



Differential springtime branch warming controls intra-crown nitrogen allocation and leaf photosynthetic traits in understory saplings of a temperate deciduous species

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Abstract

Between-branch nitrogen competition is expected to be important during spring in temperate deciduous trees as nitrogen allocation would be higher in branches from earlier budburst than in those from later budburst. Such phenology-induced branch interaction would influence plant photosynthesis, but this has not been evaluated. Warming experiments were conducted on whole crowns (warmed trees; trunks and all branches of the same tree were warmed) or parts of the crowns (warmed branches with unwarmed control branches in the same tree), with unwarmed control trees, in saplings of the deciduous species *Fraxinus lanuginosa*. Spring leaf phenology and leaf photosynthetic traits were investigated to determine how the difference in temperature affects leaf phenology and photosynthetic traits. The timing of budburst was influenced by temperature—budburst was earlier in warmed trees and warmed branches than in control trees and control branches, but budburst timing did not differ between control trees and control branches or between warmed trees and warmed branches. In contrast, leaf traits were affected by the variation in phenology within crowns—nitrogen content and photosynthetic capacity were greater in the leaves of the warmed branches than in the control branches, but they did not differ between the leaves of warmed trees and control trees. Thus, branch warming altered the distribution of nitrogen between warmed and unwarmed branches as warmed branches developed faster, resulting in intracrown variation in leaf photosynthetic traits.

Keywords Bud burst · Correlative inhibition · *Fraxinus lanuginosa* · Intracrown competition · Leaf traits

Introduction

Plants are sessile organisms, and the growth of woody plants is typically characterized by an increase in the number and size of branches within crowns. Branch growth is essential for displaying leaves, receiving light for efficient photosynthesis, and outcompeting surrounding trees in forests. As a consequence, complex crown architecture develops through intensive ramifications during growth. Investigating the physiological mechanisms of branch growth and the

associated leaf traits is important to understand how tree architecture is internally regulated.

The growth and survival of branches have often been studied through the principle of branch autonomy, that is, branches grow independently under their own light environment (Watson and Casper 1984; Sprugel et al. 1991). According to this principle, the more rapid growth of branches receiving higher light availability within the crowns can be explained by greater productivity. Previous studies have investigated the effects of various abiotic and biotic stress factors, such as light, air pollution, and herbivory, on the survival and growth of a limited number of branches, where they have scaled these effects up to whole-plant responses based on the assumption of autonomy (Sprugel et al. 1991).

Nonetheless, an increasing number of studies have shown that branch growth is not autonomous, and the survival, growth, and reproduction of branches in high light availability are enhanced at the cost of the branches under low light availability (Stoll and Schmid 1998; Takenaka

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2000; Henriksson 2001; Osada and Takeda 2003; Umeki and Seino 2003; Osada 2006). Such dominance in the survival and growth of branches under high light availability is referred to as “correlative inhibition” (Novoplansky et al. 1989). Correlative inhibition is considered to be caused by different sink strengths of branches within the crowns. In line with this, leaf photosynthetic traits, such as nitrogen content and photosynthetic capacity, are often enhanced in leaves with high light availability at the expense of leaves with low light availability (Yoshimura 2010; Sugiura and Tateno 2013).

Generally, temperate deciduous tree species drop all their crown foliage during the autumn season and produce new leaves during spring. Because spring growth is achieved using the storage of carbon and nutrients in the tree body (trunks, branches, and roots) (Klein et al. 2016), the sink strength of a given branch will strongly influence the leaf traits of not only that branch but also those of the other branches in temperate deciduous trees. For example, double-labeling of ^{13}C and ^{14}C on the leaves of different branches of deciduous walnut trees indicated that branch carbon was more or less autonomous in the growing season, while spring growth was non-autonomous (Lacointe et al. 2004). Thus, spring branch growth was not independent and the growth enhancement of a certain branch of greater sink strength must result in the suppression of other branches of smaller sink strength within the crowns. Furthermore, variation in budburst timing may influence leaf photosynthetic traits within the crowns, where earlier budbursts could lead to an increase in branch productivity and suppress other branches within the crowns. To the best of our knowledge, such phenology-induced branch interactions within the crowns of temperate deciduous trees have not been evaluated so far.

In addition, the timing of budburst and leaf-out was found to be influenced by temperature and photoperiod in a variety of temperate deciduous tree species (Körner and Basler 2010; Flynn and Wolkovich 2018). Although the relative importance of temperature and photoperiod on the timing of budburst differs among species, ambient temperature around the buds often influences the timing of budburst (Vitasse and Basler 2014). This suggests that experimental manipulation of branch temperature could be effective in evaluating the interrelationship between the leaf phenology and photosynthetic traits within the crowns of deciduous tree species. That is, if the timing of budburst is enhanced in warmed branches, then the nitrogen (N) content and photosynthetic capacity of the leaves of warmed branches would also be enhanced at the cost of the leaves of unwarmed control branches, even under similar light environments. Then, leaf phenology can be directly linked to plant-level N allocation and photosynthesis. Furthermore, this would suggest that warming effects can differ between the branches of the individual in which

whole crown was warmed and the warmed branches of the individual in which crown was partially warmed.

In this study, a warming experiment was conducted on both whole crowns and crown parts in the saplings of *Fraxinus lanuginosa* Koidz. f. *serrata* (Nakai) Murata (Oleaceae) in a cool-temperate forest in Japan. Spring leaf phenology and leaf photosynthetic traits were investigated in the leaves of warmed and control branches to evaluate the different temperature effects among branches with regards to leaf phenology and photosynthetic traits. The following two predictions were made: (1) the timing of budburst would simply be influenced by temperature, where budburst of the warmed branches and warmed trees would occur earlier than in control branches and control trees. However, the timing would not differ between the control branches of branch-warmed trees and control trees, as well as between warmed branches of branch-warmed trees and warmed trees. (2) N content and photosynthetic capacity would be affected by the variation in spring leaf phenology within the crowns due to the enhanced N allocation to the branches of earlier budburst; N content and photosynthetic capacity would be greater in the leaves of warmed branches relative to the leaves of control branches of branch-warmed trees, but they would not differ between the leaves of both warmed and control trees.

Materials and methods

Study site

The field experiment was conducted in the Tomakomai Experimental Forest, Hokkaido University, Japan (42° 40' N, 141° 36' E), where the monthly mean temperature typically ranges between − 3.2 and 19.1 °C and the annual rainfall is 1450 mm. The maximum and minimum monthly temperatures were 21.2 °C and − 10.1 °C in August and January, respectively. The forest was mainly comprised of the cool-temperate deciduous forest of approximately 20–25 m in canopy height and dominated by *Acer pictum* Thunb., *Acer amoenum* Carrière (Aceraceae), *Cercidiphyllum japonicum* Siebold et Zucc. ex Hoffm. et Schult. (Cercidiphyllaceae), *Ostrya japonica* Sarg. (Betulaceae), *Padus ssiroi* (F. Schmidt) C. K. Schneid, and *Quercus crispula* Blume (Fagaceae) (Hiura et al. 1998; Miyata et al. 2011; Osada and Hiura 2017).

Warming experiment

Fraxinus lanuginosa Koidz. f. *serrata* (Nakai) Murata (Oleaceae) is a shade-tolerant understory species of approximately 12 m at maximum height (Osada and Hiura 2019) and typically produces compound leaves of five leaflets. A total of 20 saplings of 1–2 m in height were selected within

the shaded understory in March 2016. Parts, or whole, crown branches were warmed using electric heating cables containing copper resistance wires with an output of 1000 W at 200 V (Nihon Noden, Tokyo, Japan) (Fig. 1). The cables were attached along the trunks and branches of the whole crowns in four saplings (TW: tree warmed), or along one 1st-order branch of 0.5–1.2 m in length, which directly emerged from the main trunk, and its associated higher-order branches, while excluding the other crown parts (BW: branch warmed). Warming was initiated on March 31 prior to any signs of budburst and ceased on June 2 after leaf maturation. Therefore, summer temperatures were similar between the treatments. Four saplings served as controls without cables (CO). In addition, the effects of cable loads were examined

in terms of the cables attached to whole crowns of four saplings (TC: tree cable) and selected branches, but not in other crown parts as in the BW treatment (BC: branch cable), where the cables were not heated.

To maintain the temperature of the warmed branches and trees at about 5 °C above ambient conditions, simple electronic temperature controllers (Omron, Kyoto, Japan) were connected to thermocouples (K cables) to control the power supply as reported in Nakamura et al. (2010). The thermocouples were taped to the branches with the heating cables while avoiding direct contact between the thermocouples and the heating cables, and were also taped to the controls. Branch temperature of the thermocouples was measured using a temperature sensor (Graphtec midi-logger, Graphtec Corporation, Japan) at 5-min intervals across four positions that consisted of the warmed branches of the TW treatment, warmed and control branches of the BW treatment, and branches of the CO treatment in selected saplings. Because the temperature could have been heterogeneous in the forest, the temperature was additionally measured every hour using small temperature loggers (Thermocron G, KN Laboratories, Osaka, Japan) with waterproof caps (DS9720W, KN Laboratories, Osaka, Japan) between April 9 to June 14. The waterproof caps were attached to the branches without directly touching the cables on three saplings per treatment of TW, BW_W, BW_NW, CO, BC_C, BC_NC, and TC. Temperature was also measured every 30 min between January and August in the forest understory of 1 m height using a temperature logger (Tidbit V2, Onset Computer Corporation, Bourne, MA, USA), which also served as a control. The temperature measured by the different apparatus was consistent, and thus the mean monthly temperature was calculated per treatment (Table 1).

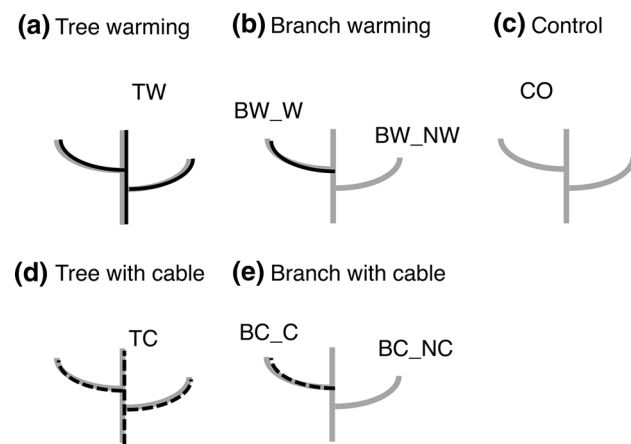


Fig. 1 The experimental design of the warming experiment. Trees and heating cables are shown in gray and black, respectively, with unheated cables are in broken lines. Electric heating cables were attached **a** along the trunks and branches of whole crowns (TW: tree warmed) and **b** along one 1st-order branch with its associated higher-order branches (BW: branch warmed), resulting in BW saplings with warmed (BW_W) and unwarmed (BW_NW) branches. Controls (**c**) were not treated with cables (CO). In addition, **d** unheated cables were attached to the whole crowns (TC: tree cable) and **e** along one 1st-order branch with its associated higher-order branches (BC: branch cable), resulting in BC saplings with cabled (BC_C) and non-cabled (BC_NC) branches

Phenology and leaf photosynthetic traits

Leaf phenology was investigated on a weekly basis between April 11 (day of year, DOY 102) to June 5 (DOY 157). The phenological time points were categorized, based on the conditions of buds or the two basal leaves that emerged from

Table 1 Seasonal changes in the mean monthly temperatures (°C) of each treatment

	J	F	M	A	M	J	J	A
CO	− 5.0	− 3.7	0.4	6.3	12.2	13.5	17.2	21.0
TW				11.9	18.0	13.6	16.8	20.6
BW_W				11.5	18.6	13.8	17.1	20.8
BW_NW				6.1	12.1	13.5	17.1	20.8
BC_C				6.0	12.0			
BC_NC				6.2	12.2			
TC				6.1	12.1			

TW tree warmed, BW branch warmed, BW_W warmed, BW_NW not warmed, CO control, TC tree cable, BC branch cable, BC_C cabled, BC_NC non-cabled

the buds, into five stages: stage 0 (dormant bud) with no visible bud development, stage 1 (budburst) with budburst and bud scales opening as well as identifiable leaf primordia, stage 2 (leaf-out) with identifiable individual leaves and a basal leaf area less than 50% of the mature leaf area, stage 3 (leaf expansion) with a basal leaf area of more than 50% of the mature leaf area, and stage 4 (end of leaf expansion) with a basal leaf area of more than 90% of the mature leaf area and almost maximum leaf expansion (Osada 2017; Osada and Hiura 2019). These stages were classified based on the conditions of the five selected buds/shoots on the tree in the TW, CO, and TC treatments, or based on those of each of the five selected buds/shoots of the BW_W and BW_NW branches in BW treatment and in the BC_C and BC_NC branches in the BC treatment. The phenological variation was found to be small within the crowns in the TW, CO, TC, and BC treatments. In addition, leaf length was measured on a weekly basis from May 9 (DOY 130) to June 5 (DOY 157) in the five selected leaves per tree or five leaves per branch of BW_W, BW_NW, BC_C, and BC_NC. As proxy for leaf chlorophyll content, soil plant analysis development (SPAD) values were measured in the same leaves using a SPAD meter (SPAD-502 Plus, Minolta, Japan) on a weekly basis from May 29 (DOY 150) to June 19 (DOY 171) as well as on July 3 (DOY 185) and August 4 (DOY 217).

Maximum photosynthetic rate (P_{\max}) was measured in the leaves of TW, BW_W, BW_NW, CO, BC_C, and BC_NC on August 4 and 5 (DOY 217 and 218) using a portable photosynthesis measurement system (LI-6400, Li-Cor, Lincoln, NE, USA). While measuring, the photosynthetic photon flux density in the chamber was maintained at $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, the temperature was 25°C , and the CO_2 concentration was $400 \mu\text{mol mol}^{-1}$. After measuring leaf photosynthesis, leaves were harvested and five discs of 5.5 mm in diameter were collected from each leaf. The discs were oven-dried at 60°C for more than a week, where the dry mass was measured. N content per area (N_{area}) and per mass (N_{mass}) in the discs was determined (NC-900, Sumitomo Chemical, Osaka, Japan), while leaf mass per area (LMA) was calculated as the ratio of disc mass to disc area.

Light availability

Photon flux density (PFD) was measured using quantum sensors (LI-190, Li-Cor, Lincoln, NE, USA) on a cloudy day (August 7) in each sapling for leaf photosynthesis measurements. PFD was measured just above the crowns of each sapling. For the BW and BC treatments, PFD was measured just above the cabled branch and the non-cabled part of each crown. At the same time, PFD was measured at the top of the canopy tower near the study site by connecting the sensor to a data logger (LI-1400, Li-Cor), where the relative PFD was calculated.

Statistics

Linear mixed models were applied to investigate the difference in the day of budburst (phenological stage 1), the day of the end of leaf expansion (phenological stage 4), the duration of leaf expansion (days from phenological stage 1–4), leaf light environment, and leaf traits among the treatments, using the lmer function in the lme4 package in the statistical software R (R Development Core Team 2018). In this procedure, individual trees were considered a random factor, and multiple comparison analyses were performed using the glht function in the multcomp package in R. In addition, the relationship between N_{area} and P_{\max} was investigated using the standardized major axis regression method using the sma function in the smatr package in R, where the difference in the relationship between treatments, indicated by the differences in the slope or shifts in the elevation along a common slope, was tested by likelihood methods (Falster et al. 2006).

Results

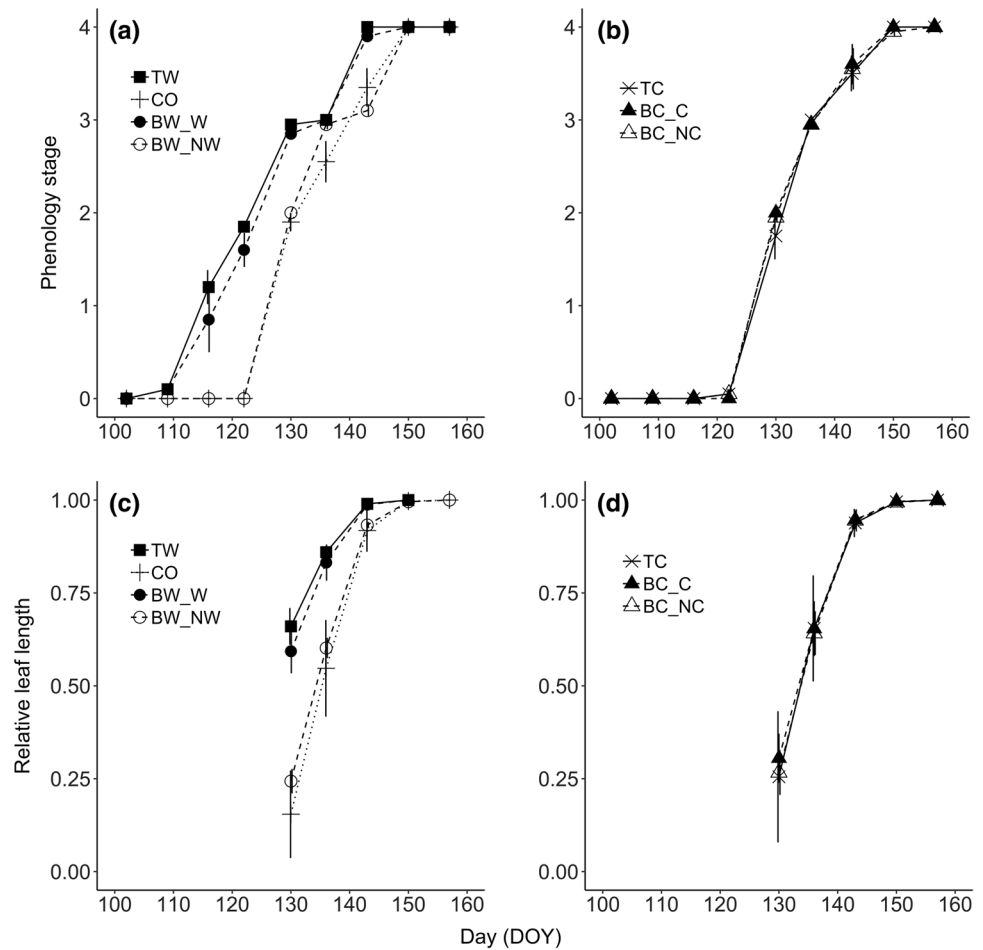
Leaf phenology

Experimental warming influenced the budburst timing of *F. lanuginosa* (Figs. 2a, b and 3, Table S1). Budburst timing in TW and BW_W was approximately 10 days earlier than in the other treatments and did not differ in CO, TC, BC_C, and BC_NC (Fig. 3, Table S1). Earlier phenologies in TW and BW_W continued until the end of leaf expansion, although the difference decreased from approximately 10 to 5 days during leaf development (Figs. 2 and 3). As a consequence, the duration of leaf expansion was longer in TW and BW_W than in other treatments (Fig. 3c). The trend of seasonal change in relative leaf length was similar to the phenological stages (Fig. 2c, d). In addition, the SPAD value continued to increase until June 19 (DOY 171) and then became stable across all treatments (Fig. 4).

Light environment and leaf traits

Relative PFD was measured to be approximately 2–5% across all trees (Fig. 5a). Although the trees were selected in the shaded understory, the light environment varied among the sample trees and differed significantly between different combinations, such as between CO and BC_C as well as between BW_NW and BC_C (Fig. 5a, Table S2). Consistent trends were not found among warmed and unwarmed treatments. Furthermore, the difference in LMA across treatments was not consistent with the light environment (Fig. 5a, b, Table S2), where LMA was greater in TW relative to BW_NW and BC_C as well as in BW_W relative to BW_NW (Fig. 5b, Table S2). N_{mass} did not differ across treatments (Fig. 5d, Table S2), but

Fig. 2 Seasonal changes in the phenology stage (a, b) and the relative leaf length (c, d) of *Fraxinus lanuginosa* in each treatment group [mean \pm standard error (SE)]. *TW* tree warmed, *BW_W* branch warmed, *BW_NW* not warmed, *CO* control, *TC* tree cable, *BC_C* cabled, *BC_NC* non-cabled



N_{area} , maximum photosynthetic rate (P_{max}), and the SPAD value displayed similar trends, and their values were only greater in BW_W relative to BW_NW and not across other combinations (Fig. 5c–f, Table S2).

In addition, P_{max} was linearly correlated with N_{area} when pooling all the leaf data (Fig. 6a). The slope and elevation between P_{max} and N_{area} did not differ between TW and CO (Fig. 6b; slope, $P=0.15$; elevation, $P=0.21$), BW_W and BW_NW (Fig. 6c; slope, $P=0.36$; elevation, $P=0.93$), or BC_C and BC_NC (Fig. 6d; slope, $P=0.91$; elevation, $P=0.24$). The shift along the common standardised major axis line was not detected between TW and CO ($P=0.49$) as well as between BC_C and BC_NC ($P=0.84$), but it was detected between BW_NW and BW_W ($P=0.041$). In other words, both N_{area} and P_{max} were greater in BW_W relative to BW_NW and had similar slopes and intercepts (Fig. 6c).

Discussion

Leaf traits were linked to spring leaf phenology, where the variations in budburst timing and leaf maturation within the crowns clearly affected the N distribution and leaf

photosynthesis in the deciduous species *F. lanuginosa* in the artificial warming experiment. As a consequence, the predictions that were supported included: (1) Budburst timing was influenced by branch temperature and budburst occurred earlier in BW_W relative to BW_NW and in TW relative to CO, but it did not differ between BW_NW and CO as well as between BW_W and TW (Figs. 2 and 3). (2) Nitrogen content and photosynthetic capacity were affected by the variation in spring leaf phenology within the crowns, and were greater in the leaves of BW_W relative to BW_NW, but did not differ between the leaves of other treatment combinations (Figs. 5 and 6).

Branch warming and seasonal changes in leaf traits

Spring leaf phenology is often influenced in a complex manner by temperatures of winter (chilling) and spring (forcing) as well as the photoperiod in temperate species (Körner and Basler 2010; Flynn and Wolkovich 2018). In this study, leaf phenology was found to occur earlier in TW and BW_W relative to CO and BW_NW, whereas it was similar between TW and BW_W as well as between BW_NW and CO (Figs. 2 and 3). Moreover, leaf phenology was

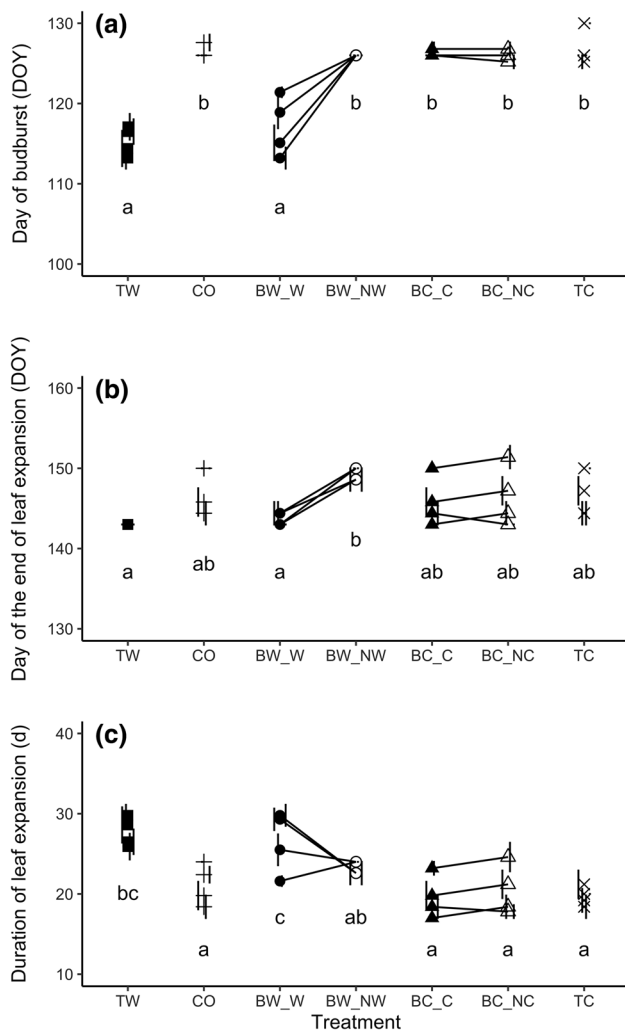
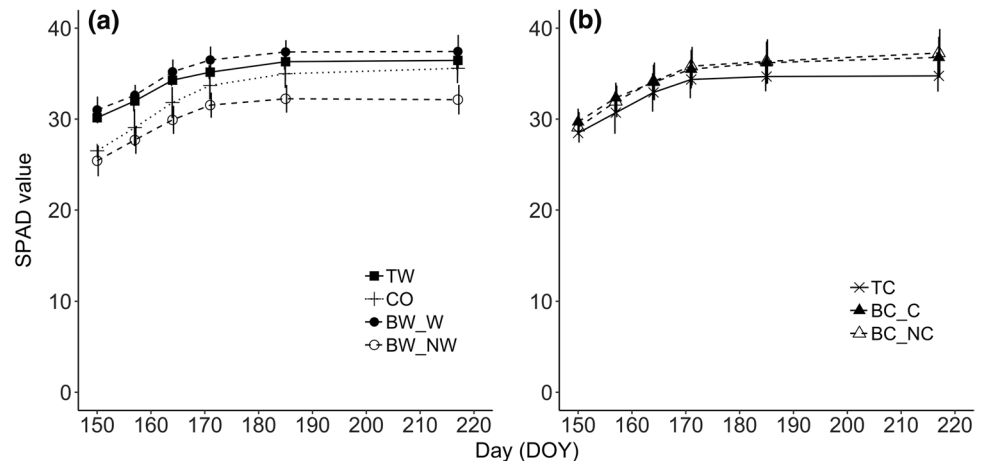


Fig. 3 Differences in **a** the days of budburst, **b** the end of leaf expansion, and **c** the duration of leaf expansion among treatments in *Fraxinus lanuginosa*. Different characters indicate significant differences at $P < 0.05$. The same tree individuals are connected by lines in BW and BC treatments. TW: tree warmed, BW branch warmed, BW_W warmed, BW_NW not warmed, CO control, TC tree cable, BC branch cable, BC_C cabled, BC_NC non-cabled

Fig. 4 Seasonal changes in the soil plant analysis development (SPAD) value of *Fraxinus lanuginosa* leaves in **a** the warmed and control treatments and **b** cabled treatments [mean \pm standard error (SE)]. TW tree warmed, BW branch warmed, BW_W warmed, BW_NW not warmed, CO control, TC tree cable, BC branch cable, BC_C cabled, BC_NC non-cabled

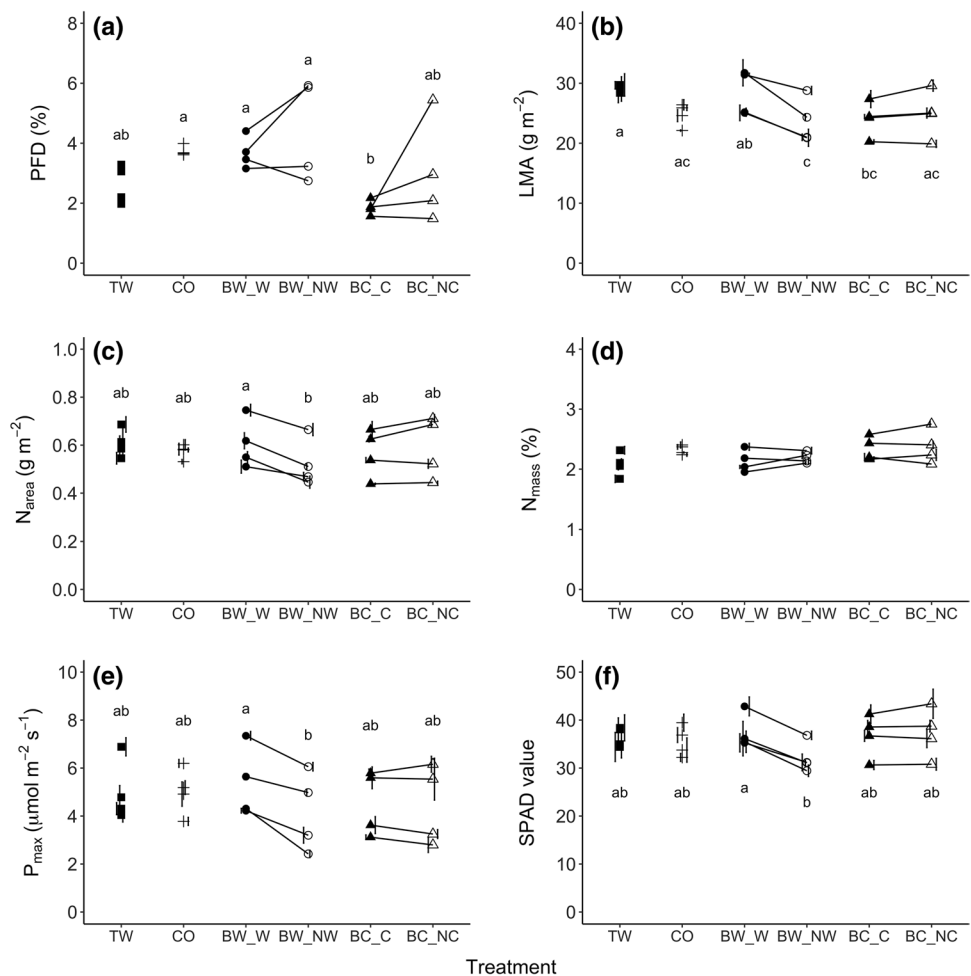


similar between TC, BC, and CO, indicating that the load of cables did not influence the results. Thus, the ambient temperature around the branches probably influenced the spring leaf phenology in *F. lanuginosa*. The temperature and/or photoperiod were considered to be perceived by the buds (Zohner and Renner 2015), and consequently the results could be consistent between the treatments that warmed the trees and branches.

Although the day of budburst was earlier in TW and BW_W than in other treatments, the duration of leaf expansion was longer in the former and thus the difference of the end of leaf expansion was smaller than that of the day of budburst (Fig. 3). The variation in the rate of leaf development is often related to leaf light environment (Ninemetts et al. 2012), but this was not the case in this study (Figs. 3 and 5). The seasonal changes in temperature might be the cause of the difference in the duration of leaf expansion, since temperature increase is accelerated with the progress of the season.

Temperate deciduous trees generally store *N* in the tree body in winter, and remobilized *N* from the storage is an important source of leaf expansion in spring (Millard and Grelet 2010). *N* remobilization usually occurs prior to *N* uptake from the soil in a range of temperate species, where *N* uptake by roots may compensate for the limited *N* (Millard 1996; Ueda et al. 2009; Millard and Grelet 2010). In this study, the trees were chosen in the shaded understory and the shoot growth rate was very low (approximately 1–4 cm/year in length). This implies that the trees would not show strong *N* requirement, or *N* uptake from the soil could have been insufficient to compensate for the decreased *N* in the BW_NW branches. Alternatively, the sink strength of BW_W branches could have been greater than that of BW_NW branches, thus, soil *N* could have been allocated preferentially to BW_W branches. It was interesting to investigate how remobilized *N* and absorbed *N* were allocated within the crowns in branch-warmed trees because *N* allocation is critical for productivity at the individual tree level.

Fig. 5 Values of **a** photon flux density (PFD) and leaf photosynthetic traits, including **b** leaf mass per area (LMA), **c** N content per area (N_{area}), **d** N content per mass (N_{mass}), **e** maximum photosynthetic rate (P_{max}), and **f** the soil plant analysis development (SPAD) of *Fraxinus lanuginosa* in each treatment in August [mean \pm standard error (SE)]. Different characters indicate significant differences at $P < 0.05$. The same tree individuals are connected by lines in BW and BC treatments. TW tree warmed, BW branch warmed, BW_W warmed, BW_NW not warmed, CO control, TC tree cable, BC branch cable, BC_C cabled, BC_NC non-cabled



Tracing experiments with ^{15}N would help in understanding the dynamics of N in relation to the within-crown variation in spring leaf phenology.

In general, leaf photosynthetic traits in temperate deciduous species, such as N_{area} and P_{max} , increased during leaf expansion in spring, became stable from late spring to early autumn, and then decreased in late autumn (Koike 1990; Muraoka and Koizumi 2005; Uemura et al. 2005). The SPAD value was highly correlated with the area-based chlorophyll content of the leaves and showed similar seasonal trends relative to N_{area} and P_{max} (Muraoka and Koizumi 2005; Uemura et al. 2005). The SPAD value was stable at the time of measuring photosynthesis (Fig. 4). Therefore, the leaf traits in summer were not influenced by the difference in leaf age between the warmed and unwarmed branches.

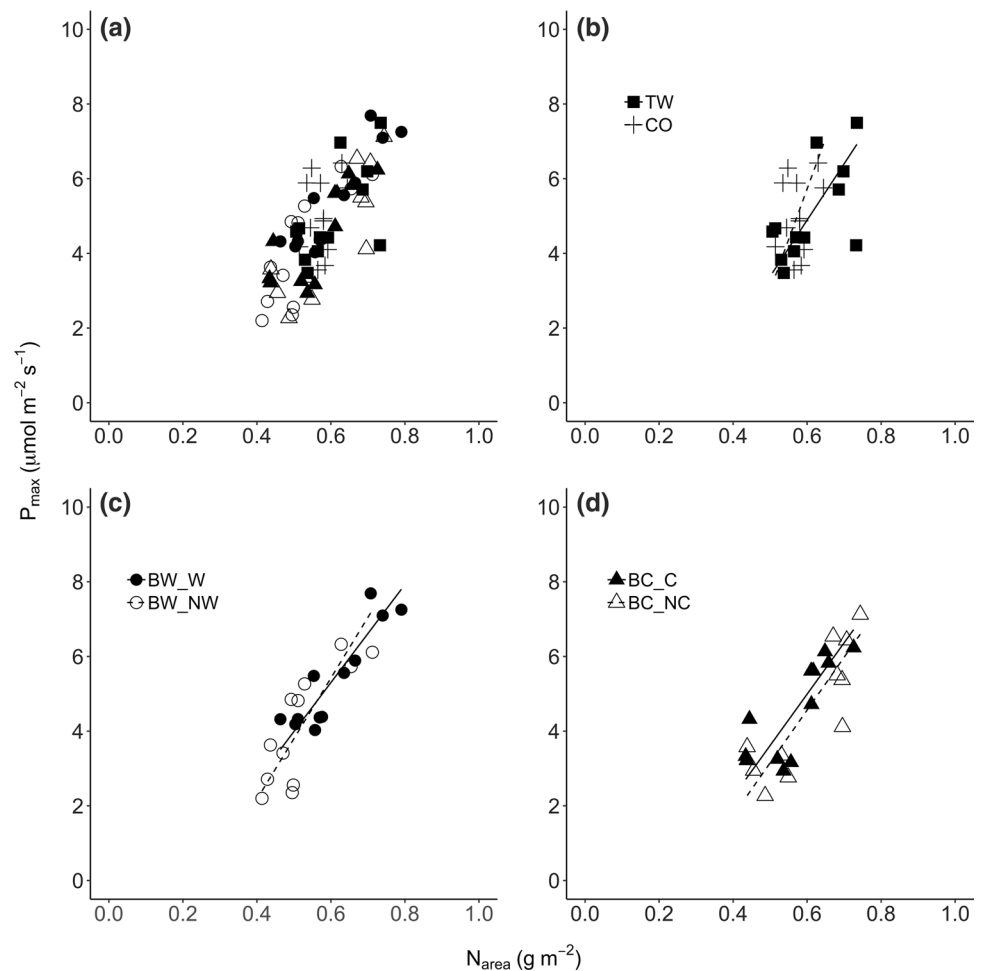
Intracrown variation in leaf traits

Correlative inhibition has been studied in a variety of tree species, where intensive growth in the branches of higher light availability occurs at the cost of growth of the branches at lower light availability (Stoll and Schmid 1998; Takenaka

2000; Henriksson 2001; Sprugel 2002; Osada and Takeda 2003; Umeki and Seino 2003; Osada 2006; Umeki et al. 2006). In this study, the trees were chosen in the shaded understory with a biased N allocation and differences in leaf photosynthesis, which were not caused by differences in light availability between branches (Fig. 5). Moreover, the warming effects on leaf traits were often influenced by temperature acclimation (e.g. Yamaguchi et al. 2016). In this study, warming was conducted before and during leaf development (from March 31 to June 2), but temperature acclimation was unclear when comparing the leaves of TW and CO in terms of the leaf traits LMA, N_{area} , and P_{max} (Fig. 5) as well as the relationship between N_{area} and P_{max} (Fig. 6). These results strongly imply that the variation in spring leaf phenology influenced the leaf traits of BW trees as a consequence of N allocation within the crowns.

N_{mass} did not differ between branches, but LMA, N_{area} , and P_{max} were greater in BW_W relative to BW_NW. These relationships were similar to light-dependent differences in leaf traits within the crowns, in which the leaves of high light availability corresponded to the leaves of BW_W, while those of low light availability corresponded to BW_NW

Fig. 6 The relationship between N content per area (N_{area}) and maximum photosynthetic rate (P_{max}) in the leaves of *Fraxinus lanuginosa* in each treatment for **a** all treatments, **b** TW and CO, **c** BW and BW_W, and **d** BC_C and BC_NC. Standard major axis regressions are depicted for each treatment by solid and dashed lines. TW tree warmed, BW branch warmed, BW_W warmed, BW_NW not warmed, CO control, TC tree cable, BC branch cable, BC_C cabled, BC_NC non-cabled



(Ellsworth and Reich 1993; Holbrook and Lund 1995; Osada et al. 2014). It is important to note that PFD was not greater in BW_W relative to BW_NW in this study (Fig. 5). Thus, earlier budburst might have enhanced not only N allocation but also carbon allocation to increase LMA, where greater sink strength in the BW_W branches might have caused similar effects to leaf traits as the branches of higher light availability within the crowns.

Notably, the studied species showed determinate shoot growth with a flush type of leaf phenology, in which the preformed leaf primordia in winter buds develop during leaf emergence in a short period of time during spring. Leaf traits differed between BW_W and BW_NW in this study (Fig. 5), which is in line with the findings of Uemura et al. (2000), who experimentally investigated the effects of previous and current-year light environments on leaf photosynthetic traits by setting shade cloths in the upper crowns of two *Fagus* species. They found that the leaf traits were partly determined at the time of bud formation in the preceding year, but the effects of current-year irradiance were greater on leaf-area-based daily carbon gain than the previous-year irradiance. Moreover, N_{area} and P_{max} of the mature leaves of

F. lanuginosa increased with higher light availability after the gap formation in the current year at the same study site (Oguchi et al. 2006). Thus, some leaf traits might have been determined before the start of the warming (March 31), but other traits could have been influenced by the warming in our study. This work illustrated that leaf traits could easily be changed in the current year by the enhanced carbon and N allocation to the warmed branches due to the greater sink strength of the earlier budburst branches and clearly affected the leaf traits of unwarmed branches. Such intracrown interactions have not been considered in most previous studies of environmental change, but should be considered because they might influence the degree of change in leaf traits, which might be limited by the total N in the tree body and physiological connectivity within crowns.

Future perspectives

One of the most important findings of this study was that the warming effects on leaf phenology and leaf traits were different. On the one hand, leaf phenology was affected by warming similarly in both TW and BW_W (Figs. 2 and 3) as

the temperature around the buds might be an important determinant of spring leaf phenology, and thus, branch warming served as a surrogate for warming the whole tree (Nakamura et al. 2010). Such results could be particularly important when studying tall trees with large crowns as artificial warming of whole crowns is quite difficult, and validate the use of cuttings in spring leaf phenology studies (Vitasse and Basler 2014). On the other hand, branch warming might not be valid to study warming effects on leaf photosynthetic traits (Yamaguchi et al. 2016) because *N* allocation within the crowns may alter the leaf traits. Thus, whole crowns should be warmed when investigating the effect of warming on leaf photosynthetic traits. It is important to investigate whether similarly biased *N* allocation can occur in tall trees with a large and complex crown architecture, because it would be restricted by physiological connectivity within crowns.

Overall, this study has clearly demonstrated that intracrown variation in the timing of budburst influenced *N* allocation and productivity. Evidence was found despite the limited number of trees used in this study due to the abundance of the species in the forest understory and the limited length (50 m) of the electric cables to use experimentally. Although this study artificially changed the timing of the budburst and the leaf emergence within the crowns of an understory species, there may be a consistent difference in the intracrown timing of budbursts in some species. For example, the timing of budburst is generally earlier in upper than in lower crowns of *Fagus crenata* (< 1 week, Osada, N. unpublished data). The difference is shorter than the experimentally induced difference of this study, yet it might be partially responsible for the intracrown variation in leaf *N* distribution patterns (Osada et al. 2014). Evaluation of spring leaf phenology and ambient environment, such as light on leaf *N* allocation and productivity at the individual tree level, would be important to predict future climate change effects on tree performance.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00442-021-04929-4>.

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