

Growing-season warming and winter soil freeze/thaw cycles increase transpiration in a northern hardwood forest

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Abstract. Climate models project higher growing-season temperatures and a decline in the depth and duration of winter snowpack throughout many north temperate ecosystems over the next century. A smaller snowpack is projected to induce more frequent soil freeze/thaw cycles in winter in northern hardwood forests of the northeastern United States. We measured the combined effects of warmer growing-season soil temperatures and increased winter freeze/thaw cycles on rates of leaf-level photosynthesis and transpiration (sap flow) of red maple (*Acer rubrum*) trees in a northern hardwood forest at the Climate Change Across Seasons Experiment at Hubbard Brook Experimental Forest in New Hampshire. Soil temperatures were warmed 5°C above ambient temperatures during the growing season and soil freeze/thaw cycles were induced in winter to mimic the projected changes in soil temperature over the next century. Relative to reference plots, growing-season soil warming increased rates of leaf-level photosynthesis by up to $85.32 \pm 4.33\%$, but these gains were completely offset by soil freeze/thaw cycles in winter, suggesting that increased freeze/thaw cycles in winter over the next 100 yr will reduce the effect of warming on leaf-level carbon gains. Soil warming in the growing season increased rates of transpiration per kilopascal of vapor pressure deficit (VPD) by up to $727.39 \pm 0.28\%$, even when trees were exposed to increased frequency of soil freeze/thaw cycles in the previous winter, which could influence regional hydrology in the future. Using climate projections downscaled from the Coupled Model Intercomparison Project, we project increased rates of whole-season transpiration in these forests over the next century by 42–61%. We also project 52–77 additional days when daily air temperatures will be above the long-term average daily maximum during the growing season at Hubbard Brook. Together, these results show that projected changes in climate across both the growing season and winter are likely to cause greater rates of water uptake and have no effect on rates of leaf-level carbon uptake by trees, with potential ecosystem consequences for hydrology and carbon cycling in northern hardwood forests.

Key words: hydrology; snow; soil freezing; soil warming; water; winter climate.

INTRODUCTION

Temperate forests in northern latitude ecosystems around the world have experienced warmer air temperatures and a smaller winter snowpack over recent decades

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(Henry 2008, Kreyling and Henry 2011). Climate models project that mean annual air temperatures in the northeastern United States will rise 2.9–5.3°C by the year 2100 relative to the 1970–1999 mean, with increased frequency of growing-season heat waves (Hayhoe et al. 2007). In addition, the snowpack will continue to shrink in depth and duration (Reinmann et al. 2019), leading to increased frequency of soil freeze/thaw cycles in winter (Campbell et al. 2010). Northern forest ecosystems currently represent a net carbon (C) sink (Pan et al. 2011), meaning they take up more carbon dioxide (CO₂) through photosynthesis than they lose through respiration, and have a large influence on water dynamics in the region through soil water uptake and evapotranspiration (Jasechko et al. 2013). At the stomatal level, there is a trade-off between C uptake and water loss, making these processes intimately linked (Cowan 1978, Wong et al. 1979). If C uptake by trees is impacted by changing growing-season and winter soil temperatures, then the

capacity for trees to take up water during the growing season may also be affected. However, the ability of these forest trees to take up C and regulate the water cycle may be altered by changes in soil temperature over the next century.

In the snow-free season, warmer air temperatures can increase rates of net C uptake (Lu et al. 2013) and net primary productivity by trees (Keenan et al. 2014), which may be linked to increases in nitrogen (N) availability from net N mineralization as soils also warm (Melillo et al. 2011). However, as air temperatures rise they may surpass the photosynthetic temperature optimum for some tree species (Aber et al. 1995) and result in reduced forest net primary productivity (Allen et al. 2010, 2015, Tang et al. 2010, Filewod and Thomas 2014, Martin-Benito and Pederson 2015, Reinmann and Hutrya 2017). In the winter, root damage caused by soil freezing (Comerford et al. 2013) reduces the ability of trees to take up water and nutrients (Campbell et al. 2014, Sanders-DeMott et al. 2018b), which can result in further reductions in net primary productivity. Reinmann et al. (2019) found that adverse impacts of soil freezing on tree physiology reduced sugar maple growth after multiple years of snow removal and increased soil freezing.

With a changing climate, the timing and magnitude of evapotranspiration (Campbell et al. 2011), which is the sum of evaporation and transpiration (hereafter referred to as “sap flow”; Penman 1948) may shift, with implications for soil moisture and hydrology in northern hardwood forests. Transpiration by trees constitutes more than two-thirds of the annual forest–atmosphere water flux in northern hardwood forests (Jasechko et al. 2013), demonstrating the magnitude of influence water uptake by trees has on whole-forest water dynamics. Sap flow by trees connects soil water to the atmosphere and is driven by air and soil temperatures, vapor pressure deficit (VPD), leaf area, photosynthetically active radiation (PAR), and soil moisture (Day et al. 1990, Bergh and Linder 1999, Oren et al. 1999, Sun et al. 2000, Yin et al. 2004, Bovard et al. 2005, Juhász et al. 2013, Chang et al. 2014, Juice et al. 2016, Harrison et al. 2020). Although warmer soils have been shown to increase rates of sap flow in some temperate tree species such as red oak (*Quercus rubra*; Juice et al. 2016), soil freezing-induced damage to roots (Tierney et al. 2001, Comerford et al. 2013) impairs the ability of some tree species, such as sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*), to take up water during the following growing season (Robitaille et al. 1995, Harrison et al. 2020). However, soil freezing in winter has also been shown to increase growing-season rates of sap flow in other species such as red oaks (Harrison et al. 2020). In addition to direct root damage, winter soil freeze/thaw cycles have been shown to increase the occurrence of embolisms in xylem, which reduces conductivity and increases crown dieback in red spruce, birch, and many other diffuse-porous tree species (Sperry et al. 1994, Schaberg et al. 1996, Cox and Malcolm 1997).

Determining the combined effects of warmer growing seasons and increased frequency of soil freeze/thaw cycles in winter on rates of sap flow and leaf-level C uptake in the growing season will help us better predict how water and C cycling may change in the future. It is possible that damage to tree roots caused by soil freeze/thaw cycles in winter may reduce growing-season rates of water and C uptake and offset any warming-induced increases (e.g., Juice et al. 2016, Harrison et al. 2020). Here, we describe the results of an ongoing experiment examining the combined effects of warmer soil temperatures in the growing season and increased frequency of soil freeze/thaw cycles in winter on growing-season rates of leaf-level C uptake and water uptake in a northern hardwood forest dominated by mature red maple trees. We measured growing-season rates of leaf-level photosynthesis and sap flow in 2015 and 2017 at the Climate Change Across Seasons Experiment at Hubbard Brook Experimental Forest (hereafter “Hubbard Brook”) in New Hampshire, United States. We expected higher growing-season temperatures to lead to greater rates of photosynthesis and sap flow, but for these warming-induced changes to be offset by a smaller snowpack and greater frequency of soil freeze/thaw cycles in winter that reduce rates of leaf-level photosynthesis and reduce whole-tree rates of sap flow.

METHODS

Study site

This work was conducted at Hubbard Brook, a U.S. National Science Foundation Long-Term Ecological Research site located in the White Mountain National Forest in central New Hampshire, United States (43°56' N, 71°45' W). Canopy vegetation at HBEF is primarily dominated by northern hardwoods, with coniferous species present on steeper slopes and at higher elevations. Soils consist of base-poor spodosols, specifically Typic Haplorthods that developed in glaciofluvial sand and gravel, and depth to bedrock is approximately 14 m (Winter et al. 2008). The climate is cool, humid, and continental, with mean annual precipitation of 1,400 mm falling evenly throughout the year. Winter air temperatures average -4.7°C (Bailey et al. 2003; years 1969–2000) and soil frost is present approximately 2 out of every 3 yr, with an average annual maximum depth of <10 cm (Campbell et al. 2010, Fuss et al. 2016).

Climate change across seasons experiment (CCASE)

We established CCASE in summer 2012 at HBEF (Templer et al. 2017) to examine the effects of a 5°C increase in temperature during the snow-free season and increase in soil freeze/thaw cycle frequency in winter projected for this region over the next century (Hayhoe et al. 2007, Campbell et al. 2010). The plots in our experiment are dominated by red maple trees (*Acer rubrum*),

which make up $63 \pm 7\%$ basal area with an understory composed of mostly American beech (*Fagus grandifolia*) saplings. The six plots are each 11×13.5 m and contain at least three mature red maple trees, a common canopy tree in northern hardwood forests and the focus of this study. The six plots were purposefully located to have similar tree species composition and aboveground biomass (Templer et al. 2016). Litterfall mass and diameter at breast height (DBH) of red maple trees were not significantly different across the six plots in 2012 (prior to the start of the experiment; $P = 0.54$ and 0.13 , respectively); all trees had a DBH >20 cm in 2015 when sap flow sensors were installed. Specifically, the DBH on which we installed sap flow sensors was 24.07 ± 2.63 cm, 30.51 ± 3.73 cm, and 32.42 ± 2.12 cm (mean $\pm$ standard error of the six trees of each treatment; $n = 3$ per plot), in the *reference*, *warmed*, and *warmed + FTC* plots, respectively, prior to the start of the experiment.

Soil temperature and snow-manipulation treatments are ongoing and began in December 2013. To summarize Templer et al. (2017), plots were established in specific locations to have similar tree basal area, soil type, slope, and aspect. We did not randomly assign treatments because of logistical constraints caused by the electrical infrastructure. There are two plots with soils warmed 5°C above ambient (“*warmed*”) between spring snowmelt and the first snowfall (April–November/December; hereafter referred to as the growing season); two plots with the same warming treatment combined with soil freeze/thaw cycles induced in winter (“*warmed + FTC*”); and two plots with ambient soil temperature (“*reference*”). The *warmed* and *warmed + FTC* plots together make up the four “treatment” plots and are equipped with heating cables that were buried by hand 10 cm deep using a flat shovel in 2012 in parallel lines spaced 20 cm apart. *Reference* plots were similarly cut to mimic cable installation disturbance, but no cable was installed.

In the *warmed + FTC* treatment plots, the first snow of winter is gently packed down to maintain albedo and minimize disturbance to the forest floor with subsequent shoveling. We induce soil freezing by removing snow within 24 h of snowfall events in winter. Soil freezing is operationally defined as soil temperature $<-0.5^\circ\text{C}$. After soils are frozen for 72 h, the heating cables are turned on to warm soils to 1°C to induce a 72-h thaw. The entire process of freezing for 72 h and thawing for 72 h total constitutes one soil freeze/thaw cycle. We induced four freeze/thaw cycles in the winters of both 2013/2014 and 2014/2015, two in 2015/2016, and one in 2016/2017. Additional details on the experimental design and implementation are described in Templer et al. (2017).

Environmental variables

Soil temperature (Betatherm type 10K3A1; $n = 6$ at 10-cm depth for all plots) and volumetric soil moisture ($\text{m}^3 \text{H}_2\text{O}/\text{m}^3$ soil volume; CS616; Campbell Scientific,

Logan, Utah, United States; $n = 4$ per plot integrated across 0–30-cm depth) were logged every 5 s, and half-hourly means were stored on a CR1000 multichannel data logger (Campbell Scientific). Air temperature ($n = 2$ sensors total) and relative humidity (RH; $n = 2$ sensors total) were measured in two locations (CS215; Campbell Scientific) and used to calculate VPD, which is the difference between saturated and actual vapor pressure.

Soil frost and snow depth were measured weekly throughout winter using frost tubes ($n = 4$ per plot; Ricard et al. 1976) and a meter stick inserted into snowpack from 1 November through 30 April for all years. We report winter data only for the two winters that precede the growing seasons of 2015 (19 November 2014 to 21 April 2015) and 2017 (29 November 2016 to 14 April 2017), as they are the focus of this study. Soil frost duration (i.e., number of days with frost) during winter was calculated as days when depth of soil frost was greater than 0 cm. Soil frost and snow depth measurements made in each plot throughout winter were each converted into a single continuous variable representing both depth and duration (Durán et al. 2014) by calculating the area under the curve (AUC) (i.e., x -axis = time measured in days) of soil frost or snow depth (i.e., y -axis = depth measured in centimeters) using the R package “pracma” (Borchers 2019).

Foliage sampling for photosynthesis and stomatal conductance

On 11 June and 3 August in 2015 and on 28 June, 28 July, and 14 August in 2017, sun-lit foliage from the top of the canopy was collected from four dominant red maple trees (three of which overlap with trees in which we measured sap flow) in each plot using a shotgun ($n = 3$ leaves per tree; 4 trees per plot). It was not possible to make in situ measurements of photosynthesis and stomatal conductance because of the height of sunlit branches in our trees and lack of accessibility to them. In our previous tests measuring rates of photosynthesis on red maple branches that were intact and still connected to the tree compared to excised branches, rates of photosynthesis did not change for at least 10 min following excision of branches from trees. Therefore, to minimize potential artifacts associated with measuring photosynthesis on excised branches in this study, we submerged the ends of excised branch shoots in water, cut 2–3 cm above the break, and measured rates of photosynthesis within 5 min of excision from the trees. We also allowed leaves to stabilize for approximately 1–2 min and then made five measurements of photosynthesis and conductance at 15-s intervals over the course of 1 min and utilized the mean of these five measurements. Photosynthesis ($\mu\text{mol CO}_2\text{-m}^{-2}\text{-s}^{-1}$) and stomatal conductance ($\text{mol H}_2\text{O-m}^{-2}\text{-s}^{-1}$) were measured on three leaves per tree using a portable photosynthesis system (LI-6400, LiCor, Lincoln, Nebraska, United States) and averaged for each tree prior to statistical analysis. Measurements were made at saturating light levels

(1,000 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) using a built-in LED light source to simulate the high light conditions at the top of the canopy, the CO_2 concentration in the reference analyzer was set to 400 $\mu\text{mol per mol}$, and relative humidity and temperature in the sample chamber were equilibrated to ambient levels. One value for conductance was removed from analysis and determined to be an outlier because it was greater than two standard deviations away from the mean. We calculated intrinsic water use efficiency (iWUE) by dividing leaf-level photosynthesis by conductance for each leaf sample ($\mu\text{mol CO}_2 \text{ mol per H}_2\text{O}$).

Sap flow measurements

Sap flow was measured continuously throughout the 2015 and 2017 growing seasons (10 May to 7 November or 2015 and 2017; total of 181 d from DOY 130–311; Appendix S1: Fig. S1) on three of the four red maple trees in each plot that we used to measure photosynthesis. We measured sap flow every other year, rather than annually, to minimize disturbance to the trees; the 2 yr included in this study had comparable environmental conditions, such as air and soil temperatures and amount of precipitation, throughout the growing season, but the winter of 2016/2017 (mean air temperatures = -2.23°C) was slightly warmer than in 2014/2015 (mean air temperatures = -6.57°C). Sap flow measurements were made using the heat dissipation method described by Granier (1987). Each sap flow sensor consisted of two probes located 10 cm vertically apart and constructed from 21-mm-long stainless steel hollow needles with copper constantan wire (type T) thermocouples that are inserted into aluminum tubes drilled into the tree bole. Sensors were installed in April of 2015 and 2017 at approximately 1.3 m height at three locations (120° from one another) on the bole of three mature red maple trees in each plot ($n = 54$ sensors total). This sampling strategy was intended to evaluate intrinsic physiological treatment responses in trees by sampling sap flux across the most hydroactive circumferential band of the xylem rather than to characterize radial depth profiles of sap flux for whole tree or site level scaling (Phillips et al. 1996, Clearwater et al. 1999). Prior to installation, the upper probe in each sensor was wound with electrically insulated constantan wire that received 185 mW of constant power to generate heat and coated with heat-conducting silicon paste. The upward movement of water dissipates the heat supplied to the upper probe so that the differential voltage measured across the copper leads is proportional to the temperature difference (ΔT_{sap}) between the two thermojunctions. Sensors were protected from precipitation using polystyrene shields sealed to the tree stem with an acid-free silicone caulk, and covered with reflective bubble wrap to protect from direct solar radiation. Half-hourly averages of differential voltages between probes measured at 30-s intervals were stored on a CR1000 multichannel data logger.

Power outages caused occasional gaps in data in 2015. Sap flow sensors were replaced for the 2017 growing season and there were no gaps in data.

Sap flow data analysis

Sap flux per unit conducting xylem area (J_s , in $\text{g H}_2\text{O}\cdot\text{m}^{-2} \text{ sapwood area}\cdot\text{s}^{-1}$) was calculated relative to the zero sap flow condition of each tree, or nighttime conditions (defined as the maximum stable difference between upper and lower probes, $\Delta T_{\text{sap max}}$). Environmental factors that affect stem water content cause variation in $\Delta T_{\text{sap max}}$ over time (Lu et al. 2004), necessitating frequent redefinition of the zero-flow state. For each day, $\Delta T_{\text{sap max}}$ was determined to have occurred when the following two conditions were met: (1) the temperature gradient between the reference and heated probes was stable for at least 2 h; and (2) the ambient VPD was calculated to be $<0.1 \text{ kPa}$. Sap flow rates (J_s) were calculated using the empirical relationship of temperature difference between the heated and unheated sensors at any given time (ΔT_{sap} ; $^\circ\text{C}$) and sap velocity (Eq. 1; Granier 1987):

$$J_s = 119 \left(\frac{\Delta T_{\text{sap max}} - \Delta T_{\text{sap}}}{\Delta T_{\text{sap}}} \right)^{1.231} \quad (1)$$

Raw data were filtered before conversion to eliminate electrical spikes and other invalid data. We averaged sap flow rates across the three sensors per tree to determine rates of instant sap flow for each tree across the entire growing season. Because of some malfunctioning probes, data from only 13 of 18 instrumented trees ($n = 3$ in the *reference*; $n = 5$ in the *warmed*; and $n = 5$ in the *warmed + FTC*) were used for analysis in 2015. However, all trees produced reliable data in the 2017 growing season. We limited our statistical analysis of sap flow rates and environmental variables to the hours 05:00 to 21:00 for the entire growing season.

Statistical analyses

All statistical analyses were conducted with R statistical software (version 3.0.3; R Development Core Team 2014). Error is reported as standard error (SE) of the mean. We considered “plot” to be the experimental unit with trees nested within plot to account for the multiple trees sampled in each plot and the repeated sampling of individual trees over time. Also, by including tree-level data within each statistical model and nesting trees within plot, we accounted for differences in the number of trees included in all analyses in 2015 and 2017. We also did not directly compare data in 2015 and 2017 and instead examined whether trends were consistent between the 2 yr.

We conducted an analysis of variance (ANOVA) with treatment as the fixed effect and plot as the random effect to determine potential differences in snow and soil

frost AUC, minimum soil temperature, maximum soil frost depth, number of soil freeze/thaw cycles within each of the 2014/2015 and 2016/2017 winters, and soil temperature at 10-cm depth in each of the 2015 and 2017 growing seasons.

We used mixed linear effects models to examine differences in photosynthesis, stomatal conductance, and the relationship between instant daily sap flow rates across the entire growing season and mean or maximum daily air temperature. We examined models as both first- and second-order linear equations and used an ANOVA of these two models to determine which line is the best fit. We used ANOVAs to compare the slopes for the relationships between air temperature and rates of sap flow on both typical and heat-stress days (see "Projected changes in sap flow") between the three treatments. For all linear models, we examined residuals plotted against predictors and confirmed there were no patterns indicating autocorrelation.

We compared daily averaged sap flow rates and VPD in all three treatments with linear mixed effects models. We examined these relationships during three time periods: leaf expansion in the early growing season (first 2 weeks of sap flow measurements; DOY 130–145 in 2015 and 2017), the entire growing season, and on heat-stress days. Heat-stress days were defined as those when the maximum air temperature was above the long-term average daily maximum air temperature in July at Hubbard Brook (23.6°C for 2001–2017; for more information see Harrison et al. 2020). Similar to Harrison et al. (2020), the remaining growing-season days that were below temperatures on heat-stress days were categorized as "typical" days.

We used the package "nlme" in R (Pinheiro et al. 2012) for all linear mixed effects models and calculated the marginal R^2 values to describe the proportion of variance explained by the fixed effects using the R package "piecewiseSEM" (Lefcheck 2016). All post hoc pairwise comparisons among treatments were calculated using Tukey's honestly significant difference tests. Because of the cost and infrastructure required to implement the experimental treatments, it was not possible to have more than two *reference*, two *warmed*, and two *warmed + FTC* plots. Therefore, we used $\alpha = 0.10$ to evaluate significant treatment effects, unless otherwise noted.

Projected changes in sap flow

We used projected regional air temperature changes by the end of this century alongside our measurements of sap flow to predict how sap flow might change in the future with warmer air temperatures and increased frequency of soil freeze/thaw cycles. VPD was not integrated into this analysis because it is not expected to change during the growing season over the next century for the northeastern United States (Ficklin and Novick 2017). Our projections are based on the number of heat-stress days (as defined above, Reinmann and Hutrya

2017, Harrison et al. 2020) during the growing season between 2001 and 2017 and those projected under low and high emissions scenarios for the years 2080–2099. The growing-season dates were limited to the same growing-season dates used to measure sap flow (DOY 130–311).

We used downscaled climate projections for Hubbard Brook calculated for 1/16° grid cells from Phase 5 of the Coupled Model Intercomparison Project (CMIP5; complete list of models can be found in Reinmann and Hutrya 2017) to estimate projected maximum daily air temperatures (DOY 130–311) for the years 2080–2099 under the Intergovernmental Panel on Climate Change (IPCC) Representative Concentration Pathway (RCP) for low (RCP 4.5) and high emission (RCP 8.5) scenarios. We then calculated the average daily instant rates of sap flow, limited to daylight hours 05:00 to 21:00 for typical and heat-stress days separately, as an average for 2015 and 2017, the 2 yr we measured sap flow. These values were then multiplied by the number of typical and heat-stress days under historic conditions (from 2001 to 2017) and under the two projected scenarios (see Table 2) and summed for the growing season (see Harrison et al. 2020 for more details).

We estimated the projected total rate of sap flow across the growing season scaled by the expected number of typical and heat-stress days in growing seasons in past (2001–2017) and future (2080–2099; Table 2) scenarios and then calculated daily rates. Although a lengthening of the growing season is projected by the end of the century, for purposes of comparison we kept the growing-season length the same for both time periods (2001–2017 and 2080–2099). We also present these results with the caveat that they do not account for changes in atmospheric CO₂ concentrations. Unlike sap flow, we did not project changes in photosynthesis over the next century, as we measured photosynthesis on five dates total, making it impossible to project rates of this process for typical and heat-stress days through the end of the century.

RESULTS

Environmental variables

The warming treatment led to increased soil temperatures in the *warmed* and *warmed + FTC* by $5.1 \pm 0.05^\circ\text{C}$ and $5.0 \pm 0.05^\circ\text{C}$, respectively, during the growing season of 2015 (21 April to 1 December 2015; Table 1, Fig. 1), and by $4.99 \pm 0.02^\circ\text{C}$ and $4.88 \pm 0.08^\circ\text{C}$, respectively, in the growing season of 2017 (14 April to 15 November 2017; Table 1) compared to the *reference* plots. Soil moisture integrated from 0- to 30-cm depth was not significantly different among plot types throughout the 2015 growing season ($P = 0.69$), the 2014/2015 winter ($P = 0.29$), the 2017 growing season ($P = 0.88$), or the 2016/2017 winter ($P = 0.32$). Snow removal in the *warmed + FTC* plots reduced snow depth, snow area under the curve (AUC), and minimum

TABLE 1. Environmental variables by experimental treatment for the 2014–2015 (19 November 2014 to 21 April 2015) and 2016–2017 (29 November 2016 to 14 April 2017) winter and growing season in 2015 (21 April 21 to 1 December 2015) and 2017 (14 April 2017 to 15 November 2017) soil temperature at 10-cm depth.

	Reference	Warmed	Warmed + FTC
2015			
Minimum soil temperature (°C)*	$-0.13^a \pm 0.029$	$-0.062^a \pm 0.14$	$-1.75^b \pm 0.028$
Maximum snow depth (cm)***	$55.30^a \pm 0.82$	$51.30^a \pm 1.10$	$18.63^b \pm 0.75$
Snow AUC (cm days)***	$3,732^a \pm 59$	$3,322^a \pm 105$	$735^b \pm 12$
Maximum frost depth (cm)*	$14.0^a \pm 1.5$	$15.2^a \pm 1.0$	$20.1^b \pm 2.9$
Frost AUC (cm days)	$1,215 \pm 149$	$1,243 \pm 105$	$1,505 \pm 123$
Number of soil freeze/thaw cycles	0	0	4
Growing-season soil temperature (°C)**	$12.73^a \pm 0.09$	$17.86^b \pm 0.16$	$17.74^b \pm 0.045$
2017			
Minimum soil temperature (°C)***	$0.11^a \pm 0.045$	$0.41^a \pm 0.021$	$-2.36^b \pm 0.11$
Maximum snow depth (cm)***	$82.55^a \pm 2.75$	$77.15^a \pm 2.15$	$16.3^b \pm 0.5$
Snow AUC (cm days)*	$4,412^a \pm 52$	$3,692^a \pm 469$	$453^b \pm 0.13$
Maximum frost depth (cm)*	$9.70^a \pm 2.3$	$10.6^a \pm 0.04$	$22.4^b \pm 0.9$
Frost AUC (cm days)*	$439^a \pm 178$	$383^a \pm 30$	$1,621^b \pm 203$
Number of soil freeze/thaw cycles	0	0	1
Growing-season soil temperature (°C)***	$13.19^a \pm 0.11$	$18.53^b \pm 0.091$	$18.48^b \pm 0.035$

Notes: Values are means with standard error (* $P < 0.05$, ** $P < 0.05$, *** $P < 0.0001$) across plots unless otherwise noted. Distinct letters within a row indicate statistically significant differences among treatments. AUC, area under the curve.

winter soil temperatures, and increased maximum soil frost depth, soil frost AUC, and number of soil freeze/thaw cycles (Table 1) in both years.

Photosynthesis, stomatal conductance, and intrinsic water use efficiency

Leaf-level photosynthesis ranged from 2.12 to 8.57 $\mu\text{mol CO}_2\text{-m}^{-2}\cdot\text{s}^{-1}$ in 2015 and 4.26–11.49 $\mu\text{mol CO}_2\text{-m}^{-2}\cdot\text{s}^{-1}$ across dates and treatments in 2017. There was a significant difference in rates of leaf-level photosynthesis across our treatments ($P = 0.08$ and <0.001 when comparing all treatments in 2015 and 2017, respectively; Fig. 2). Specifically, growing-season warming increased rates of leaf-level photosynthesis of red maples in *warmed* plots by $85.32 \pm 4.33\%$ in 2015 and by $50.29 \pm 4.28\%$ in 2017 relative to trees in the *reference* plots ($P < 0.001$ when comparing *reference* to *warmed* in both 2015 and 2017; Fig. 2). However, there were no significant differences in photosynthesis between *reference* and *warmed + FTC* plots in either year ($P = 0.66$ and 0.26 when comparing *reference* to *warmed + FTC* in 2015 and 2017, respectively; Fig. 2).

In 2015 and 2017, conductance ranged from 0.030 to 0.54 $\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 0.025 to 0.14 $\text{mol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively. Growing-season warming increased conductance in red maples by $63.72 \pm 0.28\%$ and $71.09 \pm 0.11\%$ in 2015 in *warmed* and *warmed + FTC* plots relative to trees in the *reference* plots ($P < 0.01$; Fig. 2). However, only *warmed* plots had significantly higher conductance in 2017 by $35.00 \pm 0.48\%$ compared *reference* plots in 2017 ($P < 0.01$; Fig. 2).

In 2015 and 2017, iWUE ranged from 15.14 to 91.26 $\mu\text{mol CO}_2$ mol per H_2O in 2015 and

52.69–99.25 $\mu\text{mol CO}_2$ mol per H_2O , respectively. Growing-season warming lowered iWUE in red maples by $63.72 \pm 0.28\%$ and $71.09 \pm 0.11\%$ in 2015 in *warmed* and *warmed + FTC* plots relative to trees in the *reference* plots ($P = 0.01$; Fig. 2). There were no differences in iWUE across treatments in 2017 ($P = 0.37$).

Sap flow

Across all three periods of analysis (early growing season, entire growing season, and heat-stress days) for both years, there was a positive relationship between vapor pressure deficit (VPD) and sap flow (Figs. 3 and 4). In the early growing season of 2015, there were no differences in the sensitivity of sap flow rates to changes in VPD across treatments, meaning there were no significant differences among treatments in the slope of the positive relationship between sap flow and VPD (Fig. 4 A). However, in the early growing season of 2017 sap flow rates in *warmed* and *warmed + FTC* plots were $107 \pm 0.75\%$ and $19 \pm 0.15\%$ more sensitive to changes in VPD compared to *reference* for each treatment, respectively (Fig. 4B).

Throughout the growing season, trees in the *warmed* plots were $26.65 \pm 0.73\%$ and $76.40 \pm 0.29\%$ more sensitive to changes in VPD compared to trees in the *reference* plots in 2015 and 2017, respectively ($P < 0.01$ and $P < 0.001$ for 2015 and 2017, respectively; Fig. 4C,D). In the 2017 growing season, sap flow rates in *warmed + FTC* trees were $52.03 \pm 0.25\%$ more sensitive to changes in VPD compared to *reference* ($P < 0.01$), but not in 2015 ($P = 0.11$).

During heat-stress days in 2017, trees in the *reference* plots were insensitive to changes in VPD (slope =

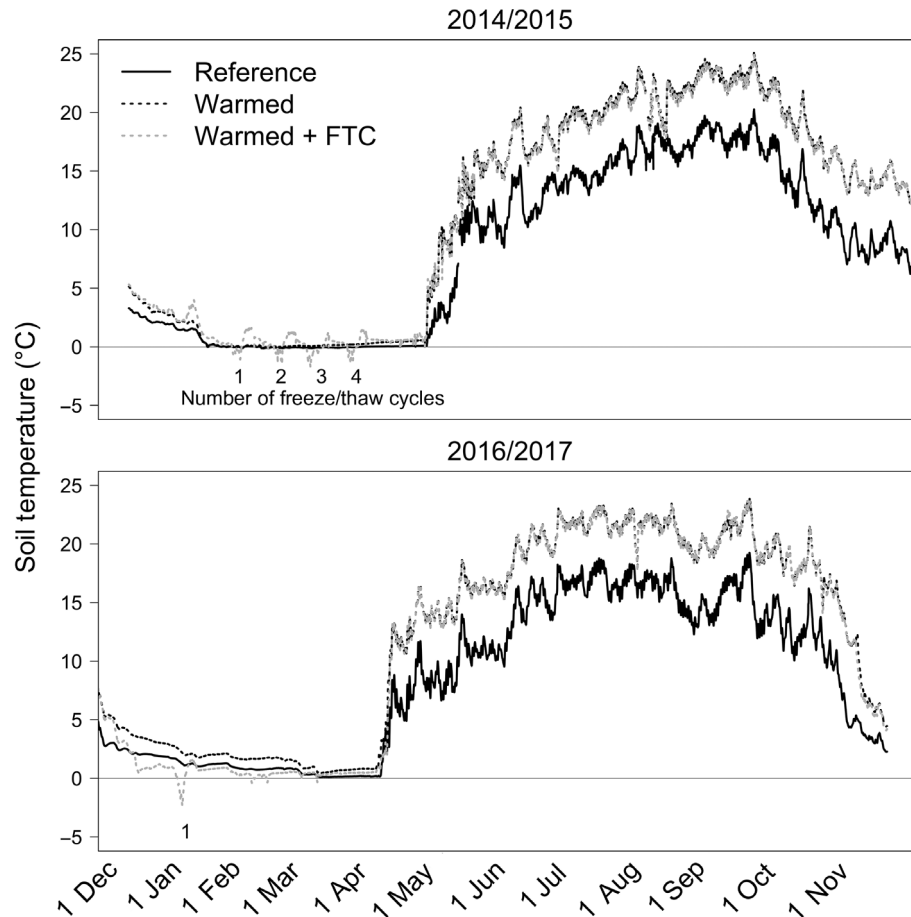


FIG. 1. Soil temperatures at 10-cm depth for three treatments for 1 December to 30 November for the 2014/2015 and 2016/2017 winter and growing season at Hubbard Brook. Numbers indicate the completed freeze/thaw cycles each winter (four in 2014/2015 and one in 2016/2017).

$0.73 \pm 3.26 \text{ g H}_2\text{O}\cdot\text{m}^{-1} \text{ sapwood}\cdot\text{s}^{-1}\cdot\text{kPa VPD}$), compared with trees in the treatment plots that were more sensitive to changes in VPD ($P = 0.01$; Fig. 4F) by $904.11 \pm 0.74\%$ and $727.39 \pm 0.28\%$ in *warmed* and *warmed + FTC*, respectively.

Rates of sap flow increased with increases in mean (Fig. 5A, B) and maximum (Fig. 5C, D) air temperature across all three plot types for both years. Trees exposed to *warmed* and *warmed + FTC* treatments had higher rates of sap flow associated with increases in mean and maximum air temperature across both years compared with *reference* trees (Fig. 5). We also observed that the slope of the relationship between sap flow and air temperature was higher in trees exposed to *warmed* and *warmed + FTC* treatments (both mean and maximum; $P < 0.001$ for all trees in both years) compared to *reference* plots (Fig. 5).

We found a slight negative relationship between rates of sap flow and soil moisture across all three treatments in both years (Fig. 6). We found a significantly larger decrease in soil moisture associated with increases in

rates of sap within the *reference* compared to *warmed* and *warmed + FTC* plots.

Projected changes in sap flow

From 2001 to 2017 there were, on average, 62 ± 4 heat-stress days throughout the growing season (DOY 130–311) per year. We project that from 2080 to 2099 there will be 52–77 additional, or 114–139 total, heat-stress days under RCP4.5 and RCP8.5 scenarios, respectively. With projected increases in air temperatures in the northeastern United States over the next century, our results suggest that red maple trees are likely to experience higher sap flow rates (Fig. 7). However, trees that experience a greater frequency of winter soil freeze/thaw cycles are likely to have lower rates of sap flow than those with growing-season soil warming alone (Fig. 7, Table 2). Our calculations of projected rates of sap flow over the next century based on measurements we made suggest that sap flow will be 42–61% (calculated based on projected sap flow

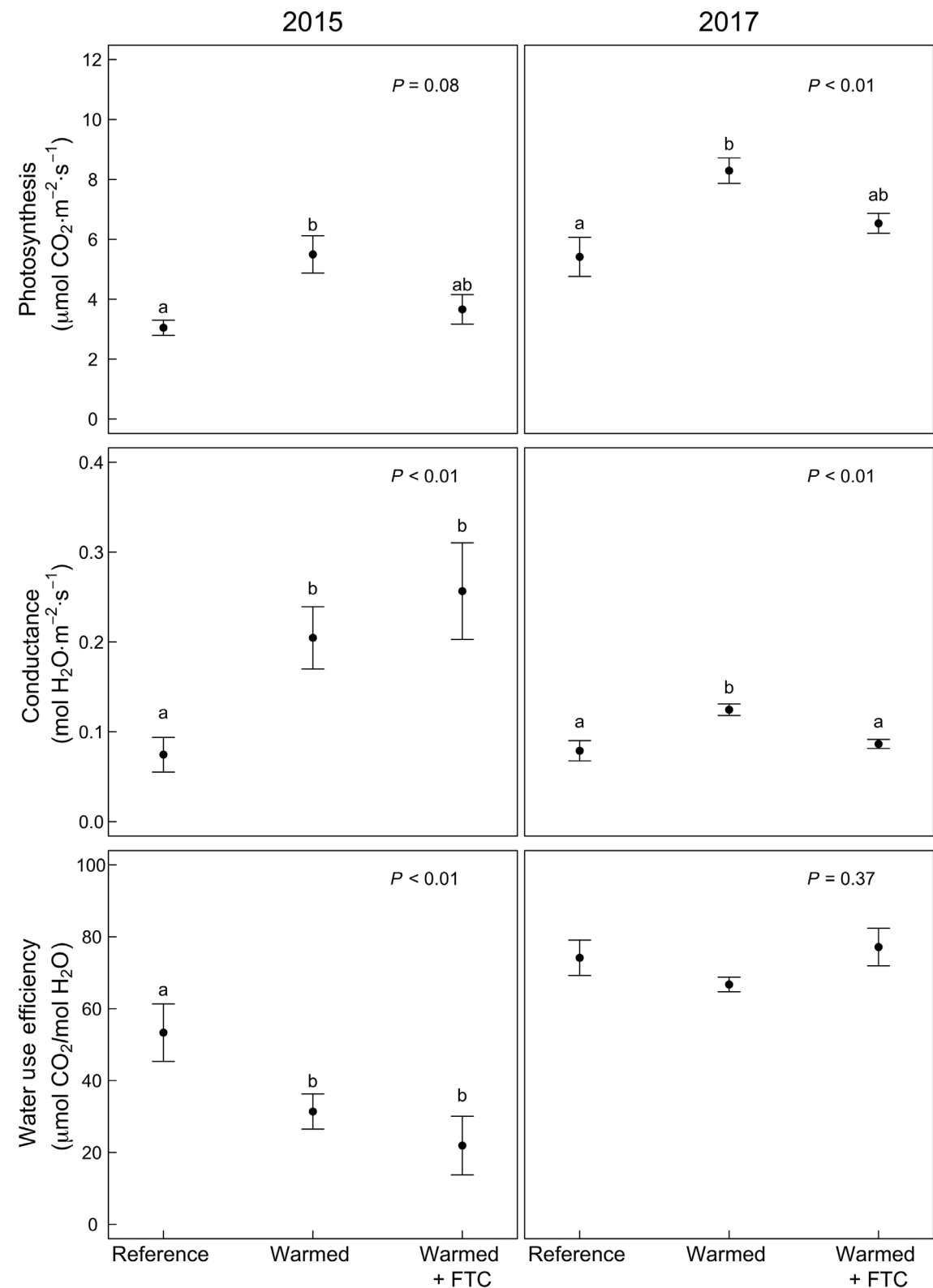


FIG. 2. Leaf-level photosynthesis, stomatal conductance, and intrinsic water use efficiency measured two and three times throughout the 2015 and 2017 growing seasons, respectively. Values are means with standard error. Statistics are shown for mixed effects models with tree nested within plot as the random variable. Different letters represent statistically significant differences among treatments.

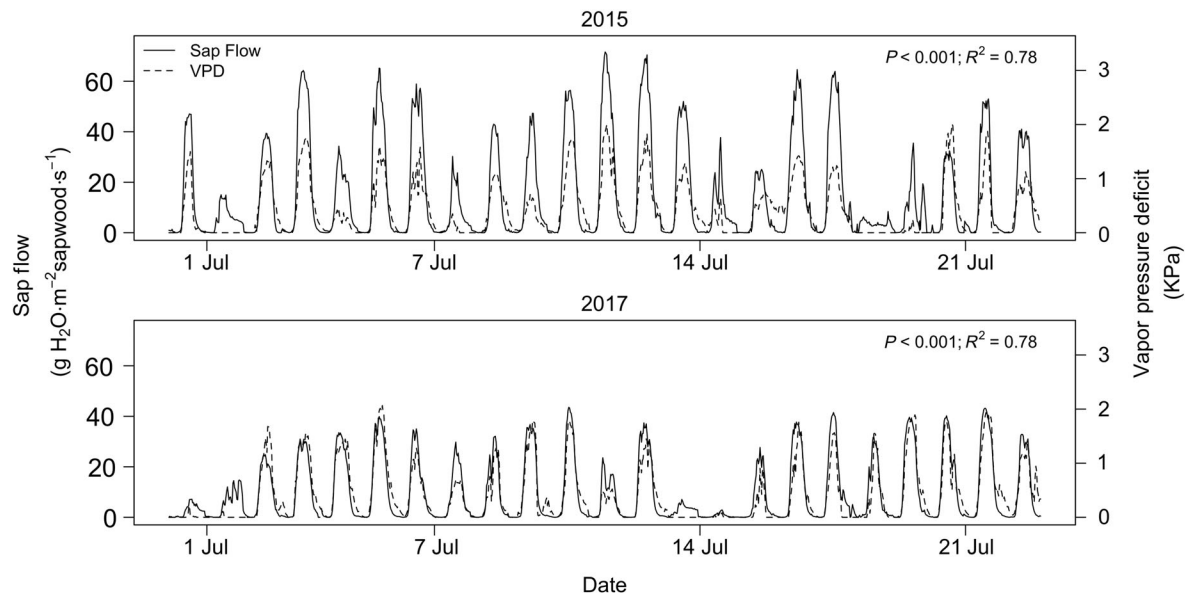


FIG. 3. Relationships between instantaneous rates of sap flow and vapor pressure deficit (VPD) during 3 weeks of the peak growing season in 2015 and 2017. Data limited to 3 weeks to make viewing data easier than the entire growing season. Figures show averages across reference plots only. The R^2 and P values provided are for the line of best fit between sap flow and VPD during the 3 weeks of data shown.

rates under RCP 8.5 in *warmed* and *warmed + FTC* plots compared to current rates of sap flow in *reference* plots; see Table 2) greater than the rates of trees under current ambient soil temperatures.

DISCUSSION

Our study demonstrates that the combined effects of the projected rise in both growing-season temperatures (Hayhoe et al. 2007) and frequency of soil freeze/thaw cycles (Campbell et al. 2010) in the northeastern United States over the next century are likely to affect leaf-level C and whole-tree water uptake differently. Although growing-season soil warming increased rates of leaf-level photosynthesis by up to 85%, soil freeze/thaw cycles in the previous winter offset these gains (Fig. 2). In both years, leaf-level conductance increased with soil warming, whereas in 2017 these high rates were offset with soil freeze/thaw cycles (Fig. 2). Overall, $iWUE$ decreased in all plots with growing-season soil warming in 2015, but not in 2017 (Fig. 2). Growing-season soil warming stimulated water uptake in 2015 and 2017, and this increase was maintained despite soil freeze/thaw cycles in the previous winter (Figs. 4 and 5). Although the trees experienced fewer freeze/thaw cycles in the winter of 2016/2017 compared to 2014/2015, our consistent results across years indicate that long-term monitoring of these plots is needed in order to understand how multiple years of treatment affect these forests. Our projections indicate that expected increases in heat-stress days over the next century will likely increase rates of transpiration for red maple trees (Fig. 7), even when considering that trees

will experience more frequent soil freeze/thaw cycles over the same time period.

Environmental variables

There were significant responses to our soil temperature manipulations in both years. Specifically, growing-season soil temperatures in both 2015 and 2017 increased by $\sim 5^\circ\text{C}$ in our warmed plots, with similar soil temperatures across both years. Our winter climate manipulation led to a reduction in winter soil temperatures, a shallower snow depth, and increased soil frost depth and number of freeze/thaw cycles in both years. However, there were more soil freeze/thaw cycles in 2015 (4) compared with 2017 (1). Our experimental definition of a freeze/thaw cycle is when soils are at or below -0.5°C for 72 h, which we then experimentally thaw for 72 h at 1°C . We were constrained in the number of freeze/thaw cycles we could induce each winter by how long air temperatures were below freezing, because soil freezing can only be induced when air temperatures are below 0°C . In 2017, winter air temperatures did not stay below freezing for as long and therefore did not allow for as many experimental soil freeze/thaw cycles as in 2015. The start of spring, which we defined here by when daily soil temperatures begin to naturally ramp up and track air temperatures (Groffman et al. 2012) were similar across both years (21 April 2015 and 14 April 2017), and there were no soil freezing events immediately before (<45 d) phenological events like bud break in either year. We therefore do not believe that differences in growing-season soil temperature or the timing of soil

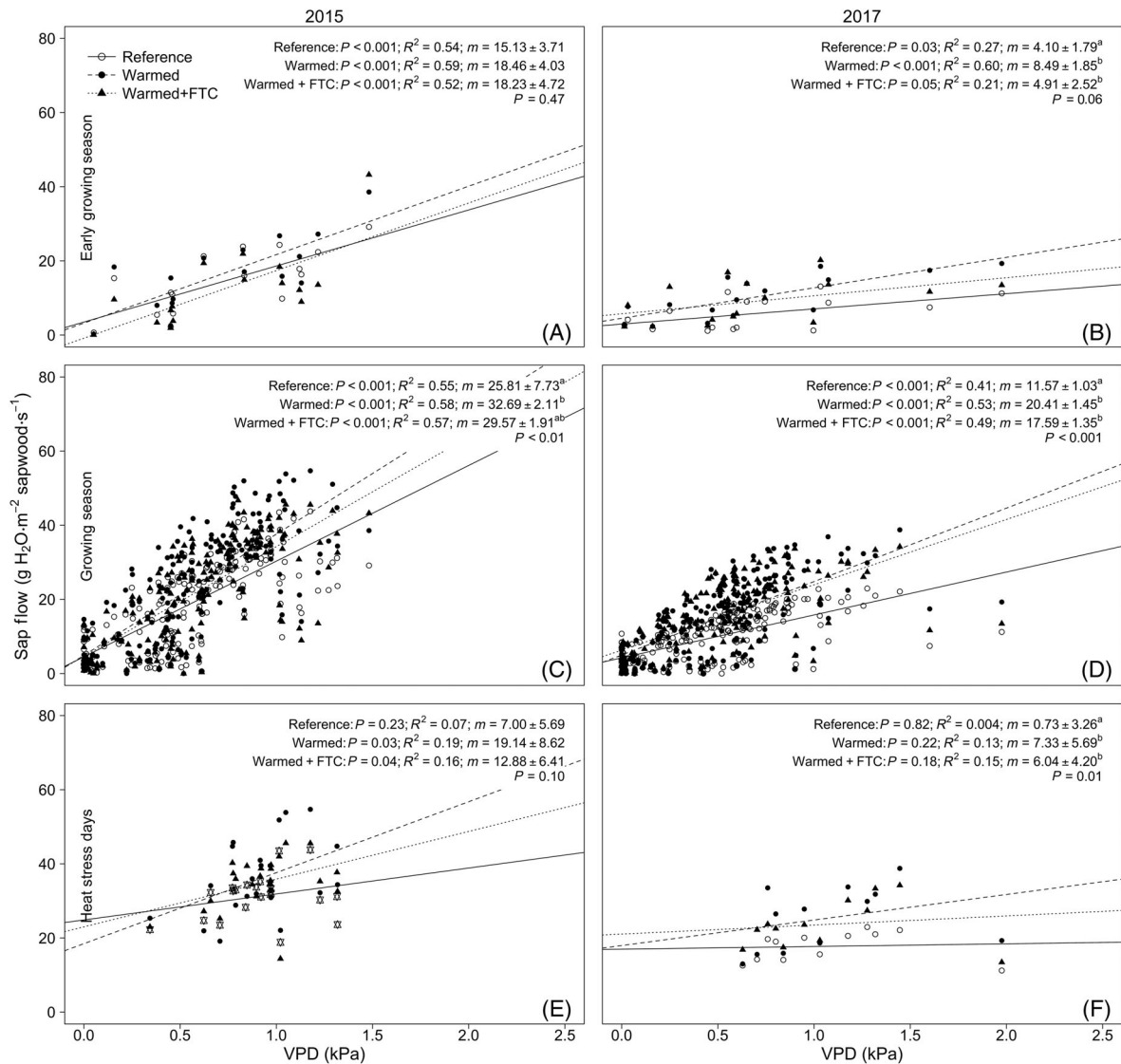


FIG. 4. Relationships between rates of sap flow and vapor pressure deficit (VPD; kPa) during three time periods in 2015 and 2017: early growing season (panels A, B), entire growing season (panels C, D), and heat-stress days (when air temperatures were $>23.6^{\circ}\text{C}$ at Hubbard Brook; panels E, F). Each dot represents the daily average rate of sap flow and VPD for daylight hours for individual trees in reference or snow-removal plots. P values are included comparing the slopes between reference and snow-removal plots.

frost events in winter caused differences in our observations between 2015 and 2017, but it is possible that the fewer soil freeze/thaw cycles in the 2016/2017 winter led to fewer significant differences between reference and treatment plots in 2017 compared to 2015.

Photosynthesis, stomatal conductance, and intrinsic water use efficiency

We found that warmer growing soil temperatures induce greater rates of leaf-level photosynthesis, consistent with past studies that show increased net C uptake and net primary productivity with warming (Lu et al.

2013, Keenan et al. 2014). In contrast, we observed no change in photosynthesis with increased frequency of soil freeze/thaw cycles. Because of the cost and infrastructure required to implement the experimental treatments in this study, we had two plots per treatment and used an $\alpha = 0.10$, resulting in a 10% chance of a false positive. Although the P value for differences in photosynthesis across treatments was greater in 2015 ($P = 0.08$) than in 2017 ($P < 0.01$), which indicates a higher probability of a false positive, the trends of increased photosynthesis with soil warming that were offset by soil freeze/thaw cycles in winter are consistent across both years. We therefore do not believe our results

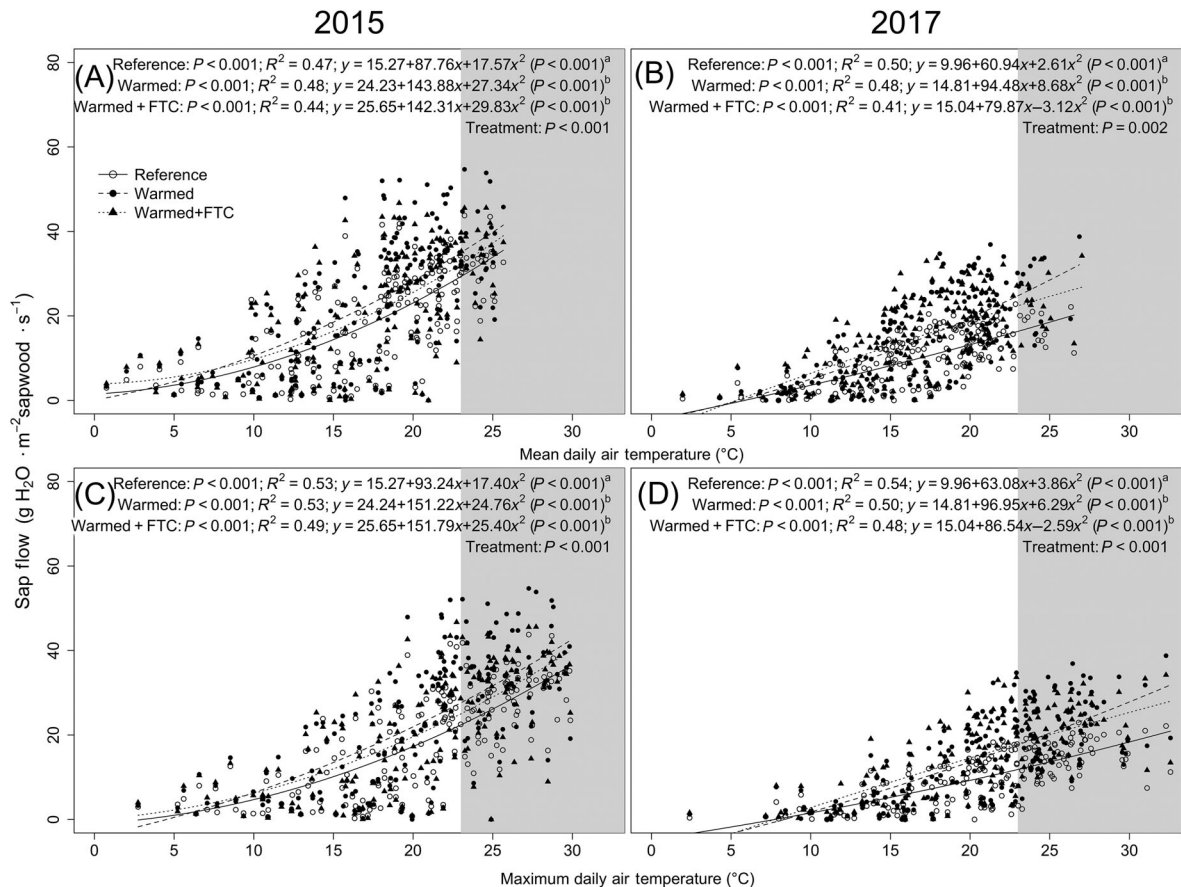


FIG. 5. Relationships between rates of sap flow and mean (panels A and B) and maximum (panels C and D) daily air temperatures (°C) throughout the entire growing season. Each dot represents the daily average rate of sap flow for individual trees in the *reference* (solid lines), *warmed* (dashed lines), or *warmed + freeze/thaw cycles* (dotted lines) plots during daylight hours (05:00 to 21:00). *P* values are shown in parentheses comparing slopes between the typical and heat-stress days, within each treatment. *R*², slopes, and equations before the parentheses are for best-fit lines that include all days (both typical and heat-stress days). Shaded region represents days that are considered heat-stress days (when air temperatures were >23.6°C).

in 2015 are a false positive, even though our *P* value is greater than 0.05 and in 2017. In addition, although there might be small effects of excision on branch photosynthesis rates, because all treatments were treated the same and our findings were consistent across years we believe our response of photosynthesis to the treatments is robust.

Our results are critical, as they demonstrate that studying the interactive effects of changing soil temperatures across seasons is essential for understanding leaf-level C uptake by hardwood trees. However, a companion study by Sanders-DeMott et al. (2018a) did not find changes in leaf-level photosynthesis in sugar maple or red maple saplings in response to growing-season warming, winter soil freezing, or the combination of these changes; the fact that those measurements were made on saplings rather than mature trees may explain some of the differences in our results. We found that the frequency of heat-stress days (days above >23.6°C) is expected to rise over the next century, which will increase

the frequency of days above the photosynthetic temperature optimum for red maple trees (>24–25°C; Aber et al. 1995, Xu et al. 2007). Specifically, by the end of the century there could be 52–77 additional days per year above the photosynthetic temperature optimum for red maples, which could decrease leaf-level C uptake by this tree species if they do not acclimate to warmer temperatures.

Our data in 2015 show that trees exposed to greater soil temperatures in the growing-season and soil freeze/thaw cycles in winter have lower iWUE compared to reference plots, and this pattern is because of increased water uptake, not changes in photosynthesis. Decreased iWUE might play a particularly important role in the northern forest water balance as growing-season droughts are projected to increase in frequency in these ecosystems over the next century (Hayhoe et al. 2007). An increase in droughts without a comparable increase in iWUE may leave trees vulnerable to water stress or desiccation. However, our results in 2017 show no significant effects of soil temperatures on iWUE, suggesting

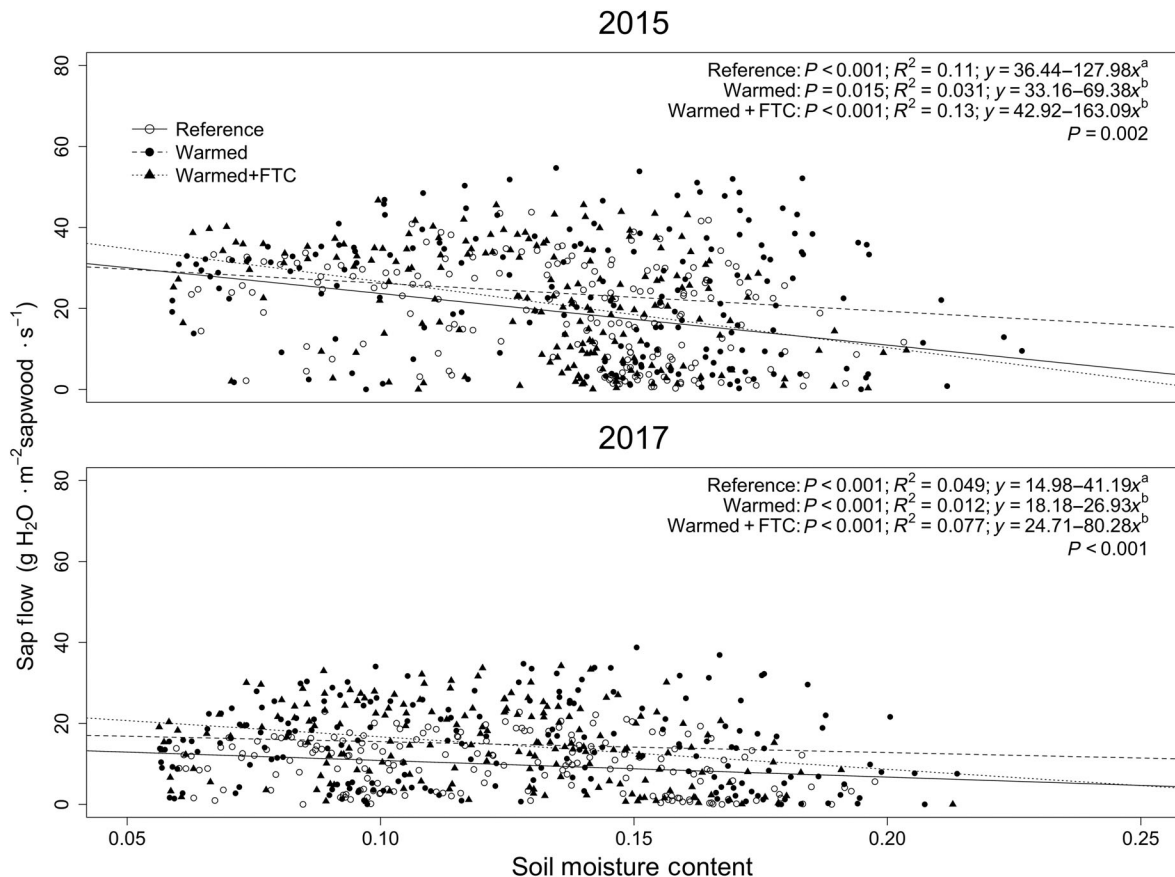


FIG. 6. Relationships between rates of sap flow and mean daily soil moisture throughout the entire growing season. Each dot represents the daily average rate of sap flow for individual trees in the *reference* (solid lines), *warmed* (dashed lines), or *warmed + freeze/thaw cycles* (dotted lines) plots during daylight hours (05:00 to 21:00). R^2 , slopes, and equations before the parentheses are for best fit lines that include all days.

trees may eventually acclimate to increased temperatures. It is also possible that iWUE will respond positively to increased CO_2 concentrations in the atmosphere (Allen et al. 2015) and greater frequency of drought (Zegada-Lizarazu and Iijima 2005).

Sap flow

Our results are consistent with past studies showing positive relationships between rates of sap flow with VPD in mixed hardwood forests (Bovard et al. 2005, Juice et al. 2016, Harrison et al. 2020), Chinese pine (Sun et al. 2000), eucalyptus (Yin et al. 2004), and white spruce (Day et al. 1990) trees. Also, as expected, trees that experienced growing-season warming experienced higher rates of sap flow throughout the growing season compared to the *reference* plots. In addition, there was a positive relationship between daily rates of sap flow with mean daily air temperatures, maximum daily air temperatures, and VPD throughout the growing season in both years across all three treatments. These results are consistent with other studies showing a positive relationship

between soil or air temperature and sap flow rates in mature red oak, Norway spruce, red maple, and sugar maple trees (Bergh and Linder 1999, Juice et al. 2016, Harrison et al. 2020). It is possible that rates of water uptake increased with warmer soil temperatures because of other changes in the forest that we did not measure, such as viscosity of water, which decreases up to 13% with an increase of 5°C temperature (Kestin et al. 1978). It is also possible that rates of water uptake increased because warmer soil temperatures increased root permeability (Wan and Zwiazek 1999, Wan et al. 2001), and/or root hydraulic conductivity (McElrone et al. 2007). In contrast to previous work at Harvard Forest (in central Massachusetts) suggesting that soil freezing in winter reduces sap flow in red maple trees (Harrison et al. 2020), we did not find that winter soil freeze/thaw cycles offset the increased rates of sap flow that we observed with growing-season warming alone.

Even though the 2 yr of our study had comparable environmental conditions in the growing season (e.g., air temperatures, amount of precipitation, and soil temperatures) and trends in sap flow across treatments were

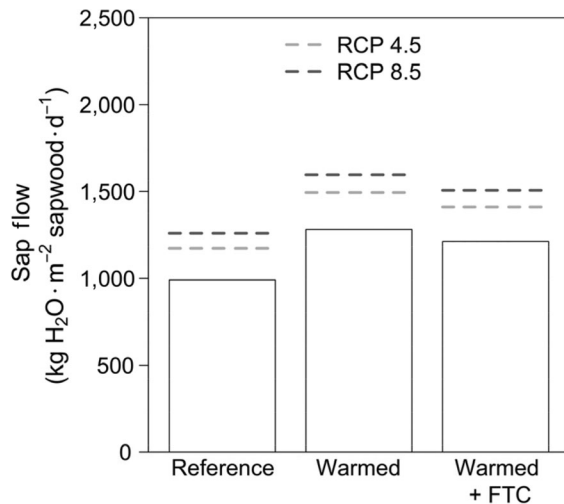


FIG. 7. Current and projected average daily rates of sap flow for the growing season under historic (2005–2018; bars) and projected maximum daily air temperatures with low (Representative Concentration Pathway [RCP] 4.5; dashed light gray) and high (RCP 8.5; dashed dark gray) emissions scenarios for the years 2080–2099.

consistent for 2015 and 2017, it is possible that slight perturbations in the factors that control stomatal conductance led to decreased stomatal conductance in 2017 and therefore overall lower rates of sap flow compared to 2015 (Fig. 2). Stomatal conductance mediates water uptake; however, understanding environmental controls on stomatal conductance is difficult and rates can vary annually based on a multitude of factors, such as light, temperature, CO_2 concentrations, humidity, leaf water status, and water potential (Buckley and Mott 2013). Because our data showed the same trends in sap flow rates across treatments for both years, our results are likely due to the soil temperature treatments we induced.

Projected changes in sap flow

With projected increases in heat-stress days relative to typical days for this region over the next century, we project that red maple sap flow rates will increase by as much as 61%. Our findings at Hubbard Brook are similar to our past study at Hubbard Brook and Harvard Forest that demonstrated a positive response of

growing-season sap flow to increased frequency of heat-stress days (Harrison et al. 2020). Our projections are larger than the projected maximum increase of 38% reported in another study that only examined the effects of a smaller winter snowpack and greater depth and duration of soil freezing in winter (Harrison et al. 2020), once again highlighting the effects of growing-season soil temperatures on sap flow rates. The higher rates of projected sap flow in red maple trees based on our measurements with rising heat-stress days demonstrate that the positive effect of growing-season soil warming compounds the positive effect of increased heat-stress days on sap flow rates, thus resulting in even higher rates than previously reported. Although we chose not to include changes in CO_2 and soil moisture availability in our projections, our results suggest that even as snow cover continues to decline in northern hardwood forests over the next century, soil freezing-induced root damage (Campbell et al. 2014, Sanders-DeMott et al. 2018b) is unlikely to offset growing-season warming-induced increases in sap flow, at least in the short term (<5 yr). However, it is possible that reductions in soil moisture caused by either drought (Hayhoe et al. 2007) or uptake of water by trees (Fig. 7) over the next century may dampen the projected increase in sap flow. Therefore, the observed reductions in soil moisture that were associated with higher rates of sap flow are likely due to increases in rates of transpiration drawing down soil water availability from near-surface soil horizons, which could increase water stress for trees in the future.

Based on results of this study, we conclude that growing-season soil warming is a more important driver of rates of sap flow by red maple trees than winter soil freeze/thaw cycles, at least in the short term, based on the intensities of each season's change projected for this region over the next century (Hayhoe et al. 2007, Campbell et al. 2010). However, tree acclimation to rising temperatures has been demonstrated globally (Niu et al. 2012), in alpine plants (Atkin et al. 2006), tall grass prairie species (Zhou et al. 2007), and northern hardwood forests (Melillo et al. 2017). It is possible that the red maple trees present in our forest will eventually acclimate to warmer temperatures and rates of sap flow may not increase as much as expected. The 26-yr-long soil warming experiment at Harvard Forest (Melillo et al. 2017) is, to our knowledge, the longest warming experiment in temperate forests. Although we are unaware of

TABLE 2. The number of typical and heat-stress days (when air temperatures exceeded 23.6°C at Hubbard Brook) under historic (2005–2018) and low (Representative Concentration Pathway [RCP] 4.5) and high (RCP 8.5) emissions scenarios (2080–2099) at Hubbard Brook and the projected sap flow ($\text{kg H}_2\text{O} \cdot \text{m}^{-2} \text{ sapwood} \cdot \text{d}^{-1}$) for each day, averaged for 2015 and 2017.

	Number of days		Reference Sap flow	Warmed Sap flow	Warmed + FTC Sap flow
	Typical	Heat stress			
Historical	120	62	990.80	1,280.54	1,212.29
RCP 4.5	68	114	1,173.13	1,493.79	1,411.08
RCP 8.5	43	139	1,260.79	1,596.32	1,506.64

any similarly long-term (>20 yr) experiments that incorporate winter soil freeze/thaw cycle events, the long-term soil warming experiment at Harvard Forest shows a multiyear fluctuations in soil carbon dioxide fluxes in response to warming (Melillo et al. 2017). The cyclical nature of these fluxes highlights the importance of long-term monitoring of sap flow as the response of sap flow might change with long-term changes in both growing-season and winter soil temperatures.

CONCLUSIONS

Our results demonstrate that the combined effects of growing-season warming and increased frequency of soil freeze/thaw cycles in winter are likely to increase rates of sap flow, but have little to no effect on rates of leaf-level photosynthesis by red maple trees, with the potential for these changes in climate to have indirect effects on hydrology and carbon cycling in northern forests. Trees experiencing soil warming in the growing season take up more water, which could lead to reductions in soil moisture over time; coupled with projected rises in droughts during the growing season (Hayhoe et al. 2007), the increase in rates of water uptake by red maples over time could increase the frequency of growing-season water stress for other co-occurring tree species in the northern forest region. The rise in leaf-level photosynthesis that we observed with soil warming was completely offset by winter freeze/thaw cycles, which shows the importance of incorporating the effects of year-round climate change on our understanding of C cycling. We therefore conclude that the projected rise in temperatures for this region will play an important role in determining future water balance and C cycling in northern hardwood forests.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3173/supinfo>

DATA AVAILABILITY

Sap flow data <https://doi.org/10.6073/pasta/6447267ffb8d00dda75534acc29e73c9> and leaf-level photosynthesis data <https://doi.org/10.6073/pasta/efef0e8cfb65463c153aa9d587004ec8> are publicly available through the Hubbard Brook Experimental Forest data archives (Templer et al. 2020a, b) and hosted by the Environmental Data Initiative.