

# Experimental branch warming alters tall tree leaf phenology and acorn production

Masahiro Nakamura<sup>a,b,\*</sup>, Onno Muller<sup>a</sup>, Shiori Tayanagi<sup>a</sup>, Tatsuro Nakaji<sup>a</sup>, Tsutomu Hiura<sup>a</sup>

<sup>a</sup> Tomakomai Research Station, Field Science Center for Northern Biosphere, Hokkaido University, Takaoka, Tomakomai 053-0035, Japan

<sup>b</sup> Nakagawa Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Otoiueppu, Nakagawa 098-2501, Japan

## ARTICLE INFO

### Article history:

Received 1 February 2010

Received in revised form 1 April 2010

Accepted 1 April 2010

### Keywords:

Canopy crane

Field experiments

Global warming

Leaf fall

Masting

## ABSTRACT

An increasing number of field experiments have been initiated worldwide to study simulated effects of global warming. To experimentally determine how tall, mature trees respond to warming, we developed a new technique that heated canopy-level branches with electric heating cables. Using a canopy crane, we attached electric cables to top canopy branches of tall, mature *Quercus crispula* Blume (18–20 m in height) trees; shoot temperature was elevated 5 °C above ambient. Branch warming extended the length of the growing season of canopy leaves by later leaf fall. Moreover, branch warming increased acorn production. Application of this technique should lead to a better understanding of how tall, mature trees respond to global warming.

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## 1. Introduction

Greenhouse gas emissions are expected to raise global mean temperature over the next century by 1.8–4.0 °C, with the largest increases at higher latitudes (IPCC, 2007). Temperature is a key factor that regulates many terrestrial processes (e.g. Shaver et al., 2000), and large changes in natural ecosystems have been attributed to global warming (e.g. Penuelas and Filella, 2001; Walther et al., 2002). However, there are still many uncertainties about the manner in which natural ecosystems respond to global warming. Within recent decades ecologists around the world have initiated field manipulations (mimicking expected global warming) to determine responses of terrestrial ecosystems to changing temperature (Shen and Harte, 2000; Rustad et al., 2001). Many techniques (e.g. greenhouses, open-top chambers, and electric infrared heaters) have been developed to experimentally warm a variety of ecosystems or ecosystem components, including tundra, grasslands, and forests (reviewed by Rustad et al., 2001).

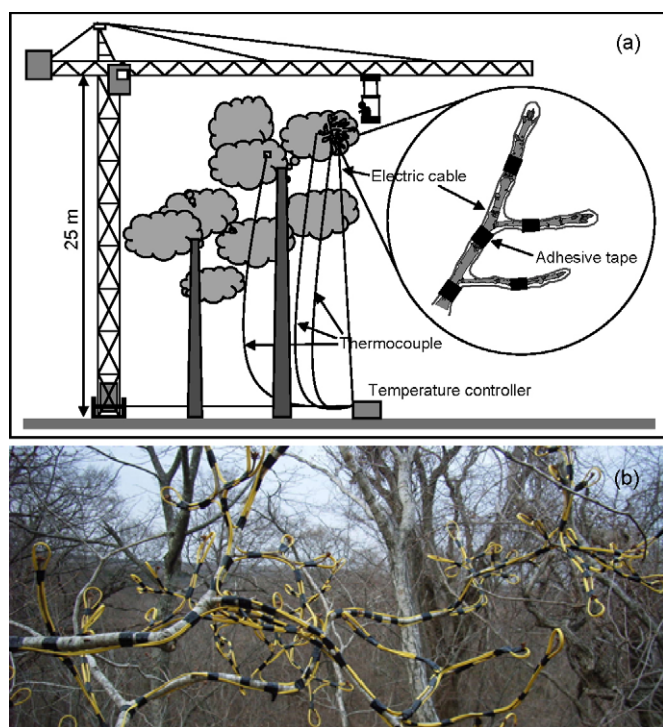
In forests, most studies have focused on effects of experimental warming on soil respiration, net N mineralization, and above-ground productivity of understory plants (reviewed by Rustad et al., 2001). Soil temperature elevation by 5 °C above ambient tem-

perature had significant short-term effects on soil organic decay and long-term effects on mineral nitrogen (Peterjohn et al., 1993; Mellilo et al., 2002). However, most biological activities and species diversity within forests are concentrated in the canopy, rather than in the understory (Basset et al., 2003; Ishii et al., 2004). This canopy effect is attributed to higher illumination in the upper forest strata, which promotes rapid rates of photosynthesis that, in turn, promote high plant productivity, thereby sustaining a community of animals that is more abundant and diverse than in the understory (Basset et al., 2003). Thus, there is a considerable interest in the ways in which canopy-level biota respond to global warming.

However, access to the canopy technically limits ecosystem-scale investigations. Thus, experiments at the upper levels of tall trees are rare (but see the web-FACE, Pepin and Körner, 2002; Körner et al., 2005). In this work (op. cit.), CO<sub>2</sub> was manipulated at the canopy level. A construction crane was used to deploy a fine web of tubes that was woven into the tree canopy and released CO<sub>2</sub>. However, to our knowledge, there has been no temperature manipulation experiment that warmed canopy strata in natural tall tree forests. To better understand how natural forest canopy responds to global warming, we developed a new technique to warm branches with electric heating cables. We worked in the Tomakomai Experimental Forest on Hokkaido using a construction crane to allow non-destructive access to the canopy. The trees we studied were tall and mature (18–20 m in height). The independent variable was branch warming (with electric cables), and the response variables were leaf flushing and fall, and acorn production.

\* Corresponding author at: Nakagawa Experimental Forest, Field Science Center for Northern Biosphere, Hokkaido University, Otoiueppu, Nakagawa 098-2501, Japan. Tel.: +81 1656 5 3218; fax: +81 1656 5 3216.

E-mail address: [masahiro@fsc.hokudai.ac.jp](mailto:masahiro@fsc.hokudai.ac.jp) (M. Nakamura).



**Fig. 1.** (a) Diagram showing branch warming procedure at canopy crane site. (b) An electric heating cable fixed to branches with adhesive tapes [enlargement of (a)].

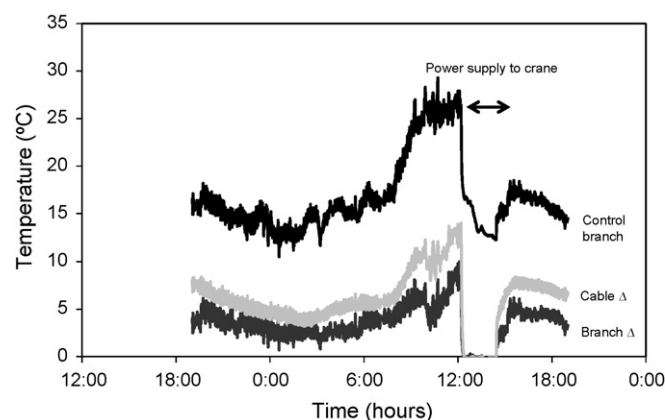
## 2. Materials and methods

### 2.1. Study area

Tomakomai Experimental Forest (TOEF; 42°40'N, 141°36'E) is located on flat land and has an area of 2715 ha. Carbon cycling and budget in this forest were investigated using the eddy covariance method and biometric technique for the long-term (Hiura, 2005; Shibata et al., 2005). The construction crane is located in a mature deciduous woodland with *Quercus crispula* Blume, *Ostrya japonica* Blume, *Acer mono* Maxim, *Cercidiphyllum japonicum* Sieb. Et Zucc., and *Tilia japonica* Simonkai (for a detailed description see Hiura et al., 1998; Hiura, 2001). Mean monthly temperatures range from −3.2 to 19.1 °C; annual precipitation is 1450 mm. The construction crane used gave us access to approximately 0.5 ha of forest canopy (jib length was 41 m at a height of 25 m). Three 18–20 m tall *Q. crispula* trees in the top of the canopy were selected for branch warming. Since 2007, these trees are also used for a soil warming experiment in which heating cables are buried around the trunk of each tree in a 5 m × 5 m plot (similar to Peterjohn et al., 1993).

### 2.2. Branch warming technique

Electric heating cables were attached with adhesive tape to upper canopy branches accessed with the crane (Fig. 1). On each tree, 1–3 thick branches ( $57.2 \pm 8.7$  mm diameter, mean  $\pm$  SD,  $n=3$ ) were selected and on these, >30 current year shoots were wired. We used 120-m-long electric heating cables (Nihon Noden, Tokyo, Japan) containing copper resistance wire and with an output of 1000 W at 200 V. To maintain branch temperature at 5 °C above ambient, we used simple electronic temperature controllers (OMRON, Kyoto, Japan) connected to thermocouples (K cables) to govern the power supply. The thermocouples were taped between the heating cables and branches, and taped at control branches with diameters similar to those of experimental subjects (Fig. 1a). When the difference in temperature between control and exper-



**Fig. 2.** Temperature elevation above ambient of a warmed branch not in direct contact with heating cable (branch  $\Delta$  °C), and temperature elevation measured with a thermocouple inserted between the heating cable and a branch (cable  $\Delta$  °C) during 3–4 October 2009. Control branch indicates temperature of an unheated shoot. The branch warming was concurrent with power supply to the crane and therefore discontinued approximately twice a week for several hours.

imental branches was  $<5$  °C, the controller relay switch opened and power was supplied to the electric cable. When the difference was  $\geq 5$  °C, the relay closed. A third thermocouple was taped to a warmed branch, but not in direct contact with the heating cable. This measured warming immediately beyond the heating cables. In spring 2008, we wired three trees and started heating continuously on 15 May 2008, after leaf flushing. With limited power supply at the crane site, heating was interrupted when the crane was in use (Fig. 2). Branch temperature data of the thermocouples on three positions were logged with a temperature sensor (Graptac midi-logger, Graptac Corporation, Japan) in October 2009.

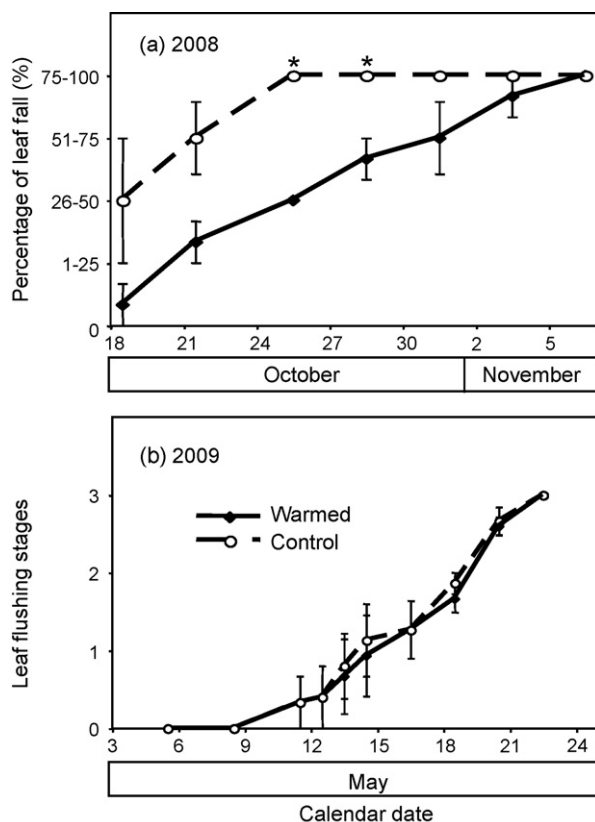
### 2.3. Response variables

In the autumn of 2008 and spring 2009, we determined leaf fall and leaf flushing, respectively, on warmed and control branches of three tall mature trees. We visually scored percentage leaf fall (0% = 0, 1–25% = 1, 26–50% = 2, 51–75% = 3, and 76–100% = 4) on every third day from 18 October to 6 November. From 5 May to 22 May, we determined the leaf flushing response to branch warming. We randomly selected five 1-year-old shoots on warmed and control branches on one canopy tree and visually scored leaf flush stages (bud stage = 1, leaf emergence stage = 2, and leaf expansion stage = 3) every third day. We analyzed data using the Wilcoxon signed rank test to compare responses of warmed and control branches on each date. Individual trees were replicates in the analysis.

To determine how acorn production responded to branch warming, we measured acorn production from 11 to 13 September 2008. We randomly selected ten warmed and control branches on each tree. We counted the number of acorns on each of 15 current year shoots per branch. Because seed production varies among tree specimens (e.g. DeSteven and Wright, 2002), individual tree effects should be considered as major contributors to variance in acorn production. Thus, we used two-way ANOVA to test the effects of branch warming, individual trees, and their interaction. Within trees, we used *t*-tests to compare between warmed and control branches. Individual branches were replicates in the analysis.

## 3. Results and discussion

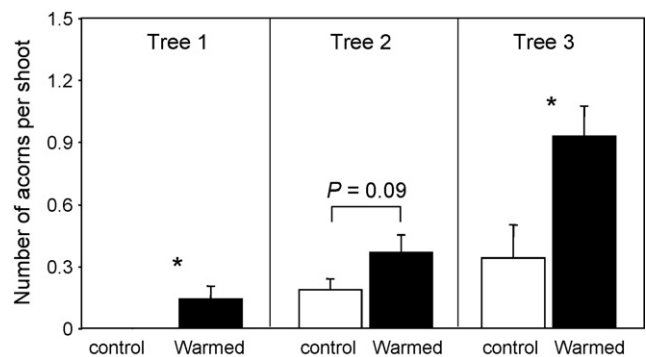
The heating cables successfully warmed canopy branches. With the feedback temperature control, extreme overheating was avoided. The temperature difference between warmed and



**Fig. 3.** (a) Percentage leaf fall of warmed (solid square) and control branches (open circle) in October 2008. (b) Leaf flushing pattern of warmed and control branches in May 2009: 1 = bud stage, 2 = leaf emergence stage, and 3 = leaf expansion stage. Values are means  $\pm$  SE. Asterisks indicate significant differences ( $P < 0.05$ ).

unwarmed branches (cable  $\Delta^\circ\text{C}$ ) ranged from 3 to 13  $^\circ\text{C}$  (average = 6.6  $^\circ\text{C}$ ; Fig. 2). The temperature elevation of warmed branches not in direct contact with heating cables (branch  $\Delta^\circ\text{C}$ ) ranged from 1 to 9  $^\circ\text{C}$  (average = 3.9  $^\circ\text{C}$ ) in October 2009. Both temperature differentials increased with steep changes in ambient temperature. To our knowledge, this is the first reported investigation to manipulate aboveground temperatures in canopies of tall trees, and we demonstrated significant effects on measured response variables.

Leaf phenology changed with branch warming. The growing season of canopy leaves was extended by later leaf fall. Leaf fall of warmed branches occurred about 10 days later than of control branches (Fig. 3a). Time of leaf emergence was unaffected by warming in 2009 (Fig. 3b). Many recent studies showed that plant phenologies respond to changes in temperature within recent global warming increases (e.g. Menzel and Fabian, 1999; Menzel, 2003; Gordo and Sanz, 2005; Doi and Katano, 2008). For example, over a 30-year period in Europe, spring events (e.g. leaf flushing) advanced 6 days, whereas autumn events (e.g. leaf coloring) were delayed by 4.8 days (Menzel and Fabian, 1999). The lack of earlier leaf flushing in our study might be due to the heating cables being one shoot length away from the buds in spring 2009 as we did not rewire the branches. Since the rewiring is relatively easy and does not induce lots of injury to branches, we recommend to, and will rewire the heating cable every year for long-term study. A preliminary study in 2006 on one tree with heating starting 3 weeks before leaf flush resulted in a 6 day earlier leaf flush (Nakamura et al., unpublished). Alternatively chilling is necessary to break winter dormancy in trees, and in its absence by continuous heating through winter, more heater units are required to promote the onset of leaf flushing (Cannell and Smith, 1986; Murray et al., 1989).



**Fig. 4.** Numbers of acorns formed per shoot on warmed and control branches (branch types). Values are means  $\pm$  SE. Asterisk shows a significant difference ( $P < 0.05$ ).

Branch warming increased acorn production in September (two-way ANOVA:  $F_{(1,54)} = 13.481$ ;  $P = 0.0006$ ; Fig. 4). Acorn production also varied significantly among individual trees ( $F_{(2,54)} = 15.982$ ;  $P < 0.0001$ ). When acorns were present, warmed branches had about double the number of acorns found on control branches. On one tree, warmed branches had about one acorn per 10 shoots, whereas there were no acorns on control branches. The whole tree formed few acorns (authors' personal observations).

In general, oaks have considerable interannual variation in acorn production, a phenomenon termed mast seeding (e.g. Sork and Bramble, 1993; Masaka and Sato, 2002). Although oaks produce many female flowers each year, the acorn set (ratio of mature acorns to female flowers) varies widely (Sork and Bramble, 1993). Masaka and Sato (2002) reported that acorn production in *Quercus dentata* decreases with low temperatures in the flowering period. This is related to pollen germination and pollen tube growth, which have high temperature requirements in oak (Hashizume, 1975). High experimental temperature in mid-May (flowering period), due to our branch warming procedure, may have promoted fertilization in the oaks.

In conclusion, we showed that branch warming changed phenologies of two major traits in a tall tree canopy. First, branch warming extended the length of the canopy leaf growing season through promotion of delayed leaf fall. Such trends in leaf phenology with climate change may, in turn, affect climate through biogeochemical and biophysical processes (Penuelas et al., 2009). Second, branch warming increased acorn production. This result aids in interpreting typical oak masting behavior. Changes in the phenologies of canopy leaves and acorn production will play major roles in forest ecosystem processes. At present, we try to reveal how the forest ecosystem, in access of the crane, will respond to global warming (e.g. photosynthesis, insect–plant interactions, and decomposition) by the new technique presented here and by soil warming. Manipulation of forest canopies is, despite its technical difficulties, essential in understanding how forests will respond to global warming.

## Acknowledgments

We thank staff members and graduate students at Tomakomai Research Station, Hokkaido University, for their support during the study. Financial support was provided by Japanese Ministry of Education, Science, Sports, and Culture of Japan to M. Nakamura (no. 21870002), T. Hiura (nos. 19657007 and 21248017), O. Muller (JSPS postdoctoral fellowship), and by Ministry of Environment to T. Hiura (Fund to technical developments).

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