a	18.15 ± 2.60 ^a
Stem	Day 1	1.30 ± 0.27 ^a	1.77 ± 0.42 ^a	1.56 ± 0.24 ^a	5.75 ± 0.36 ^a	5.39 ± 0.28 ^a	7.12 ± 0.31 ^b
	Day 3	1.05 ± 0.27 ^{ab}	0.47 ± 0.10 ^a	1.32 ± 0.19 ^b	7.83 ± 0.13 ^c	5.14 ± 0.32 ^b	6.91 ± 0.19 ^a
	Day 14	2.80 ± 0.37 ^b	1.21 ± 0.25 ^a	1.78 ± 0.20 ^a	9.29 ± 0.74 ^a	8.48 ± 0.75 ^a	9.51 ± 1.18 ^a
	Day 30	7.27 ± 0.96 ^{ab}	5.48 ± 1.09 ^a	9.25 ± 0.73 ^b	10.42 ± 1.93 ^a	14.62 ± 2.69 ^a	12.16 ± 1.77 ^a
	Day 60	14.48 ± 1.64 ^a	14.43 ± 1.22 ^a	15.13 ± 1.32 ^a	13.40 ± 2.31 ^a	17.44 ± 3.26 ^a	22.47 ± 4.27 ^a
Root	Day 1	1.71 ± 0.33 ^a	2.14 ± 0.67 ^a	1.46 ± 0.18 ^a	3.29 ± 0.37 ^a	3.88 ± 0.33 ^a	6.97 ± 0.64 ^b
	Day 3	1.06 ± 0.26 ^{ab}	0.76 ± 0.11 ^a	1.51 ± 0.27 ^b	8.13 ± 1.03 ^a	5.20 ± 0.95 ^a	6.43 ± 0.89 ^a
	Day 14	3.39 ± 0.57 ^b	1.37 ± 0.14 ^a	2.02 ± 0.31 ^a	5.73 ± 0.33 ^a	5.11 ± 0.93 ^a	5.21 ± 0.44 ^a
	Day 30	6.66 ± 1.03 ^a	4.41 ± 0.93 ^a	5.82 ± 0.76 ^a	7.11 ± 0.86 ^a	8.94 ± 0.51 ^a	8.16 ± 1.06 ^a
	Day 60	19.83 ± 2.64 ^{ab}	14.54 ± 0.37 ^a	22.28 ± 1.56 ^b	8.90 ± 1.53 ^a	13.62 ± 4.03 ^{ab}	18.32 ± 1.98 ^b

CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

n = 4

Table 2 Non-structural carbohydrate (NSC) mass (mean \pm SE) in *Amorpha fruticosa* and *Robinia pseudoacacia* seedlings was harvested at days 1 (July 7), 3 (July 9), 14 (July 20), 30 (August 5), and 60 (September 4). Different lowercase letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test

Organ	Date	NSC mass by treatment (g)					
		<i>Amorpha fruticosa</i>			<i>Robinia pseudoacacia</i>		
		CK	TD	BU	CK	TD	BU
Leaf	Day 1	0.32 \pm 0.06 ^a	0.14 \pm 0.03 ^a	0.28 \pm 0.06 ^a	1.42 \pm 0.08 ^b	0.63 \pm 0.13 ^a	0.91 \pm 0.20 ^{ab}
	Day 3	0.53 \pm 0.11 ^b	0.21 \pm 0.09 ^a	0.33 \pm 0.03 ^{ab}	1.17 \pm 0.18 ^{ab}	0.78 \pm 0.11 ^a	1.52 \pm 0.16 ^b
	Day 14	0.42 \pm 0.04 ^b	0.18 \pm 0.05 ^a	0.24 \pm 0.04 ^a	1.65 \pm 0.21 ^a	1.44 \pm 0.10 ^a	1.64 \pm 0.10 ^a
	Day 30	1.57 \pm 0.28 ^a	1.24 \pm 0.33 ^a	1.96 \pm 0.14 ^a	1.72 \pm 0.13 ^a	1.69 \pm 0.29 ^a	1.78 \pm 0.12 ^a
	Day 60	2.24 \pm 0.35 ^a	2.32 \pm 0.41 ^a	3.00 \pm 0.31 ^a	1.63 \pm 0.10 ^a	1.94 \pm 0.52 ^a	1.96 \pm 0.21 ^a
	R _{LN}	1.00	1.14	1.42	1.00	6.24	5.00
Stem	Day 1	0.09 \pm 0.03 ^a	0.08 \pm 0.02 ^a	0.07 \pm 0.11 ^a	0.47 \pm 0.02 ^a	0.28 \pm 0.04 ^a	0.47 \pm 0.10 ^a
	Day 3	0.08 \pm 0.04 ^a	0.01 \pm 0.00 ^a	0.04 \pm 0.01 ^a	0.81 \pm 0.06 ^b	0.37 \pm 0.02 ^a	0.22 \pm 0.05 ^a
	Day 14	0.09 \pm 0.01 ^c	0.03 \pm 0.00 ^a	0.05 \pm 0.009 ^b	0.76 \pm 0.17 ^a	0.62 \pm 0.14 ^a	0.67 \pm 0.08 ^a
	Day 30	0.23 \pm 0.04 ^a	0.25 \pm 0.05 ^a	0.40 \pm 0.10 ^a	0.70 \pm 0.10 ^a	0.80 \pm 0.21 ^a	0.65 \pm 0.16 ^a
	Day 60	0.53 \pm 0.12 ^a	0.51 \pm 0.06 ^a	0.63 \pm 0.11 ^a	0.74 \pm 0.16 ^a	1.03 \pm 0.30 ^a	1.10 \pm 0.28 ^a
	R _{SN}	1.00	0.98	1.27	1.00	2.78	2.33
Root	Day 1	0.20 \pm 0.04 ^a	0.16 \pm 0.08 ^a	0.14 \pm 0.04 ^a	0.17 \pm 0.02 ^a	0.21 \pm 0.04 ^a	0.33 \pm 0.08 ^a
	Day 3	0.08 \pm 0.02 ^a	0.03 \pm 0.01 ^a	0.11 \pm 0.04 ^a	0.80 \pm 0.11 ^b	0.456 \pm 0.142 ^{ab}	0.417 \pm 0.061 ^a
	Day 14	0.13 \pm 0.02 ^b	0.05 \pm 0.01 ^a	0.079 \pm 0.00 ^a	0.65 \pm 0.11 ^a	0.54 \pm 0.12 ^a	0.34 \pm 0.04 ^a
	Day 30	0.72 \pm 0.16 ^a	0.64 \pm 0.20 ^a	0.66 \pm 0.09 ^a	0.58 \pm 0.14 ^a	0.77 \pm 0.13 ^a	0.59 \pm 0.05 ^a
	Day 60	2.21 \pm 0.43 ^a	1.47 \pm 0.18 ^a	1.87 \pm 0.52 ^a	1.55 \pm 0.36 ^a	1.98 \pm 0.69 ^a	3.01 \pm 0.54 ^a
	R _{RN}	1.00	0.65	0.86	1.00	1.28	1.94
Total	Day 1	0.61 \pm 0.13 ^a	0.38 \pm 0.12 ^a	0.50 \pm 0.10 ^a	2.06 \pm 0.10 ^b	1.12 \pm 0.17 ^a	1.71 \pm 0.11 ^b
	Day 3	0.68 \pm 0.15 ^b	0.25 \pm 0.08 ^a	0.47 \pm 0.06 ^{ab}	2.77 \pm 0.32 ^b	1.61 \pm 0.26 ^a	2.17 \pm 0.26 ^{ab}
	Day 14	0.65 \pm 0.06 ^b	0.25 \pm 0.06 ^a	0.38 \pm 0.05 ^a	3.07 \pm 0.45 ^a	2.60 \pm 0.20 ^a	2.79 \pm 0.24 ^a
	Day 30	2.51 \pm 0.19 ^a	2.12 \pm 0.56 ^a	2.98 \pm 0.22 ^a	3.00 \pm 0.17 ^a	3.26 \pm 0.53 ^a	3.17 \pm 0.32 ^a
	Day 60	4.98 \pm 0.81 ^a	4.30 \pm 0.42 ^a	5.50 \pm 0.61 ^a	3.72 \pm 0.39 ^a	4.95 \pm 1.44 ^a	6.07 \pm 0.94 ^a
	Percentage mass		12%	9%	9%	55%	23%

Percentage mass represents the proportion of day 1 to day 60 NSC mass

R_{LN}, ratio of leaf NSC mass increment; R_{RN}, ratio of root NSC mass increment; R_{SN}, ratio of stem NSC mass increment; CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

$n = 4$

3 (Fig. 6c). The stem starch concentration and NSC concentration in the TD treatment of *A. fruticosa* significantly decreased at day 14 (Fig. 6c). The root NSC concentration and soluble sugar concentration in the TD treatment significantly decreased at day 1 (Fig. 6e). The starch concentration in roots was relatively stable, and there was no significant difference among defoliation treatments of starch concentration during the experiment period (Fig. 6e).

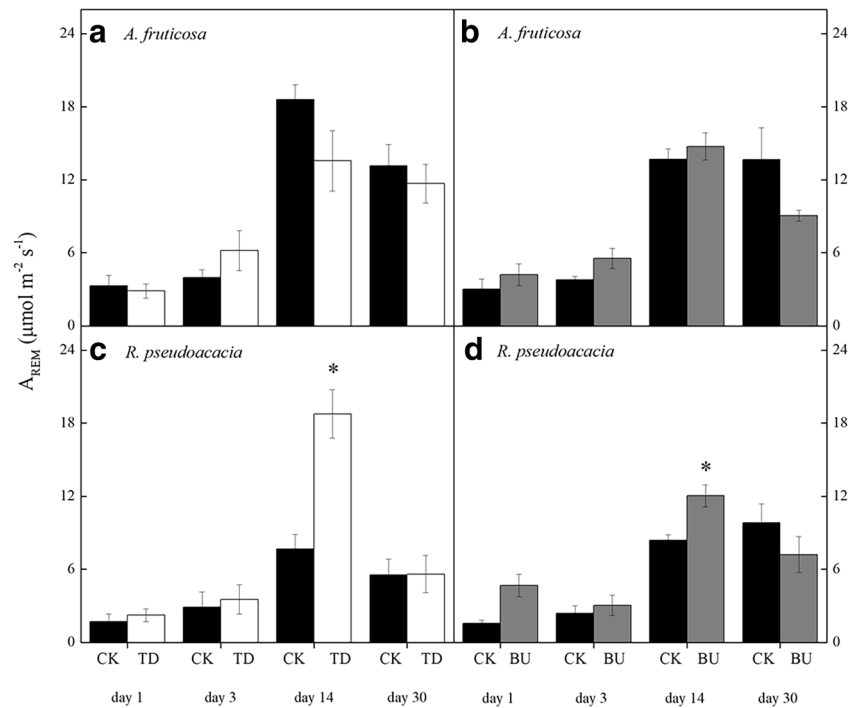
It is different in *R. pseudoacacia*, the stem SS/ST ratio significantly decreased at day 1 in both TD and BU treatments and at day 3 in the BU treatment (Fig. 7d). The stem NSC concentration, soluble sugar concentration, and starch concentration in TD and BU treatments significantly decreased at day 3 (Fig. 6d). The root NSC concentration and soluble sugar

concentration in the BU treatment significantly decreased at days 3–14, and the NSC concentration in TD significantly decreased at day 14, in *R. pseudoacacia* (Fig. 6f).

3.4 NSC mass changes

For *A. fruticosa*, NSC mass in TD was significantly lower than CK from days 3 to 14 (Table 2), NSC mass in BU was also significantly lower than CK at day 14 (Table 2). For *R. pseudoacacia*, NSC mass in TD was significantly lower than CK from days 1 to 3, and BU significantly decreased at day 3 (Table 2). In addition, the NSC mass of *R. pseudoacacia* recovered by day 14, whereas that of *A. fruticosa* recovered by day 30 after defoliation.

Fig. 3 Net photosynthetic rate of remaining leaves (A_{REM}) of *Amorpha fruticosa* (a, b) and *Robinia pseudoacacia* (c, d) under different defoliation treatments. The values shown are mean \pm SE ($n = 4$). The asterisk represents the significant difference ($p < 0.05$) between the control group and the defoliation group; CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown. CK in a and c, upper leaves in the crown were measured, whereas b and d concerned lower leaves



For *A. fruticosa*, the ratio of leaf NSC mass increment (R_{LN}) was indicated 14% higher in the TD treatment and 42% higher in the BU treatment than CK (Table 2). The ratio of stem NSC mass increment (R_{SN}) was indicated 2% lower in the TD treatment and 27% higher in the BU treatment than CK. However, the ratio of root NSC mass increment (R_{RN}) was indicated 35% lower in the TD treatment and 14%

lower in the BU treatment than CK. In addition, the ratio of stem NSC mass increment in the TD treatment of *A. fruticosa* was lower than that in the BU treatment during the experiment. For *R. pseudoacacia*, R_{LN} , R_{SN} , and R_{RN} in the TD treatment were indicated 524%, 178%, and 28% higher than CK, respectively. R_{LN} , R_{SN} and R_{RN} in the BU treatment were indicated 400%, 133%, and 94% higher than CK (Table 2).

Fig. 4 Net photosynthetic rate of refushing leaves (A_{REF}) of *Amorpha fruticosa* (a) and *Robinia pseudoacacia* (b) under different defoliation treatments. The values shown are mean \pm SE ($n = 4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

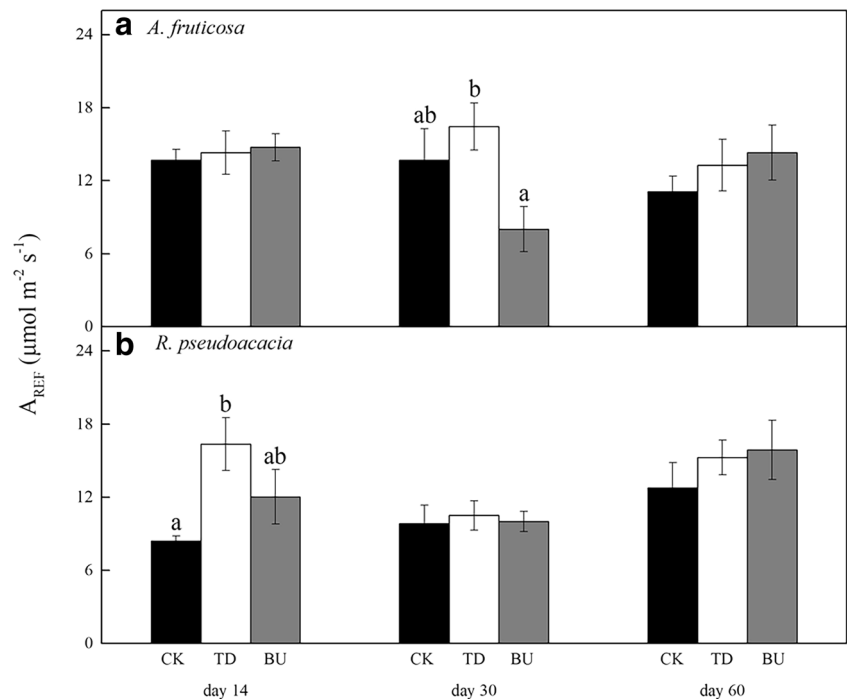
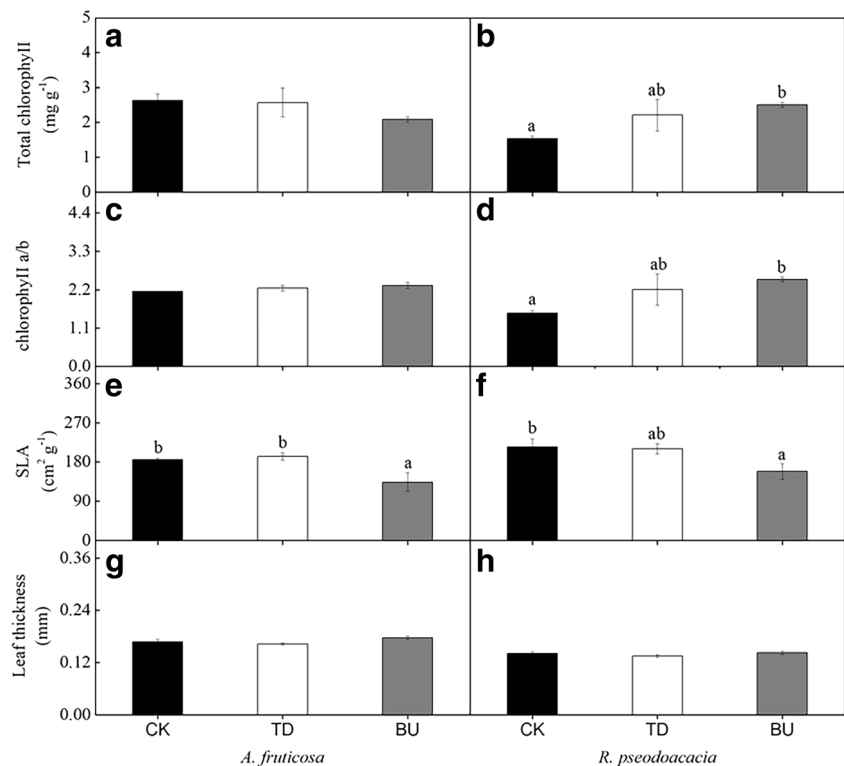


Fig. 5 Seedling total chlorophyll concentration, chlorophyll a/b, leaf thickness, and specific leaf area (SLA) of *Amorpha fruticosa* (a, c, e, g) and *Robinia pseudoacacia* (b, d, f, h) under different defoliation position treatments at day 60. The values shown are mean \pm SE ($n = 3-4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown



3.5 Temporal changes in NSC source/sink ratio

At the beginning of the experiment, 50% of seedling leaves were removed, which decreased the NSC source/sink ratio in *R. pseudoacacia* but not in *A. fruticosa* (Fig. 8). This may be related to the small size of the seedlings when the leaves were removed. Defoliation

treatment did not significantly affect NSC source/sink ratio in the TD treatment and the BU treatment of *A. fruticosa* (Fig. 8a). For *R. pseudoacacia*, the NSC source/sink in the BU treatment was significantly higher than that of CK at days 3–14 (Fig. 8b). The NSC source/sink ranged between 0.74 and 6.01 in *A. fruticosa* and between 0.53 and 2.45 in *R. pseudoacacia*.

Table 3 *F* values of three-way ANOVA in different treatments on *A. fruticosa* and *R. pseudoacacia*

Parameters	S	D	T	S×D	S×T	D×T	S×D×T
Height (cm)	296.48***	0.04	60.03***	0.68	22.81***	0.97	2.80**
Leaf SS/ST	5.79*	3.12*	54.65***	1.98	2.54*	1.39	3.49**
Stem SS/ST	5.48*	3.22*	8.60***	0.35	2.68*	5.57***	1.43
Root SS/ST	3.95	0.75	12.24***	0.03	7.65***	3.46**	1.03
Leaf concentration (mg g ⁻¹)	4.62*	1.07	10.93***	0.63	18.78***	0.58	0.61
Stem concentration (mg g ⁻¹)	70.01***	11.91***	2.25	2.52	3.90**	4.57***	1.02
Root concentration (mg g ⁻¹)	9.31**	5.79**	21.98***	0.70	21.86***	2.06*	1.90
Leaf biomass (g)	50.40***	5.59**	68.73***	0.38	14.63***	2.94**	2.86**
Stem biomass (g)	96.99***	2.89	83.61***	1.26	1.66	1.31	1.71
Root biomass (g)	15.99***	6.11**	126.84***	5.58*	17.73***	3.52**	2.46*

S, species; D, defoliation treatment; T, time

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

$n = 4$

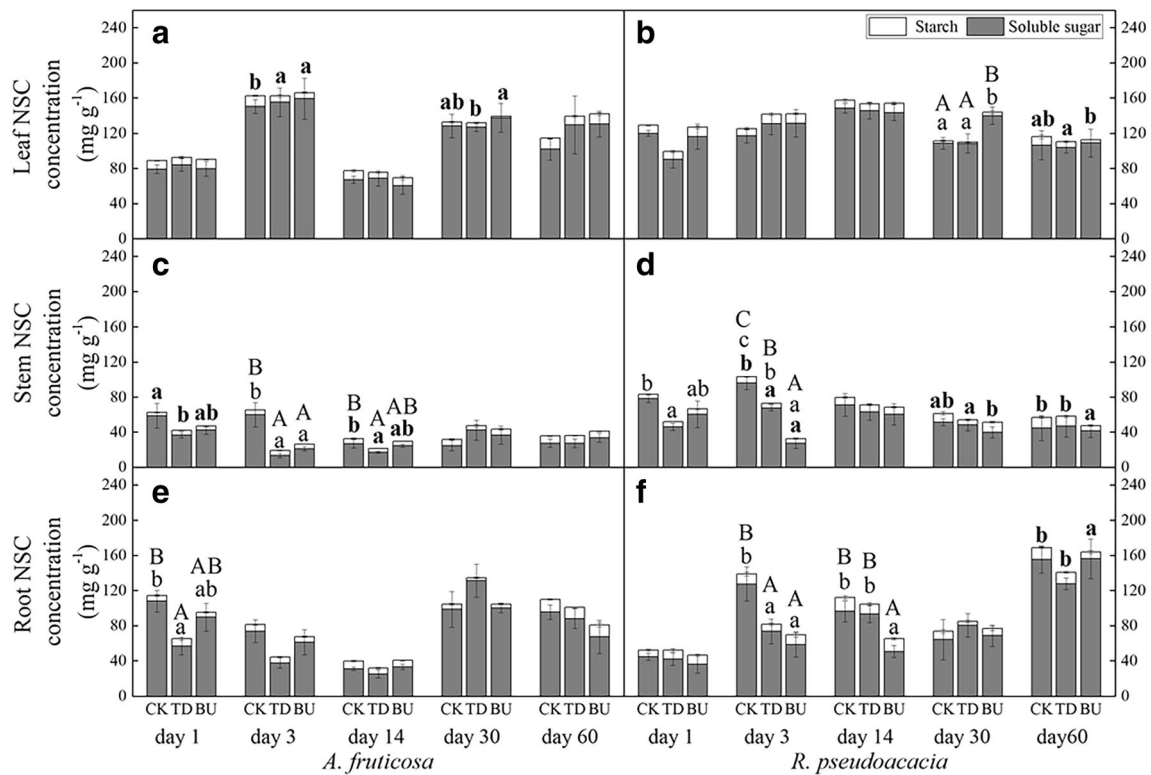


Fig. 6 NSC concentration represents the sum of soluble sugar and starch concentrations measured in three compartments (leaf, stem, root) of *Amorpha fruticosa* (a, c, e) and *Robinia pseudoacacia* (b, d, f) under different defoliation treatments through time. The values shown are mean \pm SE ($n = 4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test.

Lowercase letters without bold are used for soluble sugar concentration. Lowercase letters with bold are used for starch concentration. Uppercase letters are used for NSC concentration. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

Fig. 7 Soluble sugar/starch (SS/ST) ratio concerned three compartments (leaf, stem, root) of *Amorpha fruticosa* (a, c, e) and *Robinia pseudoacacia* (b, d, f) under different defoliation treatments through time. The values shown are mean \pm SE ($n = 4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown

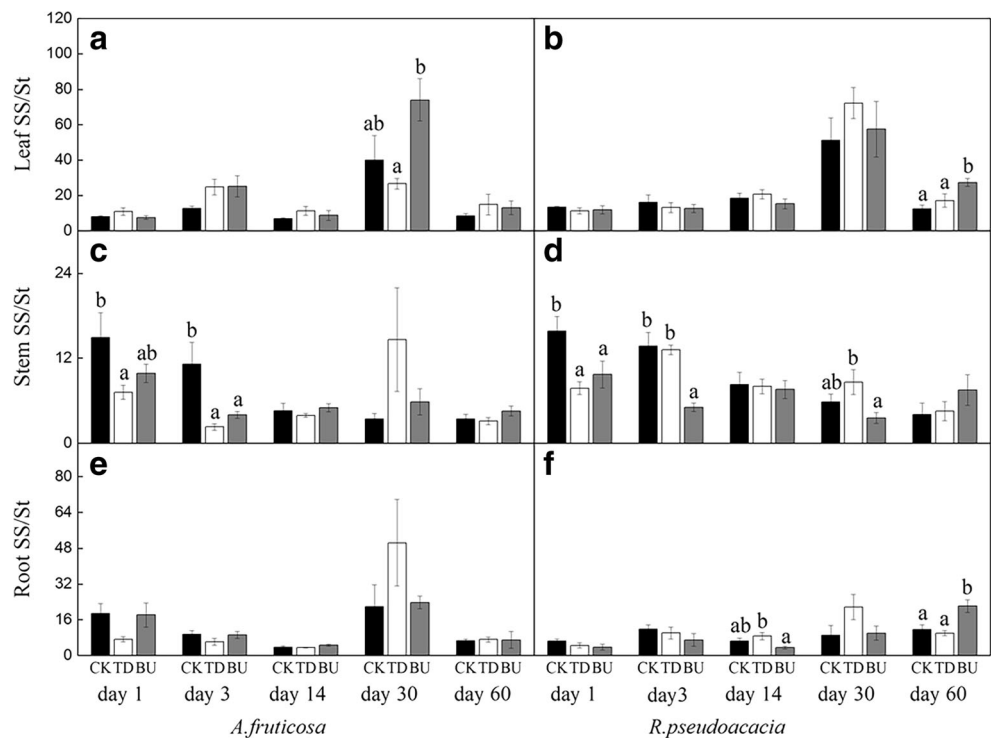
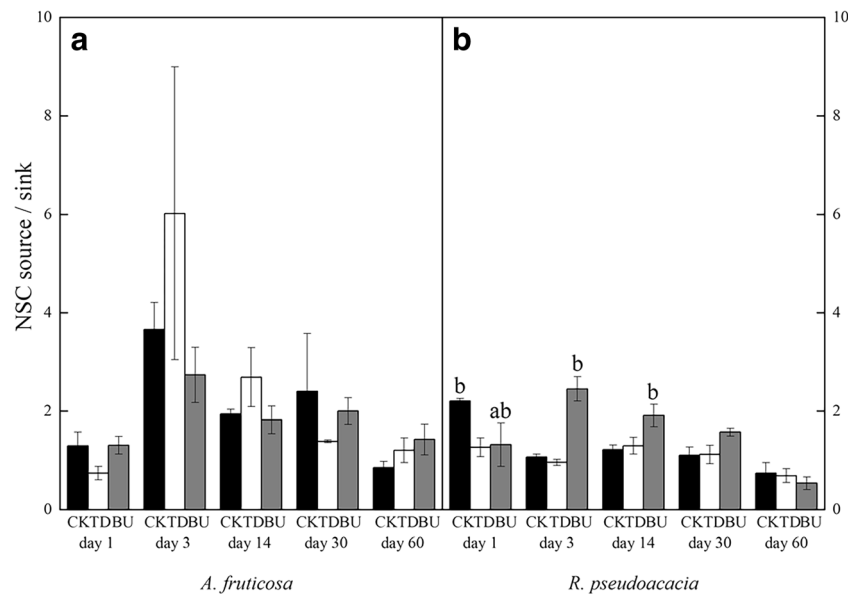


Fig. 8 The source/sink ratio of *Amorpha fruticosa* and *Robinia pseudoacacia* under different defoliation treatments through time. The values shown are mean \pm SE ($n = 4$). Different letters indicate significant differences among different defoliation treatments ($p < 0.05$) with Duncan's test. CK, no defoliation; TD, defoliation of the top down 50% of the crown; BU, defoliation of the bottom up 50% of the crown



4 Discussion

4.1 Effects of defoliation on *A. fruticosa* and *R. pseudoacacia* seedlings

The height in the defoliation treatments did not change significantly from the beginning to the end of the experiment compared with the CK treatment in both species, which was consistent with previous studies that plant height is insensitive to defoliation treatment (Pinkard and Beadle 1998). In addition, the results of our study did not support our first hypothesis that defoliation would reduce plant growth in total biomass at the end of the experiment. Instead, after 60 days of treatment, seedling total biomass did not decrease significantly in any defoliation treatment in both species, which indicates an equal compensatory mechanism (Trlica and Rittenhouse 1993). Therefore, we concluded that seedlings could return to normal levels in their early growth stage after being subjected to defoliation treatment. Our results are consistent with those reported previously in other species, which showed that moderate (50–66%) defoliation did not affect growth in *Nothofagus solandri* var. *cliffortioides* seedlings (Mikola et al. 2000). Concerning plant growth, the biomass among organs was greatly affected by the defoliation treatment in the early stage. In our study, it was found that the biomass of leaves and stems in the TD treatment decreased significantly, and the biomass of roots in the TD treatment did not change significantly at day 3 of *R. pseudoacacia* (Table 1). This indicated that in the early stage after defoliation, seedlings reduced the allocation of stem biomass in order to cope with carbon source limitation and may increase carbohydrate investment to the construction of new leaves (Eyles et al. 2011), resulting in a trade-off between carbon source organ and carbon sink organ. This trade-off type would allow seedlings to survive better under

unfavorable conditions. We also found that the root biomass increased significantly in the BU treatment compared with the CK treatment of *R. pseudoacacia* at day 60 (Table 1), which stimulated the over-compensation mechanism of root for reserves accumulating. There were no significant differences in the total biomass of the seedlings, which may be related to the strategy of storing considerable carbon that made the growth not sensitive to stress (Palacio et al. 2013).

The allocation of non-structural carbohydrates in terms of growth, storage, and defense is mainly related to the rhythm of plant growth and development (Hartmann et al. 2013). After defoliation and until the photosynthetic capacity of new leaves recovers to the control level (days 1–14), the effect of defoliation on seedlings' NSC allocation in *A. fruticosa* and *R. pseudoacacia* was relatively large. During this period, the production of new leaves required a lot of carbohydrates (Quentin et al. 2011). The SS/ST ratio reflects NSC allocation, which could illustrate the conversion dynamics between soluble sugars and starch during the experimental period (Xie et al. 2018). In the early stage of defoliation (days 1–3), stem SS/ST decreased significantly in both species, mainly due to soluble sugar decrease, which may be related to increasing investment in growth to produce new leaves. New photosynthetic products will be preferentially assigned to organs (especially new leaves and stems) near the source, which requires large amounts of energy investment (Eyles et al. 2011). Thus, at the same time, we also found that the stem soluble sugar concentration decreased significantly in defoliation treatments at day 3 in both species (Fig. 6). However, the decrease in the root NSC concentration was mainly due to the decrease in soluble sugar concentration, and the starch concentration remained relatively stable. There was also a trend of a remobilization of the carbon reserve from these organs to satisfy the carbon demand to produce new leaves. In our study, we found

that the stem starch concentration was reduced in the TD treatment of *A. fruticosa* at day 14, and *R. pseudoacacia* at day 3 (Fig. 6), which indicated that ST was converted into SS to compensate for the lack of carbohydrates after defoliation. Indeed, plants use their carbon reserves stored as starch in stems to meet soluble sugar demand for the construction of new leaves, and to compensate for the decrease of carbon synthesis because of leaves lost (Swank et al. 1981). The effect of defoliation on NSC allocation patterns had been fully recovered after 60 days, which was used to compensate for the defoliation damage (Quentin et al. 2011).

4.2 Effects of defoliation modalities on *A. fruticosa* and *R. pseudoacacia* seedlings

Seedlings are more sensitive to the removal of lower leaves (BU treatment) than upper leaves (TD treatment) in terms of leaf traits in both species. Our results showed that total chlorophyll concentration increased significantly in the BU treatment but not in TD in *R. pseudoacacia* (Fig. 5b), which indicated that defoliation led to improved nutrient content in newly developed leaves, potentially promoting photosynthetic rates (Ahmadi and Joudi 2007). Meanwhile, SLA was also significantly decreased in the BU treatment but not in the TD treatment in both species (Fig. 5 e and f), which indicated that plants could maximize the light energy utilization efficiency in their leaves by altering leaf morphology (Collins et al. 2012). Our study indicated that the leaf photosynthesis of *R. pseudoacacia* and *A. fruticosa* increased by 21% and 29%, respectively, in the BU treatment compared with the CK at day 60 (Fig. 4 a and b). Similar results were also reported in a previous study on eucalypt species (Barry and Pinkard 2013). Moreover, another study reported that decreased SLA is related to increased leaf toughness (Knapp et al. 2005); tougher leaves are more difficult for insects to ingest (Bernays 1986).

Previous studies have reported that a range of tree species were able to compensate for partial defoliation (Ågren and Willson 1992; Johnson and Lincoln 2010), through a series of compensatory mechanisms (Anten and Ackerly 2010; Chen et al. 2001), such as increasing the photosynthetic rate of remaining leaves. In our study, the net photosynthetic rate of the remaining leaves in the TD treatment was significantly higher than CK at day 14 of *R. pseudoacacia* (Fig. 3c). This may be because defoliation of the upper leaves is likely to increase the penetration of light throughout the crown (Kosola et al. 2001; Quentin et al. 2011). Meanwhile, the net photosynthetic rate of the remaining leaves in the BU treatment was also significantly higher than CK at day 14 of *R. pseudoacacia* (Fig. 3d). This may be because defoliation decreased the source size, so the increased demand for carbohydrates from the remaining leaves, in turn, increased the photosynthetic rate of these leaves (Eyles et al. 2016; Sweet

and Wareing 1966). In a study of *Populus tremuloides*, a tree species that is commonly defoliated by insects in North American forests, the compensatory photosynthetic effect was partly correlated with the rapid hydraulic responses of roots and leaves, owing to profound changes in the levels of leaf and root aquaporin expression, and this response was very fast (Liu et al. 2014). Moreover, we also found that the net photosynthetic rate of reflushing leaves in TD was significantly higher than that in BU at day 30 of *A. fruticosa*. After the upper leaves were removed, the remaining lower leaves gradually decreased in photosynthetic capacity as time passed. In order to maintain plant growth, the reflushing leaves in TD should increase the net photosynthetic rate. However, in the BU treatment, the remaining leaves were newly developed and could still perform photosynthesis normally, so the net photosynthetic rate of the reflushing leaves in BU did not increase.

Seedling carbon allocation was more susceptible to the BU treatment than the TD treatment in *R. pseudoacacia*. In the present study, it was found that the root NSC concentration under the BU treatment was significantly decreased compared with that under the TD treatment at day 14 in *R. pseudoacacia* (Fig. 6f), and a consistent trend was also found for SS/ST in *R. pseudoacacia* (Fig. 7f). Moreover, root NSC concentration in TD would recover to control levels at day 14, but the root NSC concentration in BU would recover to control levels at day 30 in *R. pseudoacacia*. This may be related to different functional traits of the top and bottom crowns (Yoshimura 2013). The supply of assimilates from leaves follows the principle of near supply; therefore, the top leaves mainly distribute photosynthetic products to new leaves and stems, and the bottom leaves mainly distribute photosynthetic products to the roots (Palit 1985). After the bottom leaves were removed (BU treatment), the allocation of assimilates to the root was reduced.

4.3 Different recovery strategies of *A. fruticosa* and *R. pseudoacacia* seedlings

Increasing the photosynthetic capacity of the remaining leaves may be the most common and important tree compensation response after defoliation (Eyles et al. 2011). In our study, we found that the net photosynthetic rate of the remaining leaves in both TD and BU treatments of *R. pseudoacacia* increased significantly at day 14 (Fig. 3 c and d). However, we did not observe an increase in the net photosynthetic rate of *A. fruticosa* during the recovery period. *R. pseudoacacia* also increased its ability to capture light by increasing the chlorophyll concentration in the BU treatment at day 60, but we did not observe an increase in chlorophyll concentration in *A. fruticosa*. Instead, increasing the carbon allocation to the above-ground parts and decreasing carbon allocation to the below-ground parts would be the recovery mechanism in

A. fruticosa. Previous studies have demonstrated that after defoliation, plants tend to allocate more biomass to the above-ground parts (Oosterheld 1992; Wiley et al. 2017).

Growth and carbon allocation recovered rapidly after defoliation in both species, but *R. pseudoacacia* recovered relatively faster than *A. fruticosa*. Our study showed that NSC mass in *R. pseudoacacia* recovered to the control level at day 14, and NSC mass in *A. fruticosa* recovered to the control level at day 30 (Table 2). Thus, *R. pseudoacacia* could be more likely to quickly recover following defoliation by insects, making it more suitable for vegetation restoration. Previous studies also found that different species had different recovery mechanisms (Mukherjee et al. 2015), and *R. pseudoacacia* recovered through more compensatory physiological mechanisms in our study. Our results indicated that these compensatory mechanisms include (1) modification of leaf traits, such as increased chlorophyll concentration; (2) increasing photosynthetic rates in the remaining leaves; and (3) the remobilization of the carbon reserve stored in long-term compartments toward new-leaf production.

Although *R. pseudoacacia* is more susceptible to pests and diseases in nature than *A. fruticosa*, *R. pseudoacacia* is usually dominant in warm temperate forests and plays an important role in soil and water conservation (Zhang and Xing 2009). Previous studies have found that *R. pseudoacacia* seedlings could survive well under drought and shaded conditions (Xu et al. 2009). Although *R. pseudoacacia* is classified as an invasive species, a nitrogen-deposition experiment showed that this species did not threaten the growth of native *Quercus acutissima* seedlings, but a positive interaction between the two species was found (Ding et al. 2012). Our results indicated the rapid recovery of *R. pseudoacacia* following defoliation, which provides further important evidence of the suitability of this species for vegetation restoration.

5 Conclusion

Our study demonstrated that physiological indicators and carbon allocation were significantly affected by defoliation, especially during the early recovery period (days 1–14). Growth dynamics were related to the change in carbon allocation, and the seedling growth in both species could be recovered to control levels at the end of the experimental period. After the leaves were removed, *A. fruticosa* and *R. pseudoacacia* adopted a positive recovery strategy; however, the mechanisms used by *R. pseudoacacia* were more diverse than those used by *A. fruticosa*. According to the results, *R. pseudoacacia* recovered quickly following defoliation, and thus might be more suitable for vegetation restoration projects. The present study provides theoretical advice for the selection of species for vegetation restoration, and more species should be included in future studies to obtain more generalizable conclusions.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

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

Abbreviations *H*, seedling height; *NSC*, non-structural carbohydrate; R_{LN} , ratio of leaf NSC mass increment; R_{RN} , ratio of root NSC mass increment; R_{SN} , ratio of stem NSC mass increment; *SS/ST*, soluble sugar/starch ratio; *TB*, total biomass; A_{REM} , net photosynthetic rate of remaining leaves; A_{REF} , net photosynthetic rate of reflushing leaves

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