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ARTICLE

Growth and physiology of a dominant understory shrub, *Hamamelis virginiana*, following canopy disturbance in a temperate hardwood forest

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Abstract: As global climatic changes increase plant susceptibility to large-scale disturbances such as drought and pathogens, understory responses to these disturbances will become increasingly important to long-term forest dynamics. To better understand understory responses to canopy disturbance, we measured changes in the growth and physiology of the dominant understory shrub, American witch-hazel (*Hamamelis virginiana* L.), in response to girdling of canopy oaks in a temperate hardwood forest of the northeastern United States. Changes in the growth and physiology of *H. virginiana* may be important to the regeneration of northeastern temperate forests, as this common shrub largely shapes the microenvironment for seedlings on the forest floor where it occurs. Canopy disturbance by girdling resulted in significant increases in light and soil nitrogen availability. In response to these environmental changes, basal-area growth of *H. virginiana* increased by an average 334%. This growth increase corresponded to significant increases in foliar nitrogen, respiration, and leaf chlorophyll and carotenoid concentrations. These findings indicate improved environmental conditions and increased growth for this understory shrub following the loss of dominant canopy trees. This study suggests that following large-scale canopy disturbance, *H. virginiana* and shrubs like it may play an important role in competing for soil N and shading seedlings of regenerating canopy species.

Key words: Black Rock Forest, canopy disturbance, foliar chemistry, photosynthesis, respiration.

Résumé : À mesure que les changements climatiques à l'échelle planétaire augmentent la sensibilité des plantes aux perturbations à grande échelle, telles que la sécheresse et les agents pathogènes, les réactions du sous-bois face à ces perturbations vont devenir de plus en plus importantes pour la dynamique forestière à long terme. Dans le but de mieux comprendre les réactions du sous-bois aux perturbations du couvert forestier, nous avons mesuré les changements dans la croissance et la physiologie de l'arbuste de sous-bois dominant, l'hamamélis de Virginie (*Hamamelis virginiana* L.), en réaction à l'annélation des chênes de l'étage dominant dans une forêt feuillue tempérée du nord-est des États-Unis. Les changements dans la croissance et la physiologie de l'hamamélis pourraient avoir des répercussions importantes sur la régénération des forêts tempérées du nord-est étant donné l'influence déterminante de cet arbuste commun sur le microenvironnement des semis au sol où il est présent. La perturbation du couvert forestier par l'annélation a entraîné une augmentation significative de la disponibilité de la lumière et de l'azote dans le sol. En réaction à ces changements environnementaux, la croissance en surface terrière de l'hamamélis a augmenté en moyenne de 334 %. Cette augmentation de croissance correspond à des augmentations significatives de l'azote foliaire, de la respiration et des concentrations de chlorophylle et de caroténoïde dans les feuilles. Ces résultats sont un indice de meilleures conditions environnementales et d'une croissance accrue pour cet arbuste de sous-bois à la suite de la perte des arbres de l'étage dominant. Cette étude indique qu'à la suite d'une perturbation à grande échelle du couvert forestier, l'hamamélis et les arbustes

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semblables pourraient jouer un rôle important en compétitionnant pour l'azote dans le sol et en ombrageant les semis des espèces de l'étage dominant qui se régénèrent. [Traduit par la Rédaction]

Mots-clés : forêt de Black Rock, perturbation du couvert forestier, chimie foliaire, photosynthèse, respiration.

Introduction

Temperate hardwood forests play an important role in regulating water and nutrients, sequestering carbon (C), and providing habitat for biodiversity. In the United States (US) and southern Canada, many temperate forests are dominated by oaks (*Quercus* spp.), with "oak–hickory" being the largest single forest type, covering 139 million acres and comprising almost one-fifth of all US forested land (USDA Forest Service 2007). Oaks currently face large-scale mortality from pathogens and regeneration failure (Lorimer 1993; Shifley et al. 2006; Fan et al. 2008), similar to the die-offs of American chestnut (*Castanea dentata* (Marsh.) Borkh.) and eastern hemlock (*Tsuga canadensis* (L.) Carrière) during the 20th century. Understanding the effects of such large-scale mortality from pathogens and the recolonization process following the loss of canopy oaks is important for predicting the stability and function of future temperate hardwood forests.

North American oaks are currently at risk due to a wide variety of pathogenic threats. These pathogens include oak wilt (Juzwik et al. 2008), leaf scorch bacteria (Barnard 2007; McElrone et al. 2008), *Armillaria* root fungus (Marçais and Bréda 2006; Fan et al. 2008; Kabrick et al. 2008), and red oak borer (Shifley et al. 2006; Fan et al. 2008; Kabrick et al. 2008), all of which are established in eastern hardwood forests. Additionally, the sudden oak death pathogen (Rizzo et al. 2002) has resulted in large-scale oak mortality in the western US (Meentemeyer et al. 2008), and the susceptibility of eastern oak species to this water mold presents a major potential threat to the vast oak forests of eastern North America (Tooley and Kyde 2003, 2007). In eastern temperate forests, competition from other shade-tolerant species (Lorimer 1993; Parker and Dey 2008), intense herbivory (predominantly by white-tailed deer; Rooney and Waller 2003), and altered fire regimes (Kruger and Reich 1997) may all contribute to suppressing the regeneration of oak seedlings, potentially amplifying the effects of these pathogens. The transition away from canopies dominated by oak species following human and natural disturbance has been well documented throughout eastern North America (Lorimer 1993; Kruger and Reich 1997). Thus, predicting which tree species will replace canopy oaks following large-scale pathogen mortality and what environmental factors govern this replacement process is key to understanding future functioning of northeastern temperate forests.

A primary competitor of canopy species' seedlings in many temperate North American forests is American witch-hazel (*Hamamelis virginiana* L.; Gleason and Cronquist 1963). This woody shrub takes up soil resources and casts shade, largely driving understory environmental conditions where it is common. Strong secondary compounds in the tissue of *H. virginiana* deter herbivory of this species (Duckstein and Stintzing 2011), allowing it to become especially dominant in forests where herbivores strongly influence understory ecology (as is the case throughout much of the eastern temperate US; Rooney 2001). Because of the large potential impact that shrubs such as *H. virginiana* can have on the growth of seedlings below their crown (Lorimer et al. 1994), responses of this species to canopy oak loss may be particularly important in determining which species replace canopy oaks.

Forest canopies largely shape the environmental conditions of the forest floor. Loss of dominant canopy trees can dramatically alter light and soil resource availability in the understory (Parker and Dey 2008), making the physiological responses of *H. virginiana* to these novel conditions important to its success in changing forests. Previous studies have shown dramatic increases in light and soil ammonium (NH_4) availability following experimentally induced mortality of canopy dominants (Jenkins et al. 1999; Lustenhouwer et al. 2012). Additional changes to water, phosphorus, soil organic matter, and microbial pools might also be expected as large components of the forest canopy turnover. For *H. virginiana*, similar environmental changes have been shown to alter growth (Hicks and Hustin 1989), leaf anatomy (Abrams and Kubiske 1990), and internal nutrient dynamics (Boerner 1985).

We designed this study to increase our understanding of the physiological responses of *H. virginiana* to canopy oak loss and to identify potential ecological consequences of these responses for temperate hardwood forests. To do this, we measured changes in several environmental and physiological variables 5 years after girdling of the dominant canopy oaks in a temperate northeastern US forest. Specifically, we aimed to document responses of growth, foliar nitrogen (N) and carbon (C), leaf pigments, photosynthesis, and foliar respiration of *H. virginiana* to canopy disturbance (Table 1). We hypothesized that large-scale canopy disturbance would increase light and soil N availability, increasing both the supply to and demand of N by the leaves of *H. virginiana*. We predicted that higher foliar N concentrations would increase photosynthesis and respiration rates, which would lead to increased growth of this important understory species.

Methods

Study site

This study, part of the Future of Oak Forests experiment, was located on the north slope of Black Rock Mountain (41.45°N, 74.01°W) at the Black Rock Forest (BRF) Consortium in the Hudson Highlands region of southeastern New York State. As a temperate forest, BRF has an annual precipitation of approximately 1200 mm and a mean annual air temperature of 9.7 °C. Temperatures at this site show strong seasonal variation, with mean monthly temperatures ranging from –2.7 °C to 23.4 °C (National Oceanic and Atmospheric Administration (NOAA) 2002). Northern red oak (*Quercus rubra* L.) and chestnut oak (*Quercus montana* Willd.) are the most widely distributed and common canopy trees, with oaks comprising approximately 67% of the hardwood basal area at BRF (Schuster et al. 2008). The understory tree community is dominated by red maple (*Acer rubrum* L.), black gum (*Nyssa sylvatica* Marsh.), black birch (*Betula lenta* L.), and sugar maple (*Acer saccharum* Marsh.) (Schuster et al. 2008; Levy-Varon et al. 2012, 2014). *Hamamelis virginiana* is, by far, the most common understory shrub species > 100 cm height at this experimental site (adult densities = 566 individuals·ha^{-1}).

Experimental design and sampling scheme

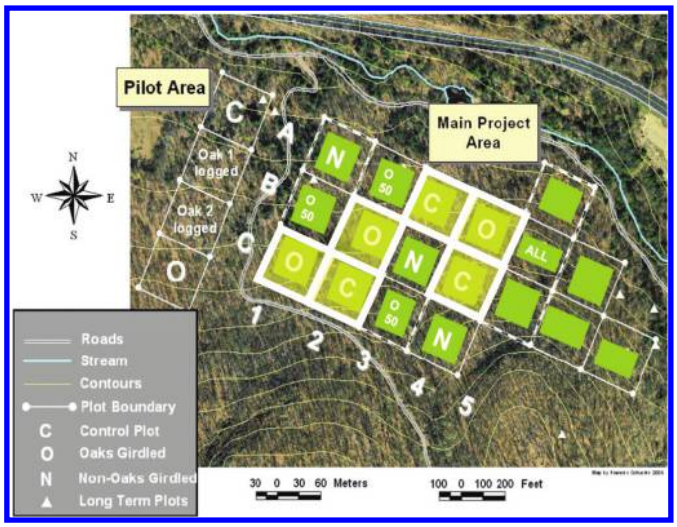
The Future of Oak Forests experiment at BRF was implemented in 2008 with the goal of understanding changes in stand-level nutrient cycling and regeneration responses following dominant canopy tree loss. This experiment comprises four treatments replicated in three blocks: 100% oaks girdled, 50% oaks girdled, 100% non-oaks girdled, and control plots where no girdling occurred (labelled "O", "O50", "N", and "C", respectively; Fig. 1). Girdling was performed on trees > 2.54 cm DBH according to the notch girdling technique described by Noel (1970), using a chain saw to sever phloem transport of carbon between leaves and roots causing gradual mortality of the tree to simulate natural death from pathogen attack. Understory oaks < 2.54 cm DBH in the 100% girdled plots were felled completely as their bark tissue was too

Table 1. Table of means (± standard error) from control and oak-girdled plots for each of the physiological measurements used in this study.

	Measurement	Units	Control plot	Oak-girdled plot
Foliar traits	SLA	$cm^2 \cdot g^{-1}$	444.56 (37.71)	405.64 (38.81)
	Carbon	%	42.11 (0.70)	44.60 (1.04)
	Nitrogen	**%**	**1.29 (0.04)**	**1.64 (0.08)**
	C:N	**Ratio**	**35.08 (1.48)**	**29.38 (1.25)**
	$\delta^{15}N$	**‰**	**−4.98 (0.26)**	**−2.43 (0.33)**
	$\delta^{13}C$	‰	−32.27 (0.14)	−32.51 (0.21)
	Chlorophyll	**$\mu g \cdot cm^{-2}$**	**13.90 (1.46)**	**20.22 (2.37)**
	Carotenoid	**$\mu g \cdot cm^{-2}$**	**1.20 (0.03)**	**1.64 (0.17)**
	Anthocyanin	$\mu g \cdot cm^{-2}$	0.20 (0.01)	0.17 (0.02)
Photosynthesis	A_{max}	$\mu mol\ CO_2 \cdot s^{-1}$	5.85 (0.56)	7.70 (0.95)
Respiration	R_{dark}	$\mu mol\ CO_2 \cdot s^{-1}$	−0.63 (0.08)	−0.84 (0.10)
	R_{10}	**$\mu mol\ CO_2 \cdot s^{-1}$**	**0.10 (0.00)**	**0.13 (0.01)**
	R_{20}	**$\mu mol\ CO_2 \cdot s^{-1}$**	**0.20 (0.01)**	**0.29 (0.02)**
	Respiratory quotient	Ratio	0.97 (0.06)	0.88 (0.07)
Growth	**BAI***	**%**	**27.79 (24.73)**	**334.46 (89.93)**
	Crown area	m^2	36.35 (5.59)	22.52 (4.09)
	Height	m	6.15 (0.47)	5.81 (0.35)

Note: Measurements shown in bold exhibited significant differences between control and oak-girdled treatments.
*Units of basal area increment (BAI) are expressed in millimetres per year; however, here we report the percent difference in mean pre- and post-girdling BAI for control and oak-girdled plots.

Fig. 1. Design of the Future of Oak Forests Experiment at Black Rock Forest. Subplot rows are labelled with letters and subplot columns are labelled with numbers. Only highlighted subplots (C1, C2, B2, B4, A3, and A4) were used in this study. [This figure is available in colour online.]



thin to reliably girdle. Mortality of girdled trees occurred 1–3 years following girdling implementation. There was no removal of leaf litter or woody debris in any plot at any point during the experiment. Girdling treatments are arranged in a complete randomized block design with blocks corresponding to slope position. Here, we sampled the control and 100% oak-girdled treatments: six plots distributed across the three experimental blocks (plots A3, A4, B2, B4, C1, and C2; highlighted in Fig. 1).

Each experimental plot is divided into nine equal 25 m × 25 m subplots. Within the central subplot of each plot, three adult *H. virginiana* individuals were selected, one closest to each of the northeastern corner, center, and southwestern corner of the subplot. This sampling scheme resulted in measurements taken on nine individuals in each of the two experimental treatments. All physiological measurements of *H. virginiana* were taken on 5 and 6 October 2013, approximately 5 years following the application of the girdling treatment. Sampling was conducted on leaves normally receiving direct sunlight and prior to any visible signs of fall leaf senescence for *H. virginiana* at this site. Data for photosynthetic rates and respiratory capacity were obtained from lab measurements of detached *H. virginiana* branches from each study plot. Measurements conducted in the lab utilized branches from the outer crown, harvested from each individual before dawn and transported to the lab with their proximal end submerged in water to preserve tissue water potential. Branches were kept outdoors in direct sunlight until measurements were taken, and all lab measurements were conducted on the same day that branches were harvested. We stress that our intent was to compare photosynthetic and respiratory capacities, not in situ rates. All detached branches were treated similarly, and no loss of photosynthetic or respiratory capacity has been observed between attached and detached branches of woody plants (A.E. Patterson, unpublished data). Although lab and field measurements were taken on the same set of 18 plants, the individual leaves used for lab and field measurements were different.

Light conditions in each plot were quantified using hemispherical photographs of the canopy of each plot over a 3-year period from 2009 to 2012. Hemispherical photographs of the canopy were taken using a fisheye lens 1 m above the ground at each of the four corners of each experimental plot during the summer of each year. Photographs were analyzed using Gap Light Analyzer software (Cary Institute for Ecosystem Studies, Millbrook, N.Y., USA). Mean daily air temperature in each plot was measured from the summer of 2008 through the end of 2012 using 107-T temperature sensors (Campbell Scientific Inc., Logan, Utah, USA) installed within radiation shelters at the center of each plot approximately 2 m above the ground.

Concentrations of soil ammonium (NH_4) and nitrate (NO_3) were determined from both organic- and mineral-layer soil samples collected every 2–3 months through the growing season from October 2007 to June 2011. These concentrations were considered to be indicators of nitrogen availability to the vegetation. Soil samples were taken with a 7.62 cm diameter soil core to a depth of 15.25 cm, separated into organic and mineral layers in the field, sieved, and extracted in 2 $mol \cdot L^{-1}$ KCl for 48 h. Extractions were analyzed for nitrate and ammonium at either the Harvard Forest (Petersham, Massachusetts) or Marine Biological (Woods Hole, Massachusetts) laboratories. Soil pH and leaf litter dry mass were measured in four of the six experimental plots used in this study (A3, A4, B2, and B4). Soil pH for the top 5 cm of mineral soil was

determined using a Kelway soil pH and moisture meter on water-moistened soil (Kel Instruments Co., Inc., Wyckoff, N.J., USA) for four samples per experimental plot.

Physiological measurements

Due to the comprehensive goals of this study, a large number of methods were used to assess the physiological changes of *H. virginiana* to experimental treatment. Here we include only basic descriptions of the methods used. Detailed methods for each measurement can be found in Appendix A.

Growth

Due to the destructive nature of growth sampling methods, growth measurements were taken on a separate set of three *H. virginiana* individuals in each of the same six treatment plots as the individuals used for physiological measurements. Basal area of each individual was calculated using the number and DBH of all stems > 1 cm, given that many individuals had multiple stems. Height of the tallest stem for each individual was measured using an 8 m sighting pole, and crown area was approximated as a rectangle with dimensions equal to the distance between the farthest leaves along the north–south and east–west azimuths.

Annual radial growth data were obtained from tree ring analysis of basal cross-sectional samples of the largest stem of each of 12 individuals (those for which annual growth rings could be reliably cross-dated; six individuals in each treatment). Cross sections were dried and sanded, and ring widths were cross-dated and quantified using a Velmex measuring system to the nearest 0.001 mm. For each individual, ring widths along two radii from pith to the outermost fully formed ring were measured. Cross-dating among all measured series was verified using the program COFECHA (Holmes 1983). Growth was calculated as basal area increment (BAI), which represents the total basal area added to the tree over a given year and was calculated using the width of the annual growth ring and the radius from the center of the stem to that growth ring. BAI data were standardized to remove the influence of relative growth rates among individuals and biological growth trends (Fritts 1976) using the dplR program in R statistical software (Bunn 2008), and the BAI values for the two radii were averaged per individual. To assess the response of BAI to canopy girdling, we also calculated the percent change in BAI between the pre- and post-girdling periods using the following equation:

$$\%\ \text{change} = \frac{\mu\text{BAI}_{\text{post}} - \mu\text{BAI}_{\text{pre}}}{\mu\text{BAI}_{\text{pre}}}$$

where μ indicates the mean BAI.

Specific leaf area

Specific leaf area (SLA) was measured for approximately 45 individual leaves per experimental treatment. Leaf area was measured using a portable area meter (LI-3000, LI-COR Biosciences, Lincoln, Nebraska, USA) on sun leaves lacking visual signs of damage haphazardly chosen from the outer canopy of each *H. virginiana* individual. Leaves were then dried at 70 °C for 72 h to achieve constant mass. SLA was calculated as leaf area divided by dry mass.

Elemental and isotopic analysis

Approximately four leaves from each *H. virginiana* individual were dried and ground using a ball-mill grinder. Leaf percent carbon (%C) and percent nitrogen (%N) were determined using an elemental analyzer (ECS 4010, Costech Analytical Technologies, Inc., Valencia, California, USA), and δ^{15}N and δ^{13}C were measured using an isotope ratio mass spectrometer (Delta PlusXP, ThermoFinnigan, Bremen, Germany) at Washington State University's Stable Isotope Core Laboratory.

Foliar pigments

Foliar chlorophyll, carotenoid, and anthocyanin concentrations were determined using biochemical analyses on four cut leaf sections from each plant. Each leaf section was ground, dissolved in acetone for chlorophyll and carotenoids and 1% acidified methanol for anthocyanins, and analyzed using a spectrophotometer (Vernier Software and Technology, Beaverton, Oregon, USA) following protocols adapted from the Carnegie Spectranomics Protocol (http://spectranomics.dge.carnegiescience.edu/Technical_Information). Pigment concentrations were converted to be expressed on a leaf-area basis ($\mu g \cdot cm^{-2}$) using the extract volume (3 mL) and the product of the sample dry mass and SLA.

Photosynthesis

Photosynthesis was measured in the lab on a single leaf from each individual using a portable photosynthesis system (LI-6400, LI-COR Biosciences). Leaves were acclimated to a steady rate of photosynthesis for up to 40 min, following which CO_2 assimilation rates (A) were measured at PAR levels varying from 0 to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to produce light response curves (Appendix A). Maximum photosynthetic capacity (A_{max}) was determined as the highest photosynthetic rate along each leaf's light response curve. In addition to plot-level light measurements taken using the hemispherical photographs described above, a plant canopy analyzer (LAI-2000, LI-COR Biosciences) was used to take leaf-level leaf area index (LAI) values above a small subset of leaves for which photosynthesis was measured. Because LAI measurements for individual leaves using the LAI-2000 required an adjacent reference area of open sky, these measurements could only be taken in one plot (C2), which contained a sufficient canopy gap. Following leaf-level LAI measurements, branches containing each leaf were transported to the lab for photosynthesis measurements using the LI-6400 as described above.

Respiration

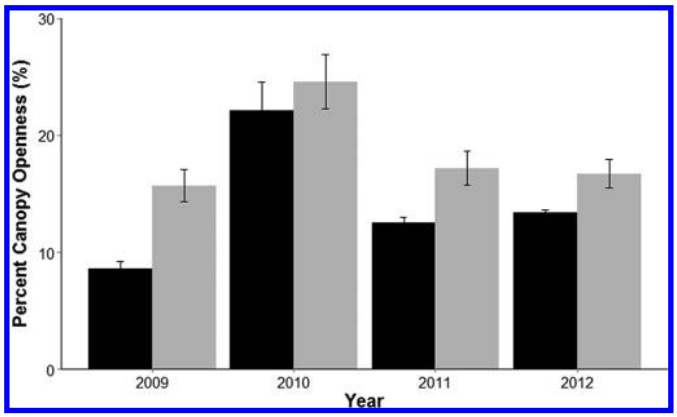
Respiration data were obtained using a custom-built plant gas exchange system consisting of a sample cuvette heated by a water bath (F25-ME, Julabo USA, Inc., Allentown, Pennsylvania, USA) coupled to an infrared gas analyzer (LI-6262, LI-COR Biosciences). Net CO_2 efflux at temperatures from 5 °C to 40 °C was measured by the infrared gas analyzer and applied to a modified Arrhenius equation to model respiration sensitivity to temperature fluctuations (O'Sullivan et al. 2013). Respiratory quotient data were obtained by dividing mean CO_2 efflux at 25 °C by O_2 uptake data obtained at 25 °C using a fast-response oxygen sensor (SO-210, Apogee Instruments, Logan, Utah, USA).

Statistical analyses

Treatment effects for all physiological measurements were evaluated using a nested one-way ANOVA with individual *H. virginiana* plants nested within each experimental plot and plots blocked by slope position (upper, mid, and lower). Where multiple leaves were measured for each plant, leaf-level values were averaged, and this mean value was used for plant-level data. Growth data were analyzed using the average growth rate (BAI) of the individuals in both treatment and control plots for the 4 years prior to and following girdling implementation (Supplementary Fig. S1[1]). Differences in soil N and growing-season temperature between control and oak-girdled plots were assessed using a repeated-measures linear mixed effects model using plot as a random factor. Because hemispherical photographs were not necessarily

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2016-0208.

Fig. 2. Mean (± SE) percent canopy openness for control (shaded bars) and oak-girdled (solid bars) plots during the growing seasons of each of the four years directly following girdling implementation (2009–2012).



taken in the exact same location from year to year, differences in growing-season canopy openness were assessed using a two-way ANOVA with treatment and year as explanatory variables and plot as a random factor. Statistical significance was assigned when $P < 0.05$, and marginally significant was assigned when $P < 0.1$. All analyses were conducted in the base, nlme, and Hmisc packages of R statistical software, version 3.2.2 (R Core Team 2015). ANOVA results for tests of environmental and physiological data are provided in Supplementary Table S1[1].

Results

Environmental changes following oak girdling

Following oak girdling, canopy openness was 29% higher in the treated plots than in the control plots on average ($P < 0.001$; Fig. 2). Canopy openness did not vary significantly by year in either control or oak-girdled plots, nor did the difference between control and oak-girdled plots vary by year ($P > 0.1$ for all). Understory air temperatures were not significantly different between oak-girdled plots and control plots during the growing season (May–October; $P > 0.1$). Mean daily growing-season temperatures were 18.75 °C and 19.10 °C in control and oak-girdled plots, respectively.

Prior to girdling, there were no significant differences in the availability of either nitrate (NO_3) or ammonium (NH_4) in the organic or mineral soil layers ($P > 0.1$ for all; Fig. 3). In the years following girdling, however, there was a >1000% increase in available NO_3 (averaged across all postgirdling sample dates) in oak-girdled compared with control plots — an effect seen primarily in the mineral soil layer (Figs. 3*b* and 3*d*). Significant treatment effects for NO_3 availability following girdling implementation occurred in both the organic ($P < 0.01$) and mineral ($P < 0.001$) soil layers. Ammonium (NH_4) availability, however, did not differ significantly by treatment after girdling in either the organic or mineral soil layers ($P > 0.1$ for both soil layers; Figs. 3*a* and 3*c*).

Soils were significantly less acidic in oak-girdled plots vs. control plots ($P = 0.024$). Mean pH for the upper 5 cm of mineral soil was 5.73 (±0.21) and 6.26 (±0.12) for control and oak-girdled plots, respectively. However, leaf litter mass, soil bulk density, and soil N concentrations did not differ significantly by treatment (data not shown).

Growth

Following the implementation of the girdling treatment in 2008, *H. virginiana* shrubs in oak-girdled plots showed an average 334% increase in annual basal area increment (BAI, $mm^2 \cdot year^{-1}$) compared with years prior to girdling (Fig. 4). This increase, averaged across years after girdling, was significantly greater than the percent difference in growth seen in control plots ($P < 0.001$). No differences were found, however, in stem number, basal area, crown area, or height of *H. virginiana* individuals between treatments ($P > 0.1$ for all).

Foliar SLA, %N, %C, $\delta^{15}N$, and leaf pigments

We did not detect any significant changes in SLA in response to girdling ($P > 0.1$; Table 1). Percent N was significantly higher for leaves in oak-girdled plots ($P = 0.001$), but %C was only marginally significantly different between experimental treatments ($P = 0.059$; Table 1). The difference in %N, however, was large enough to drive a significantly lower foliar C-to-N ratio (C:N) in oak-girdled plots ($P = 0.019$; Table 1). $\delta^{15}N$ values were significantly less negative for leaves in oak-girdled plots than those in control plots ($P < 0.001$), but no differences were found for $\delta^{13}C$ ($P > 0.1$).

Hamamelis virginiana leaves in oak-girdled plots had significantly higher total chlorophyll and bulk carotenoid content than those in control plots ($P = 0.004$ and 0.044, respectively). No significant differences, however, were found for the chlorophyll-to-carotenoid ratio across treatments ($P > 0.1$). Anthocyanin levels between leaves of control and treatment plots were also not significantly different ($P > 0.1$).

Photosynthesis

Maximum photosynthetic capacity (A_{max}) was marginally higher for *H. virginiana* plants in oak-girdled plots than in control plots when the leaves for each plant were pooled together for statistical analysis ($P = 0.089$). Additionally, photosynthesis of individual leaves was strongly positively correlated with light availability at the individual-leaf level ($R^2 = 0.826$).

Dark respiration, respiratory quotient, and respiration temperature responses

When analyzing all experimental temperatures together, dark respiration of *H. virginiana* leaves was not significantly different in oak-girdled plots than in control plots ($P > 0.1$), nor was there a difference in the respiratory quotient (CO_2 production to O_2 uptake) between treatment groups ($P > 0.1$). There was, however, a significant treatment effect on the respiratory response to temperature. Respiration of leaves from oak-girdled plots was marginally higher than those from control plots at 10 °C ($P = 0.064$) but was significantly higher at 20 °C ($P = 0.0044$), creating a significant treatment by temperature interaction ($P = 0.0205$).

Discussion

Overall, the results of this study demonstrate that the multiple changes in the understory environment following canopy disturbance have varied physiological effects on the plants that live there. Efforts combining a wide breadth of physiological metrics under a single study are exceedingly rare but may prove critical to understanding the multiple effects of future large-scale forest disturbances (Chapin et al. 1987). The ability of this study to link light and nutrient availability to changes in foliar chemistry, photosynthetic capacity, respiration, and growth provides a uniquely comprehensive description of understory responses to canopy disturbance.

As expected, death of the dominant genus of canopy trees at this site significantly increased understory light availability — an effect that has persisted throughout the experiment's duration. The peak in canopy openness in oak-girdled plots in 2010 corresponds to the delayed mortality of many girdled trees 2–3 years after the girdling event in 2008 (designed to simulate natural death by pathogens). This peak also corresponds to a relatively open-canopy year, as demonstrated by high canopy openness values for both control and oak-girdled plots, which resulted from leaf curling due to particularly dry conditions during that growing season. It is important to note that measures of canopy openness were taken at a height of 1 m, which is well below the canopy height of *H. virginiana* (5–7 m). We observed significant increases in canopy openness in oak-girdled plots below *H. virginiana* cano-

Fig. 3. Soil inorganic nitrogen availability (mean ± SE) for experimental plots prior to (unshaded region) and following (shaded region) girdling treatment implementation. Control plots are shown in solid lines and oak-girdled plots are shown in dotted lines (red online). Organic soil layer (*a*) ammonium and (*b*) nitrate and mineral soil layer (*c*) ammonium and (*d*) nitrate are shown. [This figure is available in colour online.]

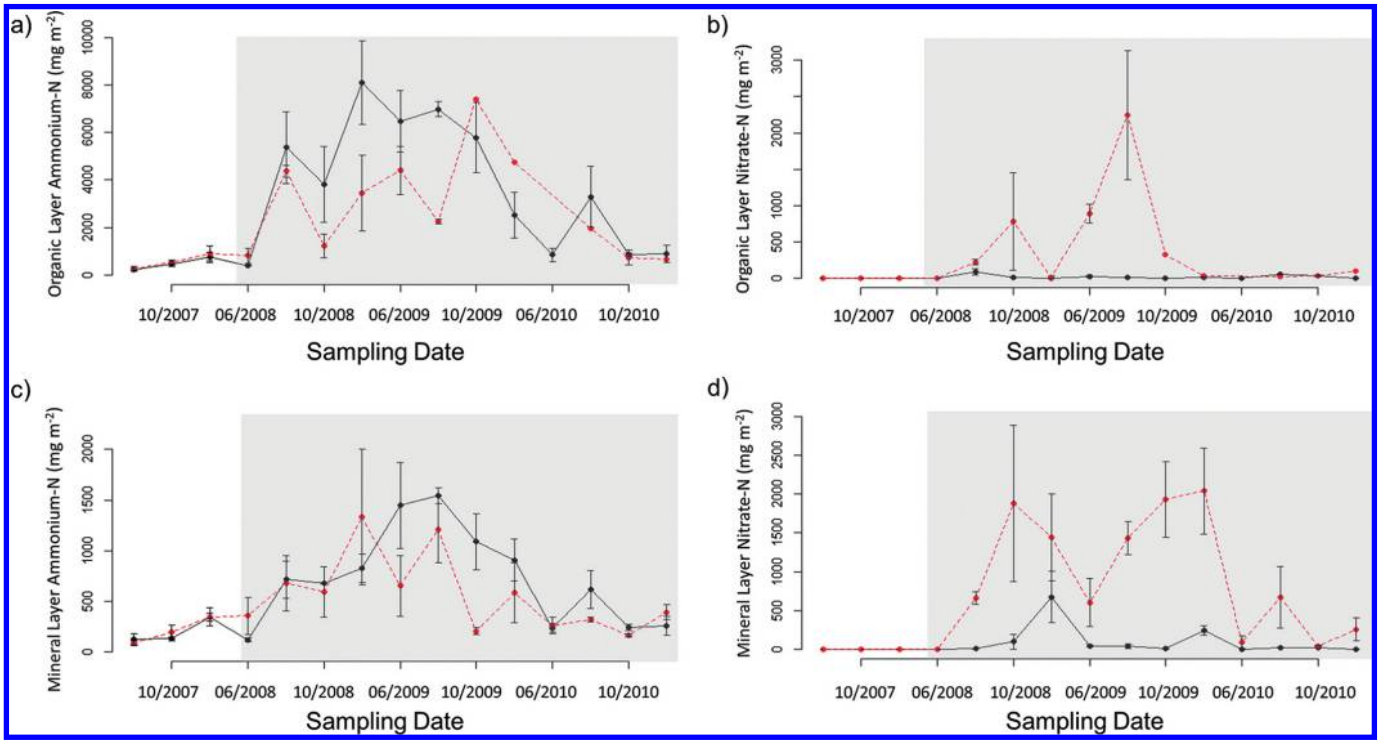
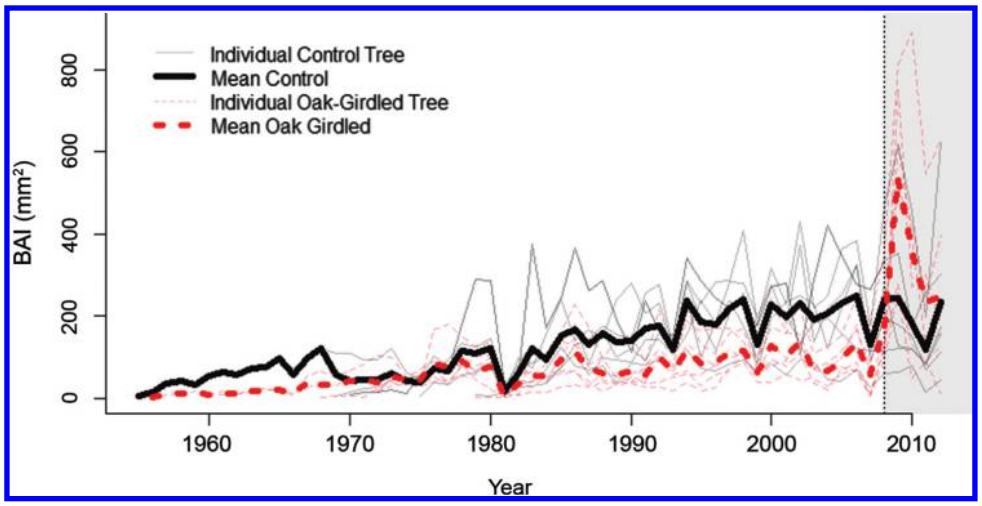


Fig. 4. Basal area index (BAI, mm^2) of *H. virginiana* individuals in control (thin grey solid lines) and oak-girdled plots (thin grey dashed lines; pink online). Thick solid and dashed (red online) lines indicate the average BAI for control and oak-girdled individuals, respectively. The shaded portion represents the time period following girdling treatment implementation. [This figure is available in colour online.]



pies despite the significantly higher growth of *H. virginiana* in these plots. This suggests that differences in the openness of the forest canopy and subsequent increases in light availability to the forest floor would be even greater than we observed in the absence of the *H. virginiana* growth effect above our canopy openness sampling height.

Belowground, dramatic increases in nitrate availability following oak girdling likely represent an initial release and subsequent cessation of nitrate uptake by oaks, which make up a large proportion of plant biomass at this site. Although not directly tested, soil temperature and CO_2 concentrations might have changed following canopy disturbance, making soil conditions more favorable for bacteria to mineralize and nitrify soil N. Increased N mineralization rates may also be partially due to the significantly lower C:N of *H. virginiana* leaves in oak-girdled plots. More neutral pH soils in oak-girdled plots are likely the result of reduced tannic acid from oak leaf litter following girdling. Physiological changes of *H. virginiana* reported in this study highlight the capacity of this species to respond to the multiple environmental changes following canopy disturbance — responses that may have important impacts on the regenerating seedling community and other understory plants among and below these shrubs.

A combination of multiple environmental changes to the understory following experimental girdling led to dramatic in-

creases in growth of *H. virginiana* shrubs under disturbed canopies at this site. The >300% increase in basal area increment indicates that *H. virginiana* has a large capacity to respond to improved understory environmental conditions following canopy disturbance. These results are qualitatively similar to, but notably smaller than, the ~600% growth increase previously observed for understory saplings of *B. lenta* in response to oak girdling at this site (Falxa-Raymond et al. 2012). Positive growth responses have also been documented in response to canopy gaps in a variety of other understory species throughout northeastern forests (e.g., Sipe and Bazzaz 1994; Finzi and Canham 2000). Allometric equations developed for *H. virginiana* (Wharton and Griffith 1993) suggest that the large BAI response seen in girdled plots relates to an even larger difference in aboveground biomass growth responses following canopy disturbance. While our data do not demonstrate differences in crown area or height between control and oak-girdled plots, it is important to bear in mind that data for these metrics were only available after girdling. It is possible that crown area increased more over the 4 years after girdling in the treatment plots than it did in the control plots but that control shrubs started out with larger crown areas initially. Considering this, basal area (BAI) increases provide the most accurate indication of aboveground growth responses of *H. virginiana* to large-scale canopy disturbance.

Significant changes in foliar N content and isotopic signature are likely the combined result of an increase in soil N availability and an increased demand for leaf N to drive photosynthesis in the high-light conditions of oak-girdled plots. The largest increase in available inorganic N in oak-girdled plots was in the form of nitrate, suggesting that this N pool may play an important role in N uptake in *H. virginiana* at this site. However, a small number of assays showing extremely low nitrate reductase in *H. virginiana* at this site, as well as a study by Ross et al. (2011), indicate that ammonium, not nitrate, is the preferred form of N for this species. Foliar $\delta^{15}N$ responses may help to reconcile this discrepancy. Several studies have shown that foliar $\delta^{15}N$ enrichment reflects increases in soil nitrification — the process by which ammonium is transformed to nitrate (Garten 1993; Pardo et al. 2002). The significant enrichment of $\delta^{15}N$ found here suggests higher nitrification rates in oak-girdled plots. Thus it is possible that decaying oak tissue is actually increasing both nitrate and ammonium inputs into oak-girdled plots and that *H. virginiana* is taking advantage of this increased ammonium availability, but that the increased ammonium in oak-girdled plots is not reflected in our soil N data due to increased nitrification rates converting the leftover ammonium to nitrate. Alternatively, the lack of large increases in soil ammonium in girdled plots may simply reflect *H. virginiana*'s preference for, and increased uptake of, this form of inorganic N. Together, changes in soil N, foliar %N, and foliar $\delta^{15}N$ suggest an increase in inorganic N uptake by *H. virginiana* following canopy disturbance.

Responses of foliar chlorophyll and carotenoid concentrations provide the mechanistic link between the increases in leaf %N and photosynthesis seen in this study. Because chlorophyll contains a large portion of the leaf's total N and serves as the initial light-gathering step in photosynthesis, increases in both foliar %N and light availability should lead to increased chlorophyll concentrations (Dillenburg et al. 1995; Sarijeva et al. 2007), as seen here. Carotenoid pigments in the leaf serve to protect the photosynthetic mechanism from high levels of irradiance (Sarijeva et al. 2007) — conditions experienced in oak-girdled plots in this experiment. Thus, the combined increases in chlorophyll and carotenoid content in the leaves of *H. virginiana* in oak-girdled plots suggest simultaneous increases in photosynthetic capacity and leaf protection against light-induced stress (Sarijeva et al. 2007).

The marginally significant increases in plant-level photosynthetic capacity seen here are likely due to large increases in light availability, coupled with increases in leaf pigment content, in oak-girdled plots. The lack of strong statistical significance may be due to the high variability in light availability between individual leaves on a single plant or to the relatively late date in the growing season when these data were collected (though no signs of senescence were present at the time of sampling). Although this study focused exclusively on sun-lit leaves in both treatments, differences in the placement and orientation of individual leaves on the upper canopy of each shrub likely led to between-leaf variability in photosynthetic capacity that partially masked plant-level treatment effects. Indeed, for a subset of leaves measured in a single plot, photosynthetic rates were found to be highly positively correlated with light levels measured directly above the leaf (R^2 = 0.826). This conclusion is further supported by the large increase in growth of *H. virginiana* individuals in treatment plots following girdling. Alternatively, increases in growth of *H. virginiana* in oak-girdled plots may not be fueled by increases in photosynthetic rates at the leaf level, but by increases in total leaf area — a metric that was not directly assessed in this study.

Even in the total absence of changes to photosynthetic capacity under disturbed canopies, the significantly higher light levels should drive higher plant-level photosynthesis in oak-girdled plots. Given the unexpected lack of a response in SLA (which has been seen elsewhere; Kloeppel et al. 1993) and crown diameter, higher light availability in girdled plots increasing whole-plant photosynthetic rates is likely the most important driver of the growth responses that we observed. Finzi and Canham (2000) demonstrated that light plays the strongest role in determining growth of saplings in canopy gaps of northeastern forests, but that soil N may also be an important factor. Given the significant changes in light availability, soil nitrate availability, and foliar %N following oak girdling, a combination of both light and N increases is likely driving the growth responses seen here.

Increases in the response of respiration to temperature strongly suggest overall higher metabolic activity of *H. virginiana* individuals in oak-girdled plots. The largest differences in respiration between plots were seen at the 20 °C reference temperature, which closely corresponds to the mean daily temperatures experienced by *H. virginiana* during the growing season at Black Rock Forest. Increases in respiration in oak-girdled plots at both reference temperatures indicates acclimation in the overall respiratory capacity, as seen elsewhere (Atkin and Tjoelker 2003; Atkin et al. 2005).

It is important to note that many of the strongest responses, of both the understory environment and *H. virginiana*, documented here demonstrate what appear to be transient temporal patterns (Figs. 3 and 4). We consider our results primarily as responses averaged across all postgirdling years, but both soil nitrate and growth data indicate dramatic decreases in girdling effects in the final postgirdling year in our dataset. Although we lack a sufficiently long time series to confirm whether these responses were truly transient, these dynamics suggest that the largest effects of *H. virginiana* on understory conditions likely occur in the initial 2–3 years following canopy disturbance. This, in addition to sampling late in the growing season, may explain why the physiological responses (i.e., photosynthesis and respiration) of *H. virginiana* did not fully reflect the dramatic increases observed in this species' growth. Additionally, while it has been shown that light is the primary driver of growth responses to canopy disturbance for many understory species (Finzi and Canham 2000), the similarity in the temporal dynamics of soil N availability and *H. virginiana* growth seen here indicates that a combination of both light and soil N are important factors in how this species responds to the mortality of dominant canopy trees.

The large increases in growth and physiological activity of *H. virginiana* in response to canopy oak death indicate that this species may play an important role in determining the environmental conditions for seedlings of regenerating canopy species as well as other understory plants — especially in the critical period directly following canopy disturbance. Greater aboveground bio-

mass and belowground nutrient uptake by *H. virginiana* may alter or mitigate the improved understory conditions for seedlings following canopy loss. Moreover, the effects of understory shade on the physiology of seedlings following canopy disturbance has been shown to have varying effects based on seedling species (Parker and Dey 2008). The shrub-layer effect on seedlings may be especially important for communities dominated by *H. virginiana* because of this species' resistance to herbivory by deer. Together, these results indicate that following potential loss of canopy oaks in eastern North America, regenerating seedling communities may become dominated by more shade-tolerant species where *H. virginiana* is abundant and responding positively to canopy opening.

Conclusion

Together, the results reported here (increased [N], chlorophyll and carotenoid content, respiration rates, and aboveground growth) suggest that *H. virginiana* plays an important role in shaping understory conditions following large-scale canopy disturbance in northeastern hardwood forests. As one of the most common understory shrubs in these forests, *H. virginiana* inherently competes with seedlings and saplings of regenerating canopy species for light and soil resources. While the increased light and N availability following canopy disturbance may favor regenerating canopy juveniles, if *H. virginiana* is successful at competing for some portion of these newly available resources in the understory, the responses of this species may help determine regeneration rate and composition of the future canopy (Lorimer et al. 1994). Our results strongly suggest that *H. virginiana* increases nitrogen uptake and aboveground growth. For seedlings of regenerating canopy species, these effects of *H. virginiana* may play a role in mitigating the increased resource availability in the understory due to canopy disturbance.

As human and climatic factors make northeastern hardwood forests prone to large-scale tree pathogen outbreaks (Ayres and Lombardero 2000; Davidson et al. 2003; Fichtner et al. 2009), understanding how these forests will respond to such mortality events is increasingly important. Despite never occupying the canopy layer, *H. virginiana* may serve a critical role in shaping canopy composition and successional dynamics following disturbance due to its role as a competitor in the regenerating understory. Given the high projected susceptibility of eastern North American forests to tree pathogens (Lovett et al. 2006), the physiological responses of *H. virginiana* to large-scale canopy disturbance have important ecological implications not only for this species, but also for the entire community of many eastern hardwood forests.

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Appendix A. Full methods for physiological measurements

Specific leaf area

Specific leaf area (SLA) was measured for approximately 45 individual leaves per experimental treatment. Leaf area was measured using a leaf area meter (LI-3000, LI-COR Biosciences). Leaves were then dried at 70 °C for 3 days to constant mass. SLA was calculated as leaf area divided by dry mass.

Elemental and isotopic analysis

Three leaves from each tree sample were oven-dried at 60 °C and ground up into a fine powder (Cianflone model 2601, Cianflone Scientific Instruments Corporation, Pittsburgh, Pennsylvania, USA). Sample powder (1–4 mg) was weighed and placed into tin capsules. Two replicates of samples were used: one complete set of samples was measured at Columbia University for leaf tissue nutrient to obtain values for %C and %N, and another complete set of samples was sent to Washington State University for leaf tissue stable isotope analysis to obtain values for $\delta^{15}N$ and $\delta^{13}C$. Nitrogen isotope signals are calculated as $\delta^{15}N$, which represents ^{15}N-to-^{14}N ratio of leaf tissue relative to that of the background atmosphere N_2. Elemental analyzer (ECS 4010, Costech Analytical Technologies, Inc., Valencia, California, USA) and isotope ratio mass spectrometer (Delta PlusXP, ThermoFinnigan, Bremen, Germany) were used for isotopic analysis.

Foliar pigments

Chlorophylls, carotenoids, and anthocyanins were extracted from a known area of leaf sample taken from leaves of the same individuals collected for spectral analysis. Dry mass of the leaf sample and its corresponding area were measured to determine SLA. Four circular punches of each leaf were taken for biochemical analysis and six were taken for calculating SLA. For chlorophyll and carotenoid measurements, samples were frozen in liquid nitrogen and ground quickly with magnesium carbonate and sand using a mortar and pestle. Leaf samples were collected in 3 mL of cold acetone and centrifuged for 2 min. The absorbance curves of the supernatants were measured using a spectrophotometer (Vernier Software and Technology, Beaverton, Oregon, USA). When the absorbance of the extract solutions exceeded one, the solutions were further diluted and absorbance was remeasured. The dilution factor was calculated from the final volume of the solution over the initial volume of the solution (3 mL). For anthocyanin measurement, the same protocol was repeated on separate punches from the same leaves, but because anthocyanins are unstable in acetone, extraction solvent was switched to 1% acidified methanol. Equations were then applied to the respective absorbance curves to measure chlorophyll, carotenoid, and anthocyanin concentrations (Lichtenthaler et al. 2001; Mancinelli and Rabino 1984; Sims and Gamon 2002). Pigment concentrations were then converted to mass per area ($\mu g \cdot cm^{-2}$) by multiplying by the initial extract volume (3 mL) and dividing by the product of sample dry mass and SLA.

Photosynthesis

Twig samples with the sun-lit leaves were taken from the branches and recut in beakers with water. Individual leaves on each twig were placed in the leaf chamber of an infrared gas analyzer (LI-6400 portable photosynthesis system, LI-COR Biosciences). Three of these gas analyzers were used, with two systems providing 24 measurements of maximum CO_2 assimilation rate (P_{Nmax}) at decreasing PAR levels ranging from 1500 to 0 $\mu m \cdot m^{-2} \cdot s^{-1}$, and the third, an older model, providing only 20 measurements. Following the placement of a leaf within a LI-COR Biosciences system, the leaves were acclimated to a steady rate of photosynthesis under the following settings: a flow rate of 500 μm, a CO_2 inflow of 400 ppm, a temperature of 25 °C, and a photosynthetically active radiation (PAR) of 1500 $\mu m \cdot m^{-2} \cdot s^{-1}$. Initial acclimation time varied from approximately 10 min to 40 min. Upon acclimation, the light curve program was run with P_{Nmax} values recorded at the following levels of PAR ($\mu m \cdot m^{-2} \cdot s^{-1}$): 1500, 1000, 500, 250, 150, 100, 90, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, and 0, with the older model eliminating measurements taken at 75, 65, 55, and 5 μm. Once the program was complete, the indi-

vidual leaves were ejected from the leaf chamber and cut, taking extreme care to cut off the petiole as well. Fresh mass (FM; in grams) of each leaf was measured using a compact scale (Fisher Science Education SLF303-US), while dry mass (DM) was estimated likewise after drying at 70 °C for at least 72 h. The LAI of all investigated leaves (in cm^2) were taken using a leaf area meter (LI-3000A portable leaf area meter, LI-COR Biosciences).

Respiration

Branch samples were stored outside in the sunlight. At the start of each trial, the leaf sample was run through a portable leaf area meter to measure leaf area (cm^2) (LI-3000C, LI-COR Biosciences). Fresh mass was recorded for each leaf sample and then samples were placed into the cuvette of the custom-built plant gas exchange system to measure respiration. Inside the cuvette, a wire attached to an adaptor was pressed against the lower leaf surface to measure temperature. The cuvette was covered with a dark cloth to stop photosynthesis and promote respiration. Using a water bath (F25-ME, Julabo USA, Inc.), the temperature inside the cuvette was heated from 5 °C to 40 °C at a rate of 1 °C·min^{-1}. Net CO_2 release from the leaf was measured in μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ using an infrared gas analyzer (LI-6262, LI-COR Biosciences). This process was repeated for each of the 18 samples. After measuring respiration, leaf samples were placed in coin envelopes, labeled, and then dried in an oven at 70 °C for 2 days. Dry mass values were recorded.

A modified Arrhenius equation was used to model respiration between 5 and 40 °C (eq. A1) (O'Sullivan et al. 2013). Equation A1 was adapted from O'Sullivan et al. and is one of seven discussed in the paper for modeling respiratory response curves and was found to maximize the r^2 value of the relationship between the measured and modeled data (2013).

$$R = R_{20} e^{\frac{E_0}{g}\left(\frac{1}{T_{20}} - \frac{1}{T_a}\right)} \quad \text{(A1)}$$

In this equation, E_0 represents a parameter related to the energy of activation and defines the shape of the response curve. Because of our chosen reference temperature of 20 °C, R_{20} is respiration at that temperature and T_{20} is simply 20 °C. Finally g is the universal gas constant (8.314 J·mol^{-1}·K^{-1}) and T_a is the ambient temperature.

Using the solver function in Excel, the square of the standard deviation was minimized by manipulating E_0 and R_{20}. From the results of the temperature response fit curve, values for E_0, R_{10}, and R_{20} were compiled for each of the 18 samples.

Growth

Within each of the six plots (A3, A4, B2, B4, C1, and C2), we selected three *H. virginiana* individuals for a total of 18 trees sampled. For each individual, we counted and measured diameter at breast height (DBH) for all stems > 1 cm. We used an 8 m sighting pole to measure the height of the tallest stem. We approximated crown area by measuring the distance between the farthest leaves or branches along the north–south azimuth and again along the east–west azimuth, ensuring that the two measurement axes crossed at the center of the crown. Crown area was then approximated as a rectangle whose area was equal to the product of those two measures. Following these measurements, we destructively sampled the *H. virginiana* individuals and collected a cross section near the base of the largest stem.

Basal cross sections were processed following standard dendrochronological procedures. The 18 cross sections were dried and sanded with progressively finer sandpaper until ring boundaries were easily distinguishable. *Hamamelis virginiana* is a diffuse porous to slightly semi-ring-porous species (Schweingruber et al. 2011) with occasional micro-rings and, therefore, requires very fine (>1000 grit) sanding. The annual rings were visually crossdated and then measured with a Velmex measuring system to the nearest 0.001 mm. Two radii, from the pith to outermost fully formed ring, were measured for each cross section. Only 12 of the 18 total cross sections, including six from oak-girdled plots and six from control plots, could be measured and analyzed. Visual crossdating was verified statistically with the software program COFECHA (Holmes 1983) using a correlation analysis window of 25 years lagged by 10 years. The raw ring-width measurements were converted to basal area increment (BAI) using the "inside-out" calculation in program dplR (Bunn 2008) in the R software environment. The BAI for both radii were averaged per individual shrub. The years directly following the girdling treatment, 2009 to 2012, were studied visually and quantitatively to identify potential growth releases in the BAI series. We quantified any changes in growth by determining the percent change in BAI from an average of 2004–2007 (no girdling effect) to 2009–2012 (full girdling effect).

Respiratory quotient

Three samples were taken from each section totaling 18 samples. Nine samples were taken from girdled plots and another nine samples were obtained from control plots. Each sample was composed of 11–35 leaves. The samples were weighted and leaf areas were measured; they were reported as the cumulated mass and the cumulated leaf area, respectively, of the leaves of the entire sample.

Leaves were placed into a glass container positioned in a water bath maintained at 25 °C after a 10 min acclimation in the open container. The carbon dioxide and oxygen concentrations were monitored thanks to adapted sensors (LI-840 CO_2/H_2O gas analyzer, LI-COR Biosciences; SO-210 fast-response oxygen sensor, Apogee Instruments). Trials were run until CO_2 reached a minimum of 3000 ppm, allowing for changes in oxygen gas levels to be significant enough to enable interpretation.

At the end of the experiment, the leaves were dried to a constant mass at 70 °C in a convection drying oven. Once dry, the leaf material was reweighed. Leaf mass per area (SLA, $cm^2 \cdot g^{-1}$) and leaf dry matter content (DMC, dry mass per unit fresh mass) was determined from the total fresh mass, total dry mass, and total leaf area.

For each experiment, we calculated the mean rate of increase of carbon dioxide concentration and the mean rate of decrease of the oxygen concentration by a linear interpolation. The data points of the first minutes (from 1 to 4 min depending on the sample) were discarded to account for the stabilization and homogenization of the gas composition in the container. The respiratory quotient was obtained by dividing the mean rate of increase of carbon dioxide concentration by the mean rate of decrease of the oxygen concentration.

Appendix references

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