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3 **Asymmetric pruning reveals how organ connectivity alters the functional**
4 **balance between leaves and roots of Chinese fir**

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20
21 **Head title:** Functional balance between branches and roots as affected by pruning

1 **ABSTRACT** The functional balance between leaves and roots is believed to be mediated by the
2 specific location of shoots and roots, i.e. differences in transport distances and degrees of organ
3 connectivity. Yet, whether tree adaptation responses to biomass removal depend on the relative
4 orientation of leaf and root pruning are still unknown. In the present study, five pruning treatments
5 were applied to *Cunninghamia lanceolata* saplings in field and glasshouse conditions, including no
6 pruning (control), half of lateral branches pruned, half of lateral roots pruned, half of same side
7 branches and roots pruned, and half of opposite side branches and roots pruned. The effects of
8 pruning on the growth, carbon storage and allocation, and physiology of leaves and fine roots on the
9 same and opposite sides were investigated. Compared to leaves, fine roots, especially when the same
10 side branches were pruned, were more limited by carbon availability and their physiological activity
11 was more strongly reduced. Moreover, opposite side pruning resulted in the lowest carbon
12 assimilation rates and growth among all treatments. The result from stable isotope labelling indicated
13 that compared to the same side organs, less C was distributed to fine roots from the opposite side
14 leaves, but N allocation from roots to leaves depended less on the relative root and leaf orientation.
15 The results collectively indicated that the functional responses of *C. lanceolata* are not only
16 supported by the source-sink balance model but are related to the interactions between leaves and
17 fine roots. We argue that the connectivity among lateral branches and roots depends on their relative
18 orientation, which is, therefore, critical for the functional balance between leaves and fine roots.

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20 **Key-words:** ecophysiology, functional equilibrium, nonstructural carbohydrates, partial pruning,
21 photosynthesis, source-sink relations, translocation.

22

1 INTRODUCTION

2

3 Balanced growth between shoots and roots that optimizes the carbon budget is crucial for the
4 survival and maximization of competitive ability and reproduction of plants. However, heterogeneity
5 among branches within a crown or root system, as induced by light, soil water, nutrients or
6 herbivores, is a common phenomenon in natural plant communities (Niinemets, 2007; Kawamura,
7 2010). On the other hand, in some planted ecosystems, asymmetric disturbance among the organs is
8 introduced through artificial manipulation (e.g., thinning) to reduce competition among neighboring
9 individuals, thereby enhancing the amount of harvestable biomass (Quentin et al., 2012; Dong et al.,
10 2016). Such imbalanced disturbances among organs usually change the whole plant growth, defense
11 and reproduction, since different organs do not grow independently (Arnold et al., 2004; De Kroon et
12 al., 2009).

13

14 Plant responses to disturbances depend on the source-sink relationships among organs (Galiano et al.,
15 2011; Wiley et al., 2017). The source-sink relationships are closely correlated with the strengths of
16 sources (the ability of the source organ to export carbohydrates) and sinks (the ability of a sink organ
17 to import and use carbohydrates). An imbalanced supply and use of carbohydrates can change the
18 source-sink balance (Paul and Foyer, 2001; Arnold et al., 2004; Pinkard et al., 2011; Savage et al.,
19 2016). Manipulations of source and sink organs (e.g., defoliation and root pruning) are often applied
20 to investigate the source-sink balance of plants, as pruning alters the availability of carbohydrates
21 and changes plant growth and allocation (Quentin et al., 2012; Wiley et al., 2017). Studies on partial
22 pruning of roots or shoots have found that modifications in the water and nutrient status,

1 nonstructural carbohydrate content and gas exchange characteristics of trees result in changes in
2 growth and biomass allocation patterns (Galiano et al., 2011; Quentin et al., 2012; Wiley et al., 2013;
3 Dong et al., 2016; Schmid et al., 2017). Generally, severe shoot pruning will decrease carbohydrate
4 reserves in roots and root growth and metabolic activity (e.g., water and nutrient uptake), increase
5 root death (Snyder and Williams, 2003; Willaume and Pagès, 2006), and ultimately will lead to the
6 growth reduction of the whole tree, especially in evergreen tree species (Schmid et al., 2017). Severe
7 root pruning, in turn, will cause a deficiency of water and nutrients, resulting in a down-regulation of
8 photosynthetic metabolism (Dong et al., 2016). Moderate pruning of shoots or roots, however, may
9 not affect tree growth and allocation (Wiley et al., 2013; Dong et al., 2016), as the growth of shoots
10 and roots may be synergistic or antagonistic depending on the extent to which leaf growth is limited
11 by carbohydrates, water and nutrients (Willaume and Pagès, 2006; Xu et al., 2008; Zhang et al.,
12 2014). When a greater share of carbohydrates is required for leaf growth, mutual competition
13 between leaves and roots for carbohydrates can lead to antagonistic responses (Willaume and Pagès,
14 2006). However, it is often unclear, whether plant growth is limited by the source or sink or both
15 (Farrar and Jones, 2000; Asao and Ryan, 2015).

16
17 Plants are complex integrated systems, in which organs interact via effective transport pathways for
18 carbon, water, nutrients and signaling molecules. Besides the properties of the sinks or sources, the
19 whole transport system (xylem and phloem) may also influence the functional balance among sink
20 and source organs (Minchin and Lacoïnte, 2005; Orians et al., 2005). Previous studies have implied
21 that the topology of vascular bundles and, accordingly, the degree of vascular connectivity, and the
22 distance of sources and sinks are two important characteristics of the long-distance translocation

1 pathways between leaves and roots (Arnold et al., 2004). Because of these linkages, the properties of
2 long-distance transport systems can affect the functional balance between organs and, further, plant
3 growth (Orians et al., 2005; Nikinmaa et al., 2014). On the other hand, a longer pathway always
4 causes a higher xylem hydraulic resistance (Mäkelä and Valentine, 2006) and greater sugar
5 concentration gradients (Paljakka et al., 2017). In trees, different anatomic characteristics of the
6 xylem or phloem between the axial and radial direction usually imply a functional difference (James
7 et al., 2003; Domec et al., 2006), and the distance from lateral branches to the same-side roots is
8 generally shorter than the distance from branches to roots on opposite locations of the same node
9 (Orians et al., 2002). Although these orientation-dependent differences in transport pathway lengths
10 can alter the connectivity among plant organs, information of how the functional balance of trees is
11 altered due to differences in the relative direction between branches and roots is still scarce.

12
13 *Cunninghamia lanceolata* (Lamb.) Hook. is a widely planted, economically important, fast-growing
14 evergreen conifer occurring in a warm monsoon climate in subtropical Asia, and it plays an
15 important role in the regional carbon cycle (Liu et al., 2000). Branch and/or root pruning are
16 common silvicultural practices used in subtropical forestry to enhance forest productivity and reduce
17 the rotation length. Previous studies have found that the leaves and roots of *C. lanceolata* are
18 sensitive to changes in nutrient and water availability (Chen et al., 2015; Dong et al., 2016), and to
19 artificial pruning (Dong et al., 2016). On the other hand, it has been demonstrated that significant
20 compensation can occur after a new functional balance is achieved (Chen et al., 2015; Dong et al.,
21 2016). In the present study, we compared growth, morphological traits of leaves, branches and fine
22 roots, nonstructural carbohydrate (NSC) and nutrient contents, and physiological properties of

1 current-year leaves and fine roots in *C. lanceolata* grown in a field experiment for two seasons. All
2 these measurements were further replicated in a carbon and nitrogen isotope labeling experiment in
3 greenhouse conditions. Our aims were to determine: (1) whether growth and NSC contents of leaves
4 and fine roots are different between partially pruned shoots and roots, (2) whether the responses of
5 lateral leaves and fine roots (e.g., morphology and physiological traits) depend on the relative
6 location of pruning, same-sided vs. opposite pruning; if yes, whether leaves or fine roots on the
7 opposite position in relation to pruning are more sensitive to partial pruning than those pruned on the
8 same side, and (3) whether carbon allocation into fine roots from shoots or nitrogen allocation into
9 leaves from roots are related to the relative location of pruning. We hypothesized that the orientation
10 of leaf and root pruning critically affects plant carbon balance, physiology and growth.

11

1 MATERIAL AND METHODS

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3 Field experiments

4

5 *Study site and materials*

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7 The study was conducted at the Hongya National Plantation Forestry Station (29°47' N, 103°18' E,
8 1100 m a.s.l.) in the Sichuan province, southwestern China. This area is characterized by a
9 subtropical humid monsoon climate with a warm and rainy summer and a dry and cold winter. The
10 average annual temperature is 16.6 °C and the average annual precipitation is 1436.5 mm. The soil is
11 mountain yellow soil. The study site is an even-aged (five years old at the beginning of the
12 experiment) monoculture plantation forest of *C. lanceolata* with a leaf area index of about 6 m² m⁻².
13 During the early growth phase, the crown architecture of *C. lanceolata* is conical (Dong et al., 2015).
14 The root system is characterized by a robust taproot with abundant lateral roots. The space between
15 the neighboring stems was about 2.0 m, and the lateral branches of adjacent saplings did not overlap
16 during the study.

17

18 *Experimental design*

19

20 We randomly selected 100 five-year-old saplings (about 2 m in height). The manipulative pruning
21 treatments started on July 22-24, 2012. At that time, the expansion growth of current-year leaves (*ca.*
22 4 months old) had been completed, the leaves were fully developed, and the trees were in a fast

1 height and diameter growing phase, whereas latewood just started to form (Liu and Wen, 2005). Half
2 of the lateral branches and lateral roots were pruned vertically with a sharp chopper after digging a
3 trench (0.5 m x 0.2 m x 0.5 m in length x width x depth) along the base of the trunk. Five treatments
4 were applied, including no pruning (NP), half of lateral branches pruned (BP), half of lateral roots
5 pruned (RP), half of same side branches and roots (both branches and roots from the same azimuthal
6 direction) pruned (SP), and half of opposite side branches and roots (branches and roots from the
7 opposite directions) pruned (OP). The orientation of pruning was randomly selected. The
8 investigated leaves and roots in NP, BP, RP, SP and OP are marked as NP_L , BP_L , RP_{SL} (same side
9 orientation with pruned roots), RP_{OL} (opposite side orientation with pruned roots), SP_L and OP_L for
10 leaves, and NP_R , BP_{SR} (same side orientation with pruned branches), RP_{OR} (opposite side orientation
11 with pruned branches), RP_R , SP_R and OP_R for roots (Fig. 1). Current-year leaves of lateral branches
12 of the same inter-node (target branches) from the mid-crown and fine roots were selected for all
13 ecophysiological measurements. In all cases, the plane of pruning was parallel to the stem and
14 taproot. Field measurements were conducted on October 5-8 in 2012 (10 weeks after pruning) and
15 September 26-28 in 2013 (60 weeks after pruning). Each treatment contained 20 individuals,
16 including four replications with five individuals in each replication. The repeated pruning treatments
17 involved the excision of new lateral branches and roots. Four individuals from each treatment were
18 chosen randomly for measurements.

19

20 *Growth and morphology of foliage and fine roots*

21

22 Four individuals per treatment (in each case, a randomly chosen individual replicate) were measured

1 for stem height and basal stem diameter. Two target branches from each tree were selected and
2 harvested between 1400 and 1600 h. Current-year lanceolate leaf samples were scanned and their
3 area was estimated from scanned images with the Image J software (National Institutes of Health,
4 USA). The leaves and branches were then oven-dried at 70 °C until a constant mass. The average
5 area and dry mass per current-year leaf, and the average length and dry mass per target branch were
6 calculated.

7
8 Roots were carefully excavated from the depth of 0-30 cm. Three intact lateral roots were harvested
9 per treatment and pooled as one root sample. Then the root samples were carefully washed and
10 transported on ice to the laboratory within a few hours. Fine roots (< 2 mm in diameter) were
11 separated and divided into two parts. One part was used for morphological analyses, and for the
12 measurements of respiration and nonstructural carbohydrates, and the other part was used for other
13 biochemical measurements. For the morphological analyses, fine root samples, measured for root
14 respiration, were scanned to estimate the length of roots with a root system analysis software
15 (WinRhizo, Regent Instruments, Inc., Québec, Canada). The dry mass of samples was measured after
16 oven-drying at 70 °C to a constant mass. The specific root length (SRL) was calculated as the ratio of
17 the root length to dry mass.

18 19 *Water potential of leaves*

20
21 Ten weeks and sixty weeks after pruning, leaf predawn water potential was determined for healthy,
22 fully-expanded current-year leaves using a *WP*₄ Dewpoint Potentiometer (Decagon Devices, Inc.,

1 Pullman, WA, USA). The leaf samples were cut with a sharp razor blade, sealed immediately in
2 small plastic bags containing moist paper towels and kept in a cooler for a short time before analyses.
3 In addition, to investigate the osmotic adjustment induced by the asymmetrical pruning, the
4 concentrations of free proline and total free amino acids of leaves were determined.

5

6 *Gas exchange measurements*

7

8 Ten weeks and sixty weeks after pruning, measurements of leaf photosynthesis and fine root
9 respiration were conducted between 0800 and 1130 h using LI-6400 portable gas exchange system
10 (Li-Cor Inc., Lincoln, NE, USA). The measurement conditions of leaf photosynthesis were as
11 follows: leaf temperature of 25 °C; relative air humidity of 60%; CO₂ concentration of 400 ± 5 μmol
12 mol⁻¹; leaf-to-air vapor pressure deficit of 1.5 ± 0.5 kPa, and photosynthetic photon flux density
13 (PPFD) of 1500 μmol m⁻² s⁻¹. Once the steady-state gas exchange rates were observed under these
14 conditions, net photosynthesis rate, stomatal conductance (g_s) and transpiration rate (E) were
15 recorded. Light-saturated photosynthesis rate (A) was measured under the saturating irradiance of
16 1500 μmol m⁻² s⁻¹, as in our pervious study (Dong *et al.* 2015). Before measurements, the sample
17 leaves were exposed with 1500 μmol m⁻² s⁻¹ PPFD provided by the LED light source of the
18 equipment for 15 min to achieve a full photosynthetic induction. Leaf dark respiration rate was
19 measured under the same condition as photosynthesis, except for the light (darkness). The respiration
20 rate was recorded when a steady-state rate was observed, but the leaves were darkened for at least 5
21 min before recording the respiration rates. After photosynthetic capacity measurements, each leaf
22 enclosed in the leaf chamber was scanned and its area was estimated with Image J software (National

1 Institutes of Health, USA).

2

3 For the respiration measurements of fine roots, three intact lateral roots were clipped from the plants
4 and the cut surfaces were sealed off with vaseline to prevent water loss and callus respiration (plant
5 organ respiration is usually higher from a wounded organ part than from a non-wounded organ part).

6 Then, the roots were immediately carefully washed in deionized water. Excess water was removed
7 by absorbent paper. A fine root sample (about 0.3 g fresh mass) was stored in an ice box and taken
8 within two hours to the laboratory and placed into a plexiglass cuvette (7.5 cm diameter× 3.5 cm in
9 length) for measurements using the LI-6400 photosynthesis system. The cuvette surface was covered
10 by a black cloth, and measurements for root respiration were carried out in the following conditions:
11 CO₂ concentration of 400 μmol mol⁻¹ and cuvette temperature of 20 °C. The temperature was
12 controlled by a central air-conditioner. After the completion of the measurements, root dry mass and
13 length measurements were conducted, as described above. To examine the physiological responses of
14 fine roots after pruning, the root vitality was measured.

15

16 *Leaf N and P concentrations*

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18 The leaves measured for gas exchange were oven-dried at 70 °C until a constant mass. Dried leaf
19 samples (0.2 g) were ground in a ball mill and used for N and P concentration measurements. The N
20 concentration was determined with a Vario MAX CN analyzer (Elementar Analysensysteme GmbH,
21 Hanau, Germany) and the P concentration by induced plasma emission spectroscopy (Optima 8300,
22 Perkin Elmer Inc., Norwalk, CT, USA).

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Nonstructural carbohydrate content of leaves and fine roots

The dried and fine-ground current-year leaf and fine root samples from each treatment were incubated in 80% (w/v) ethanol at 80 °C for 30 min and centrifuged at 5,000 g for 10 min. Total soluble sugars were determined in ethanol extracts and the residues left in the centrifuge tubes were used to determine the starch content after hydrolyzing it to glucose with 9.2 M HClO₄. Total soluble sugars and starch contents were estimated by the anthrone assay using 0.2% anthrone in concentrated H₂SO₄ as a reagent. Total soluble sugars were detected colorimetrically at 625 nm (Yemm and Willis, 1954). The amount of total nonstructural carbohydrates (NSC_T) was calculated as the sum of soluble sugars and starch.

Isotope labeling experiments

Study site and materials

To examine the effect of partial pruning on the carbon and nutrient translocation and allocation, two-year-old *C. lanceolata* saplings with full root systems and attached soil were carefully excavated from the site of the field experiment. The saplings were transplanted to a greenhouse (average day/night temperature about 25/15 °C and average relative humidity about 60% during the treatment period) at the Chengdu Institute of Biology, Chinese Academy of Sciences. Individual saplings were grown in the center of plastic pots (volume about 60 L) filled with 50 kg homogenized yellow soil

1 from the experimental site. During the second growing season in the greenhouse, the saplings with
2 approximately the same crown size and height were chosen for the experiments.

3
4 A similar experimental design as in the main experiment (NP, BP, RP, SP and OP) was used. The
5 pruning treatment began on May 10-11 in 2013 when the new leaves were fully expanded. On
6 August 20-23 (15 weeks after pruning), three trees from each treatment were randomly chosen for
7 ^{13}C and ^{15}N labeling experiments, and three trees from each treatment were used as controls
8 (unlabeled).

9

10 *^{13}C and ^{15}N labeling procedure*

11

12 The ^{13}C labeling procedure was conducted according to Zhang *et al.* (2005) with some modifications.
13 One healthy lateral branch from the middle crown per individual was chosen. 0.5 g $\text{Ba}^{13}\text{CO}_3$ (93%
14 abundance of ^{13}C ; Shanghai Engineering Research Center of Stable Isotopes, China) was added into
15 a 25 ml glass vial. After the branch was sealed and the CO_2 concentration had decreased to near
16 equilibrium, a pulse of $^{13}\text{CO}_2$ was released by injecting 3 ml 70% H_2SO_4 from a syringe through a
17 gas port into the $\text{Ba}^{13}\text{CO}_3$ solution. A similar procedure with an equal amount of $\text{Ba}^{12}\text{CO}_3$ was
18 carried out for the control trees. The branch was exposed to $^{13}\text{CO}_2$ (labeled) or $^{12}\text{CO}_2$ (unlabeled) in a
19 transparent polycarbonate bag. The labeling proceeded in a sunny day from 0900 to 1100. Three
20 lateral fine root samples (sub-samples from three intact and attached lateral roots per individual)
21 from three individuals (one pooled sample per tree) were harvested 72 h later from ^{13}C labeled and
22 control individuals.

1

2 For ^{15}N labeling, five intact and attached lateral roots per individual were chosen. The roots were
3 placed into a $100\ \mu\text{M N L}^{-1}\ \text{K}^{15}\text{NO}_3$ (99% abundance of ^{15}N ; Shanghai Engineering Research Center
4 of Stable Isotopes, China) solution (20 mg per plant) in a glass vial with a black cover. A similar
5 procedure with an equal amount K^{14}NO_3 was conducted for control trees. In addition, the solutions
6 contained $10\ \text{mg L}^{-1}$ of ampicillin to minimize microbial activity and $0.2\ \text{mM CaCl}_2$ to maintain the
7 function of roots (Warren and Adams 2007). Three current-year leaf samples from lateral branches in
8 the mid-crown of three individuals were harvested 72 h later from ^{15}N labeled and control
9 individuals.

10

11 *Carbon and nitrogen isotope analysis*

12

13 $\delta^{13}\text{C}$ of the fine root samples and $\delta^{15}\text{N}$ of current-year leaf samples from labeled and non-labeled
14 individuals were measured with a combined system of an elemental analyzer (Flash EA1112 HT;
15 Thermo Fisher Scientific, Inc., USA) and an isotope ratio mass spectrometer (DELTA V Advantage;
16 Thermo Fisher Scientific, Inc., USA). We determined excess $\delta^{13}\text{C}$ (‰) of fine roots and $\delta^{15}\text{N}$ of
17 current-year leaves as deviations from the baseline values ($\delta^{13}\text{C}_{\text{baseline}}$ or $\delta^{15}\text{N}_{\text{baseline}}$; values for each
18 tree from each treatment were calculated by averaging the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of the non-labelled
19 individuals) as excess $\delta^{13}\text{C} = \delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{baseline}}$ and excess $\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{baseline}}$,
20 respectively (Kagawa et al., 2006a).

21

22 Excess ^{13}C of fine root samples (from the three target roots) or excess ^{15}N of current-year leaf

1 samples (from one target branch) was also calculated according to procedures described by Kagawa
2 et al. (2006b). To reveal different amounts of ^{13}C and ^{15}N translocated into specific tree parts, the
3 relative ratios of excess ^{13}C in target fine roots and ^{15}N in target current-year leaves of each tree part,
4 and the excess ^{13}C in the labeled current-year leaves of each target lateral branch or ^{15}N of the fine
5 roots of the target lateral root were calculated.

6 Relative ratio of Excess ^{13}C or Excess ^{15}N was calculated as follows:

7 Relative ratio of excess ^{13}C = excess $^{13}\text{C}_{\text{target fine roots}}/\text{excess } ^{13}\text{C}_{\text{labeled current-year leaves}}$;

8 Relative ratio of excess ^{15}N = excess $^{15}\text{N}_{\text{target current-year leaves}}/\text{excess } ^{15}\text{N}_{\text{labeled fine roots}}$.

9

10 *Statistical analysis*

11

12 The differences in the effects of pruning treatments on growth, and on morphological and
13 physiological data (the mean of each leaf/branch/root from each side in each individual in each
14 treatment used) were analyzed with one-way analysis of variance (ANOVA). A post hoc test
15 (Tukey's test) was conducted if the differences were significant ($P < 0.05$). Our previous studies
16 showed that the allocation, chemical and physiological traits of *C. lanceolata* saplings were not
17 significantly different among azimuthal directions in non-pruned control plants (Dong et al., 2015,
18 2016). Thus, for branches, leaves and fine roots in the controls (no pruning treatment), we averaged
19 the ecophysiological data (dry mass per branch and leaf, leaf nutrient concentration, gas exchange
20 traits, fine root mass distribution among the quadrants) of an individual. Before ANOVA, the data
21 were checked for normality and homogeneity of variances, and log-transformed to correct deviations
22 from these assumptions when needed. All statistical analyses were carried out using the SPSS 18.0

1 for Windows statistical software package (SPSS Inc., Chicago, IL, USA). Principal component
2 analysis (PCA) of eco-physiological traits in the field experiment was further undertaken to reveal
3 the most discriminatory effects on position-related pruning.

1 RESULTS

2

3 *The effects of lateral pruning on growth, and on dry mass and morphology of branches*

4

5 At the whole tree level, increments in basal diameter and height were significantly different among
6 the five treatments both 10 weeks and 60 weeks after pruning (Fig. 2). Height growth decreased due
7 to pruning, and the lowest values (66.7% and 37.8% lower 10 weeks and 60 weeks after pruning,
8 respectively, compared to control plants) were observed when half of opposite side branches and
9 roots were pruned (OP; Fig. 2). The greatest basal diameter increase was observed in trees with half
10 of lateral roots pruned (RP). Compared to non-pruned (NP) trees, those with half of lateral branches
11 pruned (BP) had a lower basal diameter increment (27.3%) after 60 weeks, while their height
12 differences were not significant. Opposite side pruning had a greater negative effect on the growth of
13 basal diameter and height compared to the same side pruning (SP; Fig. 2).

14

15 At the branch scale, the average branch mass (30.3% and 40.7% higher 10 weeks and 60 weeks after
16 pruning, respectively, compared to control plants) and length (14.8% and 22.3% higher 10 weeks and
17 60 weeks after pruning, respectively, compared to control plants) were highest in the BP treatment
18 but lowest in the OP treatment (Table 1). In the RP treatment, both branch mass and length of RPs
19 (lateral branches with the same orientation with pruned roots) were lower (20.2% in dry mass and
20 15.1% in length) than those of RPO (lateral branches with the orientation opposite to pruned roots) 10
21 weeks after pruning but they were similar 60 weeks after pruning. There were no significant
22 differences in branch mass and length between SP and NP at either time point (Table 1).

1

2 *The effects of lateral pruning on dry mass, morphological, nutrient and physiological traits of leaves*

3

4 At the leaf level, the average dry mass (M_L) and area (A_L) per leaf were the lowest (47.1% and 52.7%
5 lower mass, and 36.8% and 46.5% lower area 10 weeks and 60 weeks after pruning, respectively,
6 than in control plants) in OP, and M_L was lower (25.5% and 16.2% lower 10 weeks and 60 weeks
7 after pruning, respectively) in RPs than in NP. In the RP treatment, M_L of RPs was slightly lower
8 than that of RP_O (Table 1).

9

10 Leaf nitrogen and phosphorus concentrations observed in BP treatments were higher than those in RP
11 (Table 1). The leaf phosphorus concentration of the OP treatment was highest 10 weeks after pruning
12 but lowest 60 weeks after pruning (Table 1). The leaf nitrogen concentration was similar in SP and
13 OP treatments 10 weeks after pruning, while later it was lower in OP than in SP (Table 1). In addition,
14 N and P were not significantly different between RP_O and RPs, and between SP and NP.

15

16 The branch-pruning treatment increased the light-saturated photosynthetic rate (A ; Fig. 3a), but it did
17 not affect the leaf respiration rate (R_L ; Fig. 3b), while the OP treatment decreased A and increased R_L
18 after a longer post-pruning period. Ten weeks after pruning, stomatal conductance (g_s) was slightly
19 higher in the case of pruning compared to no pruning, while g_s values were similar among treatments
20 60 weeks after pruning (Fig. 3c). Pruning always increased the leaf transpiration rate (E ; Fig. 3d),
21 while it decreased the water-use efficiency (E_w), except for E_w in BP when measured 10 weeks after
22 pruning (Fig. S1b). The transpiration rate was greater in the BP treatment than in the non-pruned

1 treatment, and E in BP_S was higher than that in BP_O 10 weeks after pruning (Fig. 3d). In addition, E
2 in OP was higher than that in SP 10 weeks after pruning, while E values were similar 60 weeks after
3 pruning. Pruning also resulted in reduced leaf water potential (Ψ), except in BP (Table 1). The lowest
4 E_W and Ψ values were found in OP, while E_W and Ψ values in BP_S and BP_O treatments were similar.
5 In addition, the nitrogen use efficiency (E_N) and phosphorus use efficiency (E_P) (Fig. S1c, d), and the
6 amounts of free proline and free amino acids (FAA) of leaves (Fig. S2) were affected by pruning.

8 *The effects of lateral pruning on dry mass, morphology, physiology and nutrient traits of roots*

9
10 Specific fine root length (SRL) varied significantly among pruning treatments: SRL was highest in
11 RP (24.0% and 36.4% higher 10 weeks and 60 weeks after pruning compared to control plants) but
12 lowest in OP (35.0% and 43.4% lower 10 weeks and 60 weeks after pruning compared to control
13 plants) (Fig. 4a). SRL values in the BP treatment were lower for those pruned from the same (BP_S) or
14 opposite (BP_O) side than in the control trees (no pruning): 25.1% or 31.7% lower 10 weeks after
15 pruning, and 47.0% or 14.1% lower 60 weeks after pruning. SRL of BP_S was 38.2% lower than that
16 of BP_O 60 weeks after pruning (Fig. 4a).

17
18 Analyses of differences in root vitality indicated that RP increased root vitality but BP decreased it
19 (Fig. 2b; Fig. S3). The highest fine root respiration rate (R_R) was observed in RP, and the lowest one
20 in OP (Fig. 2b). Sixty weeks after pruning, R_R of BP_S was slightly lower than that of BP_O , and it was
21 significantly lower in OP than in SP. The SP treatment did not change root vitality, but in OP the
22 vitality was lower than in the other treatments. A greater difference in root vitality was observed

1 between RP_S and RP_O 60 weeks after pruning (Fig. S3).

2

3 *The effects of lateral pruning on leaf and root nonstructural carbohydrates*

4

5 Ten weeks after pruning, leaf soluble sugars and total nonstructural carbohydrate (NSC_T) contents
6 per mass in various pruning treatments were not significantly different from those in the control
7 plants, while 60 weeks after treatments, pruning decreased leaf sugar and NSC_T contents, and they
8 both were lowest in BP (Fig. 5a). In the RP treatment, leaf sugars and NSC_T were lower in RP_S than
9 in RP_O 10 weeks after pruning, while these differences were not observed 60 weeks after pruning
10 (Fig. 5a). Compared to SP, leaf soluble sugars and NSC_T were slightly higher in the OP treatment.
11 The starch content was the lowest in SP 10 weeks after the treatment, and in OP 60 weeks after the
12 treatment (Fig. 5a).

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14 In fine roots, starch and NSC_T increased in the RP treatment, especially after a longer period
15 following pruning (Fig. 5b). In the BP treatment, nonstructural carbohydrates (except starch 10
16 weeks after pruning) were lower in BP_S than in BP_O, and BP_O was similar with NP. When half of
17 both lateral branches and roots were pruned, the contents of nonstructural carbohydrates of fine roots
18 (except starch 10 weeks after pruning) in OP were lower than those in SP treatments, while sugar,
19 starch and NSC_T contents of SP were not significantly different when compared to NP (Fig. 5b).

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21 *The effects of lateral pruning on excess $\delta^{13}C$, excess ^{13}C and RR ^{13}C in fine roots, and excess $\delta^{15}N$,
22 excess ^{15}N and RR ^{15}N in leaves*

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Both the excess $\delta^{13}\text{C}$ and excess ^{13}C of fine roots located *on* the same side were significantly higher than those in the roots opposite to the labeled branch (Figs. 6a, b). Compared to the non-pruned control roots, the excess $\delta^{13}\text{C}$ of fine roots located on the same side as the labeled branch was lower in BP, while there were no significant differences in $\delta^{13}\text{C}$ of fine roots located on the opposite side relative to the labeled branch among NP, BP and RP (Fig. 4a), and the OP treatment increased the fine root $\delta^{13}\text{C}$ (Fig. 6a). Excess ^{13}C and the relative ratio of ^{13}C ($\text{excess } ^{13}\text{C}_{\text{target fine roots}}/\text{excess } ^{13}\text{C}_{\text{labeled current leaves}}$) in fine roots (both for the same and opposite sides of the labeled branch) in the RP treatment were the highest among treatments (Fig. 6b, c), while these values on the same side and opposite side were similar among NP, BP and OP, except for the relative ratio of ^{13}C in OP (Fig. 6b, c).

The excess $\delta^{15}\text{N}$, excess ^{15}N and relative ratio of ^{15}N ($\text{excess } ^{15}\text{N}_{\text{target current leaves}}/\text{excess } ^{15}\text{N}_{\text{labeled fine roots}}$) in current-year leaves were similar between the same and opposite locations relative to the labeled roots (Fig. 6d-f). These three traits were similar in SP and NP, while the lowest values of excess $\delta^{15}\text{N}$ and excess ^{15}N were observed in the OP treatment (Fig. 6d-f). BP and OP treatments decreased leaf excess $\delta^{15}\text{N}$, while RP and SP did not influence leaf excess $\delta^{15}\text{N}$ (Fig. 6d). Excess ^{15}N decreased in pruning treatments, except for SP (Fig. 6e). The lowest relative ratio of ^{15}N in current-year leaves among pruning treatments was observed in RP (Fig. 6f).

Relationships among studied traits in shoots under different pruning treatments

1 The PCA showed a clear delineation based on trait combinations in different pruning treatments (Fig.
2 7). The pruning treatments were well separated from each other at 10 weeks (Fig. 7a) and 60 weeks
3 (Fig. 7b). The PCA model with two components explained 57.11% and 67.48% of the observed total
4 variance at 10 weeks and 60 weeks. PC1 was strongly influenced by height growth, leaf area and
5 mass, photosynthetic rate, transpiration rate and water potential. PC2 was strongly influenced by
6 DBH growth, leaf NSC and phosphorus concentrations.

1 **DISCUSSION**

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3 We found that partial branch and root pruning changed tree growth, carbon and nitrogen allocation,
4 water and nutrient status, and physiological processes of leaves and fine roots, similarly as observed
5 in some previous studies on trees (e.g., Li et al., 2002; Eyles et al., 2013; Aguadé et al., 2015; Wiley
6 et al., 2017). However, the responses varied depending on the relative lateral positions of pruned
7 branches and roots. There was a significantly higher photosynthate allocation to lateral fine roots
8 from the same side branches compared to the opposite side branches, while nitrogen allocation to
9 lateral leaves was not significantly different between the same side and opposite side roots.
10 Furthermore, with increasing time after branches or roots were partially pruned, differences in
11 morphology and physiology between the same and opposite side fine roots increased, but the
12 differences between the same and opposite side current-year leaves decreased. Our results thus
13 demonstrated that (1) the position of pruning has a strong influence on growth, photosynthesis, and
14 carbon storage and remobilization, as well as on water- and nutrient-related physiological responses;
15 (2) compared to leaves, carbon availability for fine roots is more strongly affected by partial pruning.

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17 *The responses of growth, and leaf and fine root traits to partial lateral branch and root pruning*
18 *(shoot pruning vs. root pruning)*

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20 Once the distribution of shoots or roots is altered, adjustments in physiology, morphology and
21 growth will begin, and these adjustments may happen at the organ or whole plant level until a new
22 balance is reached (Pinkard et al., 2011; Eyles et al., 2013; Dong et al., 2016). Despite the growth of

1 branch and root apical parts and secondary stem thickening being characteristic sinks of
2 photoassimilates in summer, we observed decreases in growth (Fig. 2), root respiration rate (R_R) (Fig.
3 4b) and root vitality (Fig. S3), and lower root NSC contents (Fig. 5b) when branches were partially
4 pruned. Overall, our results show that the source limitation induced by branch pruning results in
5 carbon depletion and lowered metabolic capacity in roots, and, consequently, in decreased uptake of
6 water and nutrients (see also Kosola et al., 2002; Kobe et al., 2010). The results were supported also
7 by the nitrogen isotope labelling study (Fig. 6d, e). In addition, the decrease in growth may be
8 beneficial for maintaining the nonstructural carbohydrate concentration of leaves (Wiley et al., 2013).
9 Our finding that growth (height and DBH increments) has a negative relationship with leaf NSC
10 (Fig. 7) may explain the observation that leaf NSC decreased slowly (Fig. 5a). On the other hand,
11 after partial branch pruning, plants can have compensatory responses by increasing the
12 photosynthetic rate, biomass and length of remaining branches (Paul and Foyer, 2001; Pinkard et al.,
13 2011; Eyles et al., 2013; Asao and Ryan, 2015). In our study, partial branch pruning (about 50% leaf
14 area lost) did not significantly affect height and DBH increments (except for DBH that decreased by
15 27.3% at 60 weeks). These results are related to the increased remaining leaf area and photosynthetic
16 rate (Fig. 7), which derived from the increased leaf nitrogen and phosphorus concentrations in the
17 remaining foliage, and enhanced nitrogen- and phosphorus-use efficiencies (Table 1; Fig. S1c, d).
18 These results further confirm the onset of plant compensatory responses after branch pruning.

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20 Under only partial root pruning (RP), the basal stem diameter growth was greater, despite the
21 decreased height growth. When branch and leaf growth were compared to the non-pruned control
22 plants, a lower dry mass per branch and leaf (mainly from the same direction with root pruning) was

1 detected in RP 10 weeks after pruning, while RP did not affect the growth of branches and leaves 60
2 weeks after pruning (Table 1). Our results indicate that the physiological (e.g., root respiration rate
3 and root vitality) and morphological (e.g., specific fine root length) compensation responses of fine
4 roots can maintain the water and nutrient supply of leaves, and this conclusion was further supported
5 by the results from the nitrogen isotope labelling (Fig. 6d). The photosynthetic down-regulation after
6 partial pruning of roots may be mainly caused by the limitation of the carbon sink storage capacity
7 (initial root and shoot height growth decreased) rather than by water or nitrogen limitation at the
8 whole-plant level (Farrar and Jones, 2000; Paul and Foyer, 2001; Eyles et al., 2013; Dong et al.,
9 2016). In addition, we suggest that the increased basal stem diameter in RP is mainly caused by
10 greater carbohydrate availability for stem growth, as lateral pruning of roots restricts carbohydrate
11 translocation to roots (Asao and Ryan, 2015).

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13 Our results clearly demonstrate that branch pruning treatment caused a negative effect on fine root
14 growth and metabolic activity, although it increased the growth and photosynthesis of remaining
15 branches. On the other hand, a positive effect on the growth of remaining fine roots and metabolic
16 activity was detected in the root pruning treatment. These responses highlight the acclimation
17 changes in the source-sink balance of carbon allocation after pruning. Excess $\delta^{13}\text{C}$, excess ^{13}C and
18 the relative ratio of ^{13}C in fine roots to that in labeled leaves were lower in branch pruning treatments
19 than in root pruning treatments (Fig. 6a-c), clearly indicating that photoassimilate partitioning to
20 roots increased in response root pruning. Overall, the data also suggest that the carbon status
21 (respiration and NSC concentration) of heterotrophic fine roots changes more strongly than that of
22 autotrophic leaves during asymmetric pruning of shoot and roots.

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The bidirectional responses of branches, leaves and fine roots under partial pruning of branches or roots (BP, RP)

It has been demonstrated in non-pruned *C. lanceolata* plants that the dry mass of branches and leaves, gas exchange characteristics, nutrient concentrations and water use traits of leaves and fine roots are similar bilaterally (Dong et al., 2015, 2016). However, when half of the lateral branches or roots were pruned, differences in morphological and physiological responses between branches located at the same or opposite direction in current-year foliage, and in fine root traits became significant. Half of the branches being pruned induced greater decreases in fine root traits (SRL, R_R , vitality and NSC) on the branch-pruned side than on the opposite side (Fig. 4; Fig. S3; Fig. 5b), while half of the roots being pruned did not lead to differences between the root-pruned side and the opposite side in most leaf traits (e.g., biomass, area, physiological characteristics and nutrient concentrations) (Table 1; Fig. 3). These results indicate that the ecophysiological responses of *C. lanceolata* within an individual are related to the relative position between lateral branches and roots, and leaf and fine root responses are different during asymmetric disturbances depending on the position, which suggests that the xylem is better connected than the phloem (Thorn and Orians, 2011). The results indicate that the plant transport system (xylem and phloem) plays a crucial role in determining the responses of roots and leaves, as has also been shown in some previous studies (Minchin and Lacoite, 2005; De Kroon et al., 2009; Savage et al., 2016).

Thorn and Orians (2011) have found that *Ocimum basilicum* accumulates more ^{15}N (applied to one

1 half of the root system) in orthostichous (growing at an angular distance of 0 degrees) leaves than in
2 leaves from the opposite sector. This result suggests that tighter vascular links exist between roots
3 and branches on the same side. In our study, excess $\delta^{13}\text{C}$ (Fig. 6a) and excess ^{13}C (Fig. 6b) in fine
4 roots were always higher on the same side than on the opposite side relative to the lateral branches,
5 and the pattern did not change with pruning (Fig. 6a, b). However, excess $\delta^{15}\text{N}$ (Fig. 6d) and excess
6 ^{15}N (Fig. 6e) in current-year leaves were not different between the same and opposite side positions
7 relative to the pruned lateral roots, despite the mean values being slightly higher on the same side
8 than on the opposite side. The values of excess ^{13}C and excess ^{15}N characterize new carbon and
9 nitrogen allocation within the plant (Kagawa et al., 2006a, b). Thus, the nitrogen isotope labeling
10 results suggest that nitrogen allocation among leaves from different target branches was not related
11 to their orientation. Different allocation patterns between nitrogen and photosynthates in leaves and
12 fine roots may be related to the anatomy and physiology of xylem and phloem (Savage et al., 2016)
13 rather than to the source-sink relationships. Therefore, the results of carbon and nitrogen allocation
14 partially support our expectations.

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16 *The responses of branches, leaves and fine roots at the same and opposite side positions under*
17 *partial pruning of branches and roots (SP vs. OP)*

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19 The integrated transport system at the whole plant level has an adaptive evolutionary advantage
20 related to a high growth rate (Orians et al., 2005; Zanne et al., 2006; Schenk et al., 2008) and biotic
21 resistance (Aguadé et al., 2015). The functional integration (transport of water, sugars and nutrients)
22 between branches and roots within a tree can be mediated by vascular connections (Orians et al.,

1 2002, 2005; Zanne et al., 2006). The key result of our study on *C. lanceolata* is that the smallest
2 effects on growth and the largest effects on leaf and fine root morphology and physiology (e.g., stem
3 increment, gas exchange traits, leaf water and nutrients, and root carbon status) were detected when
4 opposite side branches and roots had been pruned (OP), while branch and fine root traits were not
5 affected when half of branches and roots from the same side had been pruned (SP). These results
6 provide the first evidence for the view that trees can maintain the functional balance between lateral
7 branches and roots in orthostichous positions, while the functional linkage in opposite side branches
8 and roots is relatively easily disturbed. The evidence comes from isotope labelling experiments, in
9 which both the excess ^{15}N of leaves and excess ^{13}C of fine roots were significantly lower under
10 opposite side pruning than under same side pruning (Fig. 6). This phenomenon may be due to
11 differences in resistance (related to the distance between source and sink organs and architecture of
12 structure) between transport pathways in xylem and phloem in different lateral positions (Orians et
13 al., 2002; James et al., 2003; Thorn and Orians, 2011). Thus, the distinct performance of branches,
14 leaves and fine roots between OP and SP supports the view that leaf and fine root responses are
15 related to the position of pruning (Thorn and Orians, 2011). Our results also suggest that nutrients
16 can be transported in transverse directions via xylem (James et al., 2003; Snyder and Williams,
17 2003).

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19 Some of the compensatory responses observed here might reflect physiological adjustments of leaves
20 that enhance the stress resistance of foliage (Niinemets et al., 2015). In this study, the decreased
21 growth under OP is caused by pruning effects on both photosynthesis and carbon assimilation
22 efficiency (photosynthesis rate divided by respiration rate, Fig. 4a), while g_s (Fig. 3c) was not

1 significantly influenced (although a slight increase was observed 10 weeks after the treatment). This
2 evidence suggests that the decreased photosynthesis rate under OP may have resulted from the
3 reduced maximum carboxylation activity of Rubisco (Flexas et al., 2016). However, a higher
4 transpiration rate (E) was found in OP compared to SP and the control treatment (no pruning).
5 Higher E increases water loss, but higher E is compatible with a greater mass flow from roots to
6 leaves and is expected to result in a greater nutrient uptake. This possibility is supported by similar
7 nitrogen and higher phosphorus concentrations in leaves (Table 3). We observed greater amounts of
8 soluble sugars, proline and total free amino acids (FAA) in leaves in OP, while the leaf water
9 potential was lower in OP than in SP (Table 1; Fig. S2). These responses suggest that the OP
10 treatment caused a greater water stress than SP (Lei et al., 2006). Thus, our results indicate that in OP,
11 *C. lanceolata* has a higher metabolic cost of leaves (higher rates of respiration and transpiration,
12 lower carbon fixation efficiency and water use efficiency), which may be related to nutrient uptake
13 (Dong et al. 2015).

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1 **CONCLUSIONS**

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3 To our knowledge, the current study is the first investigation on the responses of both branches and
4 roots to different types of lateral pruning, specifically on the functional balance of carbon, water and
5 nutrients in branches and roots of tree saplings. We showed that pruning of a half of the shoot and/or
6 root system significantly alters tree growth, branch morphology, current-year leaf and fine root
7 structure, and physiology in *C. lanceolata* and that these effects are related to the horizontal direction
8 of pruning, same-side vs. opposite-side pruning. Our experiments support the source-sink interaction
9 model of branches and roots, and emphasize that the functional balance of carbon distribution
10 between branches and roots is more readily achieved for the same-side pruning compared with
11 opposite-side pruning of roots and leaves. These results provide novel insight into the functional
12 balance of tree carbon, nutrient acquisition and distribution, and water acquisition and use, and into
13 effects of standard forest management practices on forest carbon storage. Further studies are required
14 to examine the mechanisms of the coupling of carbon and nutrient transport through xylem and
15 phloem between leaves and roots, and to test the observed patterns in other species.

16

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20

21 **Conflict of interest** The authors declare that they have no conflicts of interest.

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Table 1. Effects of different pruning treatments on the structural characteristics of branches and structural characteristics, nutrient concentrations and water potential of current-year leaves in *C. lanceolata* in 2012 (10 weeks after pruning) and 2013 (60 weeks after pruning).

	Treatment	M_B (g branch ⁻¹)	L_B (cm branch ⁻¹)	M_L (mg leaf ⁻¹)	A_L (cm ² leaf ⁻¹)	Nitrogen (mg g ⁻¹)	Phosphorus (mg g ⁻¹)	Water potential (MPa)
2012	NP	26.7±0.2b	56.2±0.7b	15.3±0.4a	1.25±0.03ab	18.9±0.5ab	1.14±0.03b	-0.73±0.03ab
	BP	34.8±1.4a	64.5±1.3a	13.3±0.5ab	1.33±0.06a	20.8±1.1a	1.35±0.02a	-0.54±0.04a
	RP _S	19.7±1.3c	45.0±1.5c	11.4±0.9b	1.08±0.06b	16.1±0.3c	1.10±0.04b	-0.97±0.04c
	RP _O	24.7±0.3b	53.0±1.1b	13.5±0.2ab	1.27±0.06ab	15.4±0.2c	1.14±0.03b	-0.81±0.03bc
	SP	25.6±1.2b	56.8±1.3b	14.5±0.9ab	1.30±0.07ab	16.7±0.2bc	1.18±0.04b	-0.92±0.04c
	OP	16.8±0.8c	38.9±2.4c	8.1±0.3c	0.79±0.01c	17.6±0.2bc	1.37±0.03a	-1.5±0.07d
	<i>F</i>	41.27***	39.96***	17.96***	14.45***	14.35***	12.55***	58.01***
2013	NP	36.6±0.9b	79.1±1.0b	14.8±0.6a	1.27±0.04ab	17.1±0.9ab	1.21±0.05bc	-0.87±0.04ab
	BP	51.1±0.7a	96.6±3.0a	13.1±0.4a	1.22±0.02ab	18.4±1.3a	1.48±0.07a	-0.59±0.09a
	RP _S	35.7±1.9b	73.7±1.7b	12.4±0.2a	1.09±0.02b	16.3±0.5ab	1.35±0.02ab	-1.0±0.05b
	RP _O	36.5±1.1b	79.2±1.6b	13.7±0.8a	1.22±0.08ab	13.9±0.3bc	1.16±0.03bc	-0.89±0.01ab
	SP	36.7±1.1b	78.6±1.0b	13.7±0.4a	1.33±0.03a	16.9±0.6ab	1.31±0.04ab	-1.2±0.1b
	OP	28.0±1.3c	63.5±0.9c	7.0±0.6b	0.68±0.06c	12.5±0.7c	1.02±0.05c	-1.8±0.1c
	<i>F</i>	38.27***	40.12***	27.41***	25.33***	7.85***	12.56***	27.34***

M_B , average branch dry mass; L_B , average branch length; M_L , average current-year leaf dry mass; A_L , average current-year leaf area. Values (means ± SE, $n = 4$) followed by the same letters in the same column are not significantly different at $P < 0.05$ according to Tukey's tests. All factorial analyses (ANOVA) are significant at $P \leq 0.001$ (denoted as ***). Treatments are as defined in Fig. 1.

Figure legends

Figure 1. Illustration of the experimental design of *Cunninghamia lanceolata* pruning treatments. NP (no pruning), BP (half of lateral branches pruned), RP (half of lateral roots pruned), SP (half of same side, i.e. the same azimuthal direction, branches and roots pruned) and OP (half of opposite side, i.e. opposite azimuthal direction, branches and roots pruned). Leaves from the same internode in lateral branches in mid-crown were chosen for analyses. Codes for the branch and root samples taken are also shown. NP, no pruning; BP_S, the same side, half of lateral branches pruned; BP_O, the opposite side, half of lateral branches pruned; RP_S, the same side, half of lateral roots pruned; RP_O, the opposite side, half of lateral roots pruned; SP, half of the same side lateral branches and roots pruned; OP, half of the opposite side lateral branches and roots pruned.

Figure 2. Weekly increments of basal stem diameter (a) and tree height (b) of *C. lanceolata* in each treatment, as defined in Fig. 1, in 2012 and 2013. Different letters denote significant differences ($P < 0.05$) according to Tukey's tests among different pruning treatments at each time.

Figure 3. Light-saturated photosynthetic rate (a), dark respiration rate (b), stomatal conductance (c) and transpiration rate (d) of current-year leaves of *C. lanceolata* in each treatment, as defined in Fig. 1, in 2012 and 2013. Different letters denote significant differences ($P < 0.05$) according to Tukey's tests among different pruning treatments at each time.

Figure 4. Specific fine root length (SRL) and fine root respiration rate (R_R) of *C. lanceolata* in each treatment, as defined in Fig. 1, in 2012 and 2013. Different letters denote significant differences among treatments at $P < 0.05$ according to Tukey's tests. Error bars represent standard errors.

Figure 5. Soluble sugars (circles), starch (triangles) and total nonstructural carbohydrate ($\text{NSC}_T = \text{soluble sugars} + \text{starch}$; squares) contents per dry mass in current-year needles (a) and fine roots (b) of *C. lanceolata* in each treatment, as defined in Fig. 1, in 2012 and 2013. Different letters denote significant differences among treatments at $P < 0.05$ according to Tukey's tests.

Figure 6. Excess $\delta^{13}\text{C}$ (a) and excess ^{13}C in target fine roots (b), relative ratio of excess ^{13}C in target fine roots (excess ^{13}C in target fine roots/excess ^{13}C in the labeled current-year leaves; c), excess $\delta^{15}\text{N}$ (d), and excess ^{15}N in target current-year leaves (e), and relative ratio of excess ^{15}N in target current-year leaves (excess ^{15}N in target current-year leaves/excess ^{15}N in the labeled fine roots; f) for samples with the same side orientation (light gray bars) and opposite side orientation (black bars) in *C. lanceolata* in each treatment, as defined in Fig. 1, 15 weeks after the treatments (mean \pm SE, $n = 3$). The ^{13}C -related traits of RP in same or opposite side positions mean that the labeled branch is from the same or opposite side as the corresponding root, respectively; ^{15}N -related traits of BP in same or opposite side positions mean that the labeled root is from the same or opposite side as the corresponding branch, respectively.

Figure 7. PCA based on eco-physiological traits in shoots in each treatment (as defined in Fig. 1; NP, black circle; BP, white square; RPs, white inverse triangle; RPo, white inverse triangle; SP, white star; OP, black star) in 2012 (a) and 2013 (b). DBH and H, basal stem diameter and height increment per week, MB and LB, dry mass and length per branch; ML and AL, dry mass and area per leaf; A, photosynthetic rate; g_s , stomatal conductance; R_d , dark respiration rate of leaf; E, transpiration rate; N and P, leaf nitrogen and phosphorus concentration; SSL, STL and NSCL, leaf sugars, starch and NSC concentration; WP, leaf potential.

Figure 1

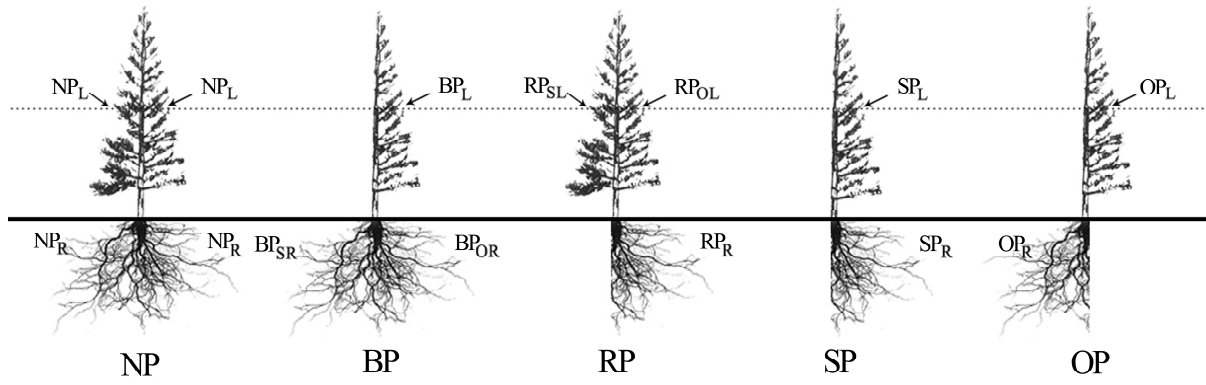


Figure 2

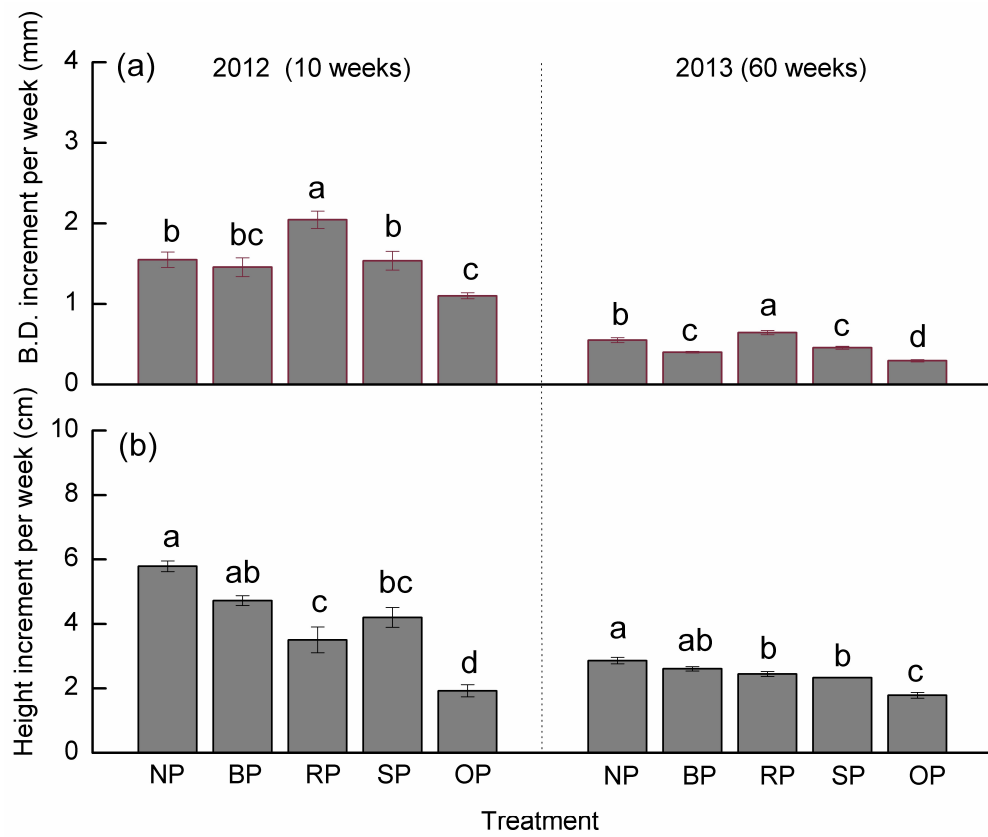


Figure 3

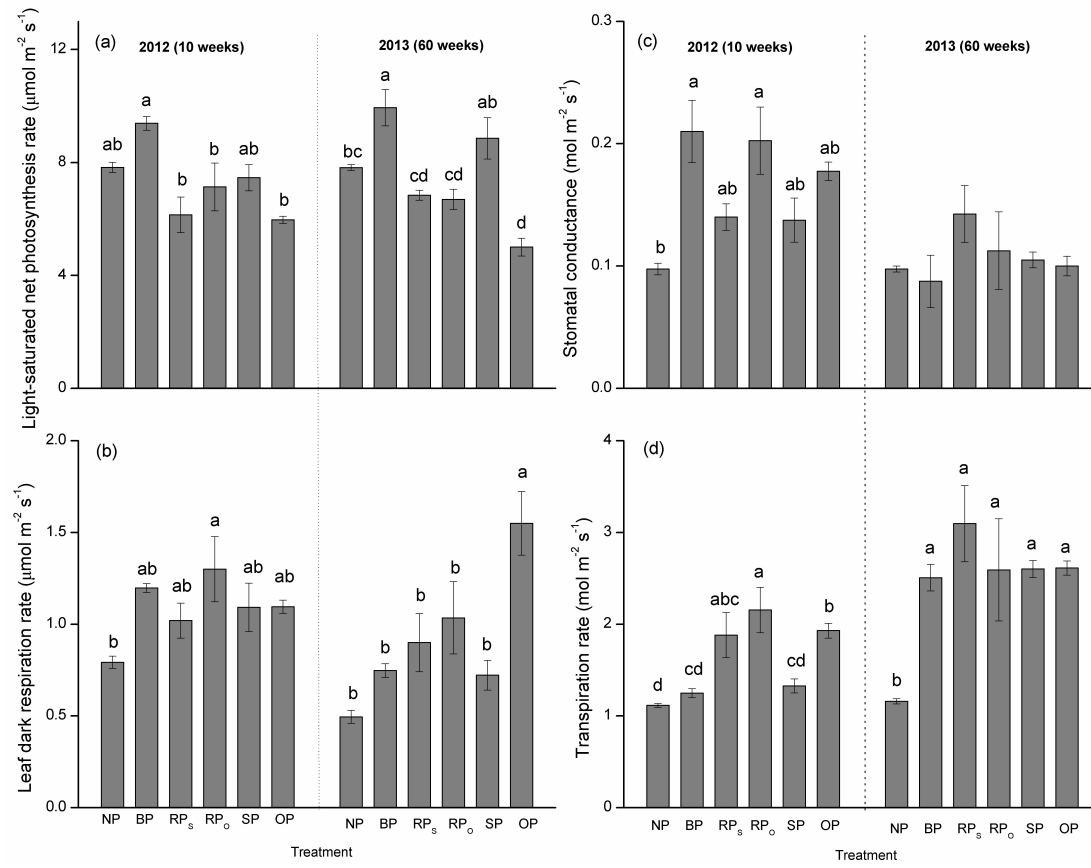


Figure 4

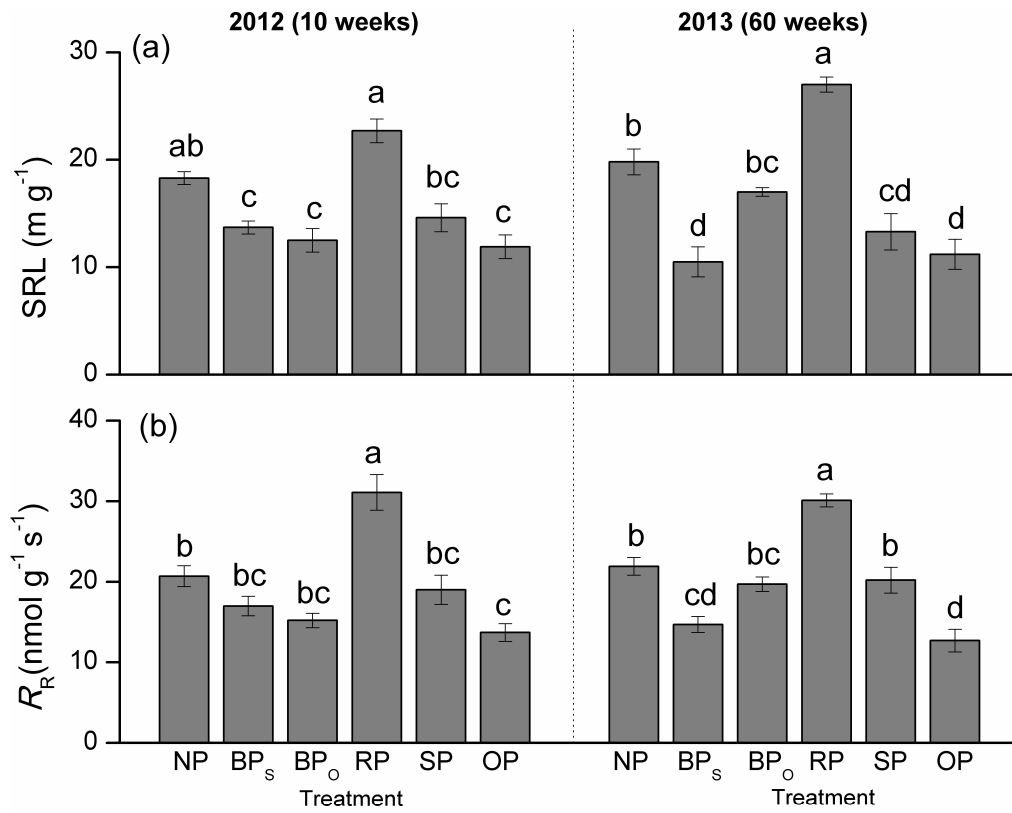


Figure 5

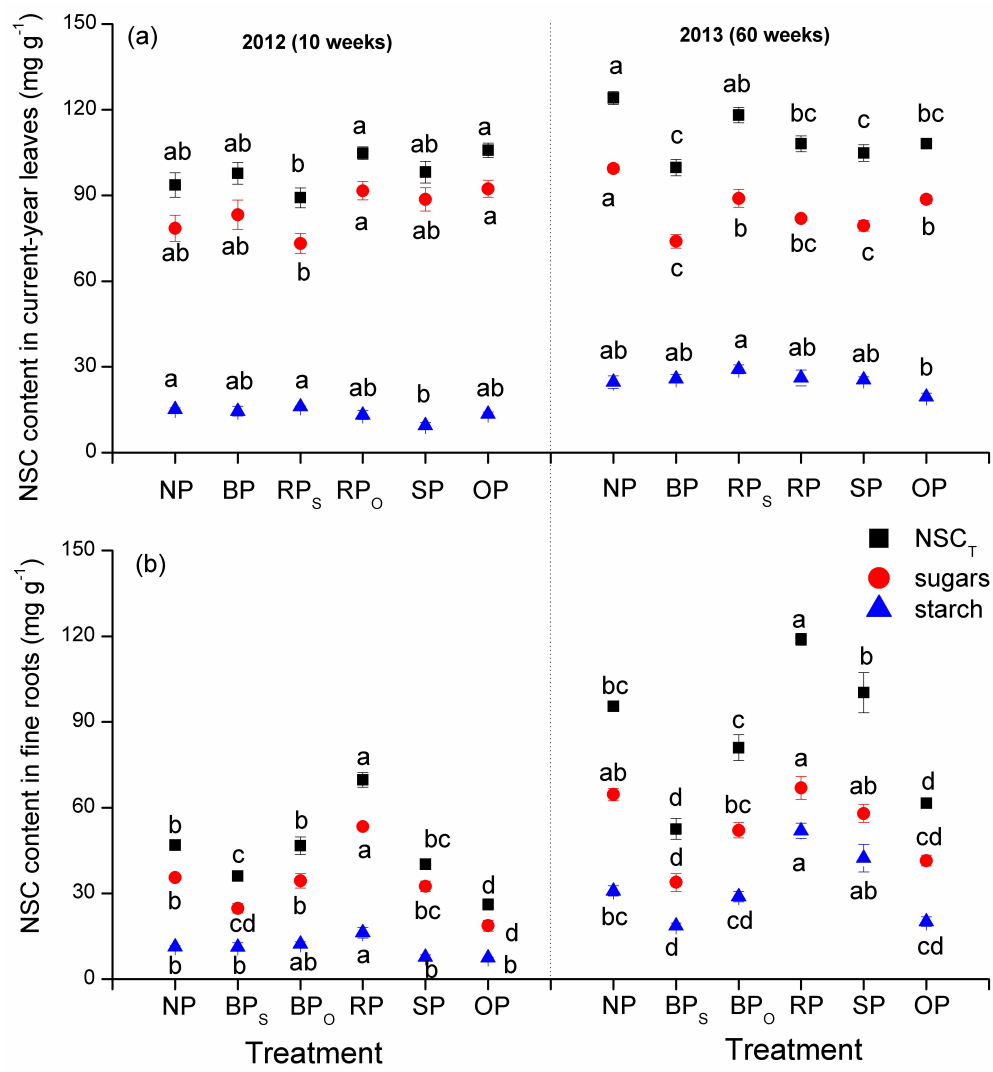


Figure 6

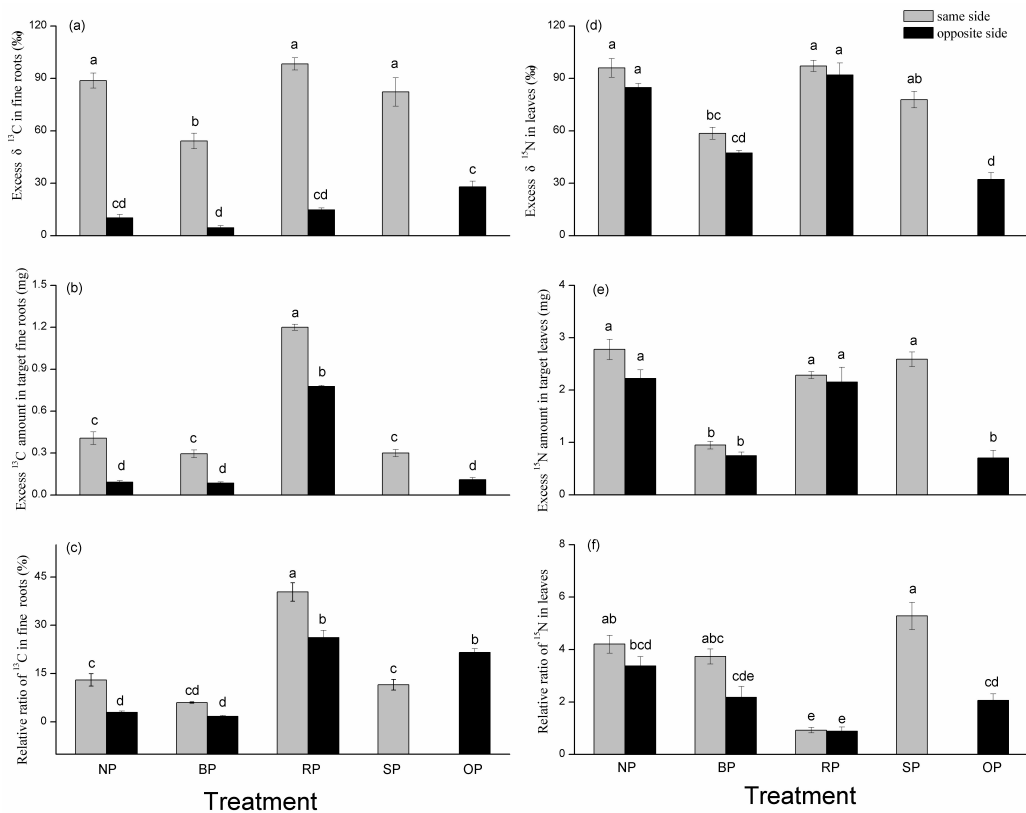


Figure 7

