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

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- 2 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 like |
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| 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), in response to girdling of canopy oaks in a |
| northeastern U.S. temperate hardwood forest. 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. |

Keywords: Black Rock Forest, canopy disturbance, foliar chemistry, photosynthesis, respiration

Introduction

Temperate hardwood forests play an important role in the regulation of water and nutrients, sequestration of carbon (C), and providing habitat for biodiversity. In the U.S. and southern Canada, many temperate forests are dominated by oaks (*Quercus* spp.), where "oakhickory" is the largest single forest type, covering 139 million acres and comprising almost one fifth of all U.S. forested land (US 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 U.S. (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 U.S.; 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 (NH₄) 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 U.S. 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. 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. 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 (NOAA 2002). 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). *H. 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

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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, Figure 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 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 - 6 plots distributed across the 3 experimental blocks (plots A3, A4, B2, B4, C1, and C2, highlighted in Figure 1).

| Each experimental plot is divided into 9 equal 25 m x 25 m subplots. Within the central |
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| subplot of each plot, three adult <i>H. virginiana</i> individuals were selected, one closest to each of |
| the northeast corner, center, and southwest corner of the subplot. This sampling scheme resulted |
| in measurements taken on 9 individuals in each of the 2 experimental treatments. All |
| physiological measurements of <i>H. virginiana</i> were taken on October 5 and 6, 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 <i>H. virginiana</i> at this site. Data for photosynthetic rates and respiratory capacity |
| were obtained from lab measurements of detached <i>H. virginiana</i> 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 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. |
| Environmental Conditions – Light conditions in each plot were quantified using hemispherical |
| photographs of the canopy of each plot over a 3-year period from 2009-2012. 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, NY, 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., UT, USA) installed within radiation shelters at the center of each plot approximately 2 m above the ground.

Concentrations of soil ammonium (NH₄) and nitrate (NO₃) were determined from both organic- and mineral-layer soil samples collected every two-three months through the growing season from October 2007 – 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 2M KCl for 48 hours. Extractions were analyzed for nitrate and ammonium at either the Harvard Forest (Petersham, MA) or Marine Biological (Woods Hole, MA) Laboratories. Soil pH and leaf litter dry mass were measured in 4 of the 6 experimental plots used in this study (A3, A4, B2, and B4). Soil pH taken for the top 5 cm of mineral soil and was determined using a Kelway soil pH and moisture meter on water-moistened soil (Kel Instruments Co., Inc. Wyckoff, NJ, USA) for four samples per experimental plot.

Physiology 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 1.

<u>Growth</u> – Due to the destructive nature of growth sampling methods, growth measurements were taken on a separate set of 3 *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-section samples of the largest stem of each of 12 individuals (those for which annual growth rings could be reliably cross-dated; 6 individuals in each treatment). Cross-sections were dried, sanded, and ring widths 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 % change in BAI between the preand post-girdling periods using the following equation:

$$\% Change = \frac{\mu BAI_{post} - \mu BAI_{pre}}{\mu BAI_{pre}}$$

where µ indicates the mean BAI.

| Specific Leaf Area – Specific Leaf Area (SLA) was measured for approximately 45 individual |
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| leaves per experimental treatment. Leaf area was measured using a Li-Cor Li-3000 Leaf Area |
| Meter (Li-Cor Biosciences, NE, 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 hours to achieve constant mass. SLA was calculated as leaf area divided by dry mass. |
| Elemental and Isotopic Analysis – Approximately 4 leaves from each <i>H. virginiana</i> individual |
| were dried and ground using a ball-mill grinder. Leaf %C and %N were determined using an |
| elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA) and $\delta^{15}N$ and $\delta^{13}C$ 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 4 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, OR, |
| 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 ($\mu g/cm^2$) using the extract volume (3 mL) and the |
| product of the sample dry weight and specific leaf area. |
| <u>Photosynthesis</u> – Photosynthesis was measured in the lab on a single leaf from each individual |
| using an LI-6400 portable photosynthesis system (Li-Cor Biosciences, NE, USA). Leaves were |
| acclimated to a steady rate of photosynthesis for up to 40 mins, following which CO_2 |
| assimilation rates (A) were measured at PAR levels varying from 0 to 1500 μ mol m ⁻² s ⁻¹ to |
| produce light response curves (Appendix 1). Maximum photosynthetic capacity (A_{max}) was |

| determined as the highest photosynthetic rate along each leaf's light response curve. In addition |
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| to plot-level light measurements taken using the hemispherical photographs described above, an |
| LAI-2000 Plant Canopy Analyzer (Li-Cor Biosciences, NE, USA) 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 individuals 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 an F25-ME water bath (Julabo, PA, USA) and coupled |
| to an LI-6262 infrared gas analyzer (Li-Cor, NE, USA). 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 SO-210 Fast Oxygen Sensor (Apogee Inst., UT, USA). |
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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.

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 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 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).

Results

Environmental Changes Following Oak Girdling

Following oak girdling, canopy openness was 29% higher in the treated plots than control plots on average (P < 0.001; Figure 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 (NO₃) or ammonium (NH₄) in the organic or mineral soil layers (P > 0.1 for all, Figure 3). In the years following girdling, however, there was a >1000% increase in available NO₃ (averaged across all post-girdling sample dates) in oak-girdled compared to control plots – an effect seen primarily in the mineral soil layer (Figure 3b, d). Significant treatment effects for NO₃

| availability following girdling implementation occurred in both the organic $(P < 0.01)$ and |
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| mineral ($P < 0.001$) soil layers. Ammonium (NH ₄) availability, however, did not differ |
| significantly by treatment post-girdling in either the organic or mineral soil layers ($P > 0.1$ for |
| both soil layers, Figure 3a, 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 mm²/yr) compared to years prior to girdling (Figure 4). This increase, averaged across years post-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, δ^{15} N, and Leaf Pigments

We did not detect any significant changes in SLA in response to girdling (P > 0.1; Figure 5). 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; Figure 5). The difference in %N, however, was large enough to drive a significantly lower foliar C:N ratio in oak girdled plots (P = 0.019; Figure 5). δ^{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 δ^{13} C (P > 0.1).

 $H.\ virginiana$ leaves in oak-girdled plots had significantly higher total chlorophyll and bulk carotenoid content than did those in control plots (P=0.004 and 0.044, respectively). No significant differences, however, were found for the chlorophyll: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 than control plots (P > 0.1), nor was there a difference in the respiratory quotient (CO₂ production to O₂ 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 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* canopies 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 uptake of nitrate by oaks, which make up

a large proportion of plant biomass at this site. Although not directly tested, soil temperature and CO₂ 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 increases 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 *Betula 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 post-girdling. It is possible that crown area increased more

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over the four years post-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 δ^{15} N responses may help to reconcile this discrepancy. Several studies have shown that foliar δ^{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 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 of *H. virginiana*, documented here demonstrate what appear to be transient temporal patterns (Figures 3 & 4). We consider our results primarily as responses averaged across all post-girdling years, but both soil nitrate and growth data indicate dramatic decreases in

girdling effects in the final post-girdling 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 biomass 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 shrublayer 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, Fitchner 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 the entire community of many eastern hardwood forests.

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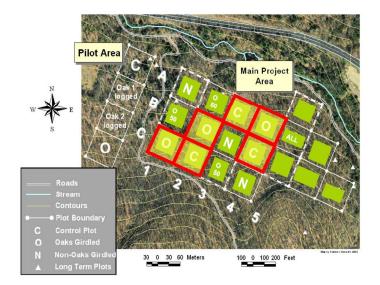
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Table 1. Table of means (± standard error) from control and oak-girdled plots for each of the physiological measurements used in this study. Measurements shown in bold exhibited significant differences between control and oak-girdled treatments.

| | Measurement | Units | Control | Oak-girdled |
|----------------|-------------------|-------------------------|----------------|----------------|
| | SLA | cm ² /g | 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) |
| Foliar Traits | $\delta^{15}N$ | δ/‰ | -4.98 (0.26) | -2.43 (0.33) |
| | δ^{13} C | δ/‰ | -32.27 (0.14) | -32.51 (0.21) |
| | Chlorophyll | $\mu g/cm^2$ | 13.90 (1.46) | 20.22 (2.37) |
| | Carotenoid | $\mu g/cm^2$ | 1.20 (0.03) | 1.64 (0.17) |
| | Anthocyanin | $\mu g/cm^2$ | 0.20 (0.01) | 0.17 (0.02) |
| Photosynthesis | A_{max} | μmol CO ₂ /s | 5.85 (0.56) | 7.70 (0.95) |
| | R _{dark} | μmol CO ₂ /s | -0.63 (0.08) | -0.84 (0.10) |
| | R_{10} | μmol CO ₂ /s | 0.10 (0.00) | 0.13 (0.01) |
| Respiration | $\mathbf{R_{20}}$ | μmol CO ₂ /s | 0.20 (0.01) | 0.29 (0.02) |
| | Respiratory | ratio | 0.97 (0.06) | 0.88 (0.07) |
| | quotient | | | |
| | BAI* | % | 27.79 (24.73) | 334.46 (89.93) |
| Growth | Crown Area | m^2 | 36.35 (5.59) | 22.52 (4.09) |
| | Height | m | 6.15 (0.47) | 5.81 (0.35) |

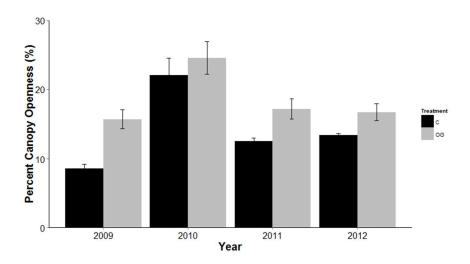
^{*}Units of Basal Area Increment (BAI) are mm²/yr, however, here we report the % difference in mean pre- and post-girdling BAI for control and oak-girdled plots.



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, A4, and A5) were used in this study.

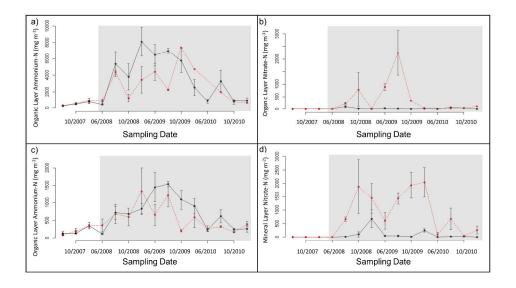
338x190mm (300 x 300 DPI)





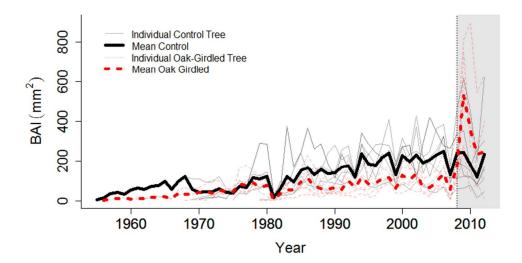
Mean (± SE) percent canopy openness for control (grey) and oak-girdled (black) plots during the growing seasons of each of the four years directly following girdling implementation (2009 – 2012).

338x190mm (300 x 300 DPI)



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 black and oakgirdled plots are shown in dotted red. Organic soil layer a) ammonium and b) nitrate, and mineral soil layer c) ammonium and d) nitrate are shown.

338x190mm (300 x 300 DPI)



Basal Area Index (BAI mm2) of H. virginiana individuals in control (thin grey dashed lines) and oak-girdled plots (thin pink dashed lines). Thick solid black and dashed red lines indicate the average BAI for control and oak-girdled individuals, respectively. The shaded portion represents the time period following girdling treatment implementation.

291x174mm (72 x 72 DPI)

- 1 Taylor et al. Appendix 1. Full methods for physiological measurements
- 2 Specific Leaf Area Specific Leaf Area (SLA) was measured for approximately 45 individual
- 3 leaves per experimental treatment. Leaf area was measured using a Li-Cor Li-3000 Leaf Area
- 4 Meter (Li-Cor Biosciences, NE, USA). Leaves were then dried at 70° C for 3 days to constant
- 5 mass. SLA was calculated as leaf area divided by dry mass.
- 6 Elemental and Isotopic Analysis Three leaves from each tree sample were oven-dried at 60C
- 7 and ground up into a fine powder (Cianflone model 2601, Cianflone Scientific Instruments
- 8 Corporation, Pittsburgh, PA, USA). 1-4 mg of sample powder was weighed and placed into tin
- 9 capsules. Two replicates of samples were used: one complete set of samples were measured at
- 10 Columbia University for leaf tissue nutrient to obtain values for %C and %N, and another
- complete set of samples were sent to Washington State University for leaf tissue stable isotope
- analysis to obtain values for δ^{15} N and δ^{13} C. Nitrogen isotope signals are calculated as δ^{15} N,
- which represents ¹⁵N:¹⁴N ratio of leaf tissue relative to that of the background atmosphere N₂.
- 14 Elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA) and isotope ratio mass
- spectrometer (Delta PlusXP, ThermoFinnigan, Bremen, Germany) were used for isotopic
- analysis.
- 17 Foliar Pigments –
- 18 Chlorophylls, carotenoids, and anthocyanins were extracted from a known area of leaf sample
- 19 taken from leaves of the same individuals collected for spectral analysis. Dry weight of the leaf
- 20 sample and its corresponding area was measured to determine specific leaf area. Four circular
- 21 punches of each leaf were taken for biochemical analysis and six for calculating specific leaf
- area. For chlorophyll/carotenoid measurements, samples were frozen in liquid nitrogen and
- 23 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 minutes. The absorbance curves of |
|---|
| the supernatants were measured using a spectrophotometer (Vernier Software & Technology, |
| Beaverton, OR, USA). When the absorbance of the extract solutions exceeded one, the solutions |
| were further diluted and absorbance was re-measured. 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 ($\mu g/cm^2$) by multiplying by the initial extract volume (3 mL) and dividing by the |
| product of sample dry weight and specific leaf area. |
| <u>Photosynthesis</u> – Twig samples with the sun-lit leaves were taken from the branches and recut in |
| beakers with water. Individual leaves on each twig was placed in the leaf chamber of an infra-red |
| gas analyzer (LI-6400 Portable Photosynthesis System, LI-COR). Three of these LI-COR 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 μ m m ⁻² s ⁻¹ , and the |
| third, an older model, providing only 20 measurements. Following the placement of a leaf within |
| a LI-COR 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 μm m ⁻² s ⁻¹ . Initial acclimation time varied |
| |
| from approximately ten minutes to forty minutes. Upon acclimation, the light curve program was |
| from approximately ten minutes to forty minutes. Upon acclimation, the light curve program was run with P_{Nmax} values recorded at the following levels of PAR ($\mu m m^{-2} s^{-1}$): 1500, 1000, 500, |

- 47 250, 150, 100, 90, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 0 with the older
- 48 model eliminating measurements taken at 75, 65, 55, and 5 μm. Once the program was complete,
- 49 the individual leaves were ejected from the leaf chamber and cut, taking extreme care to cut off
- the petiole as well. Fresh weight (FW) in grams of each leaf was measured using a compact scale
- 51 (Fisher Science Education SLF303-US), while dry weight (DW) was estimated likewise after
- drying at 70 °C for at least 72 hours. The leaf area index (LAI) of all investigated leaves were
- taken using a leaf area meter (LI-3000A Portable Area Meter, LI-COR) in cm².
- Respiration Branch samples were stored outside in sunlight. At the start of each trial, the leaf
- sample was run through a leaf area meter to measure leaf area (cm²) (Licor 3000C, Lincoln,
- USA). Fresh mass was recorded for each leaf sample and then samples were placed into the
- 57 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 in order to stop photosynthesis and
- 60 promote respiration. Using a waterbath (Julabo F25-ME, Allentown, USA) the temperature
- 61 inside the cuvette was heated from 5 °C to 40 °C at a rate of 1 °C per minute. Net CO₂ release
- 62 from the leaf was measured in μmol CO₂ m⁻²s⁻¹ using an infrared gas analyzer (Licor 6262,
- Lincoln, USA). This process was repeated for each of the eighteen samples. After measuring
- respiration, leaf samples were placed in coin envelopes, labeled, and then dried in an oven at 70
- °C for two days. Dry weight values were recorded.
- A modified Arrhenius equation was used to model respiration between 5-40 °C (Eq.1)
- 67 (O'Sullivan et al. 2013). Equation 1 was adapted from O'Sullivan et al. is one of seven discussed

88

in the paper for modeling respiratory response curves and was found to maximize the r² value of the relationship between the measured and modeled data (2013).

$$R = R_{20} e^{\frac{E_0}{g} (\frac{1}{T_{20}} - \frac{1}{T_a})}$$
 (Equation 1)

- In this equation, E_0 represents a parameter related to the energy of activation and it defines the
- shape of the response curve. Because of our chosen reference temperature of 20 °C, R₂₀ is
- respiration at that temperature and T₂₀ is simply 20 °C. Finally g is the universal gas constant
- 74 $(8.314 \text{ J mol}^{-1} \text{ K}^{-1})$ and T_a is the ambient temperature.
- Using the solver function in Excel, the square of the standard deviations 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 eighteen samples.
- 78 $\underline{\text{Growth}}$ Within each of the six plots (A3, A4, B2, B4, C1, and C2), we selected three H.
- 79 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 eight-meter sighting
- 81 pole to measure the height of the tallest stem. We approximated crown area by measuring the
- 82 distance between the farthest leaves or branches along the north-south azimuth and again along
- 83 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
- 85 those two measures. Following these measurements, we destructively sampled the *H. virginiana*
- 86 individuals and collected a cross-section near the base of the largest stem.
 - Basal cross-sections were processed following standard dendrochronological procedures (Stokes and Smiley 1968). The 18 cross-sections were dried and sanded with progressively finer

| sandpaper until ring boundaries were easily distinguishable. H. virginiana is a diffuse porous to |
|--|
| slightly semi-ring-porous species (Schweingruber 2011) with occasional micro rings, and |
| therefore requires very fine (>1000 grit) sanding. The annual rings were visually cross-dated 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 cross-dating 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). |
| <u>Respiratory Quotient</u> – 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 have been weighted and leaf |
| area measured; both were reported as the cumulated leaf area and cumulated weight 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 minute acclimation in the open container. The carbon dioxide and oxygen |
| concentrations were monitored thanks to adapted sensors (IRGA Li 840 infrared gas analyzer, |

| LiCor, Lincoln NE; and SO-210 Fast Oxygen Sensor, Apogee Inst. Logan Utah). 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 in a 70 °C |

At the end of the experiment, the leaves were dried to a constant mass in a 70 °C convection drying oven. Once dry, the leaf material was reweighed. Leaf mass per area (SLA, cm² g⁻¹), and leaf dry matter content (DMC, dry mass per unit wet mass) was determined from the total fresh mass, total dry mass, total leaf area (Patterson 2012).

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 one to four minutes depending of the sample) were discarded in order 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.

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

Contents: Table S1, Fig. S1

Table S1. Table of ANOVA results for tests of environmental and physiological data

| Measurement | Residual Degrees | F-statistic | P-value |
|--------------------------|------------------|-------------|----------|
| | of Freedom | | |
| SLA | 84 | 0.5044 | .4796 |
| Foliar %C | 13 | 4.2628 | .0595 |
| Foliar %N | 13 | 17.2942 | .001123 |
| Foliar C:N | 13 | 7.1246 | .01929 |
| Foliar δ ¹⁵ N | 13 | 40.0591 | .0000262 |
| Foliar δ^{13} C | 13 | 0.7878 | .3909 |
| Chlorophyll | 12 | 12.2476 | .004385 |
| Carotenoid | 12 | 5.0555 | .04412 |
| Anthocyanin | 12 | 2.8061 | .11975 |
| \mathbf{A}_{max} | 12 | 3.4209 | .08915 |
| R_{dark} | 12 | 2.2025 | .1636 |
| R_{10} | 11 | 4.2259 | .06434 |
| R_{20} | 11 | 12.7651 | .004373 |
| Respiratory Quotient | 12 | 1.1136 | .3121 |
| BAI | 6 | 90.665 | .0000765 |
| Crown Area | 12 | 0.0729 | .7917 |
| Height | 12 | .2632 | .6173 |
| Canopy Openness | 122 | 13.332 | .000385 |
| (Treatment) | | | |
| Canopy Openness | 122 | 0.735 | .392875 |
| (Treatment x Year) | | | |
| Leaf Litter dry weight | 12 | 1.1142 | .3120 |
| Soil pH | 12 | 6.6751 | .02394 |
| Soil Bulk Density | 28 | 0.2469 | .62318 |
| Soil Moisture | 22 | 2.5126 | .127208 |

Figure S1. Plot of Basal Area Increment (BAI) for only the 4 years prior-to and following girdling treatment implementation.

