

# Effects of climate warming on photosynthesis in boreal tree species depend on soil moisture

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**Climate warming will influence photosynthesis via thermal effects and by altering soil moisture<sup>1–11</sup>. Both effects may be important for the vast areas of global forests that fluctuate between periods when cool temperatures limit photosynthesis and periods when soil moisture may be limiting to carbon gain<sup>4–6,9–11</sup>.** Here we show that the effects of climate warming flip from positive to negative as southern boreal forests transition from rainy to modestly dry periods during the growing season. In a three-year open-air warming experiment with juveniles of 11 temperate and boreal tree species, an increase of 3.4 °C in temperature increased light-saturated net photosynthesis and leaf diffusive conductance on average on the one-third of days with the wettest soils. In all 11 species, leaf diffusive conductance and, as a result, light-saturated net photosynthesis decreased during dry spells, and did so more sharply in warmed plants than in plants at ambient temperatures. Consequently, across the 11 species, warming reduced light-saturated net photosynthesis on the two-thirds of days with driest soils. Thus, low soil moisture may reduce, or even reverse, the potential benefits of climate warming on photosynthesis in mesic, seasonally cold environments, both during drought and in regularly occurring, modestly dry periods during the growing season.

A changing climate will influence plants by altering temperature, precipitation and soil moisture, as well as their variability and seasonality<sup>1–11</sup>. In temperate and boreal climates, temperatures switch seasonally from cold (and limiting to biological processes) to warm and periodically dry, during which time moisture can be limiting<sup>2–6,9–11</sup>. Both the ‘law of the minimum’ and multiple limitation theory<sup>12–14</sup> provide a conceptual basis for predicting climate warming interactions with soil moisture. Although higher temperatures may alleviate enzymatic limits on the biochemistry of photosynthesis, realized rates of CO<sub>2</sub> assimilation may decrease if and when low soil water causes stomatal closure and limitation of the CO<sub>2</sub> substrate for photosynthesis. As growing season conditions in temperate and boreal forests are likely to become effectively drier than in the past<sup>3,8,9</sup>, because climate warming will increase evapotranspiration more than precipitation<sup>3,9</sup> and increase variability in the amount of precipitation per event<sup>1,9</sup>, the importance of water availability to forest responses to rising temperature may increase in the future<sup>3–6,9–11,15–18</sup>.

Mid- and high-latitude plants will therefore probably experience both positive and negative effects of climate warming on photosynthesis within and across years—we propose that these will be positive when soil moisture is ample but negative when soils are drier<sup>4–6,9–11,15–17</sup>. Whether such effects are in aggregate positive or negative is likely to depend on the balance of time that warming alleviates low temperature limitations to plant function as opposed to causing limitations to function through decreased soil moisture. However, direct tests of the effects of climate warming across a range of soil moisture conditions, caused by seasonal or interannual variation or by manipulations of temperature or moisture, are rare, and it remains unclear how plant responses to climate warming will be influenced by these indirect effects of soil moisture<sup>4–6,9–11,16–18</sup>.

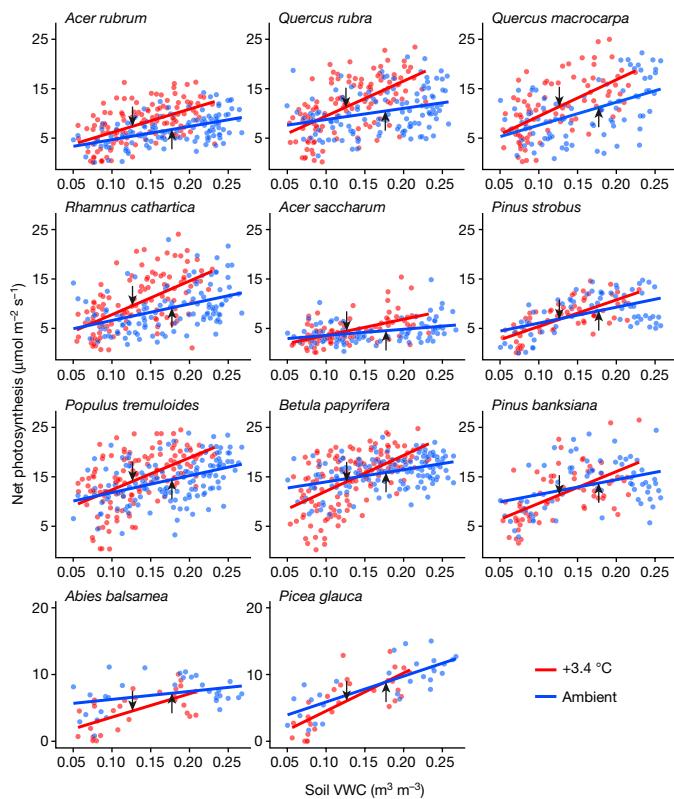
Here we provide evidence from 11 co-occurring boreal and temperate tree species (Fig. 1) in support of the overarching hypothesis that low soil moisture status has a dampening effect on the photosynthetic enhancement that results from experimental warming. This moisture regulation of the response to climate warming was consistent for all 11 species and occurred in response to reductions in soil moisture due to typical seasonal variation and in response to further reductions in soil moisture due to experimental warming. Results are from the free-air B4WarmED experiment<sup>19–22</sup>, in which juveniles (3–5 years old at the time of measurements) of local ecotypes of the 11 tree species were grown under ambient and seasonally elevated (+3.4 °C, April–November) temperatures from 2009 to 2011 at two southern boreal sites in Minnesota, USA (Extended Data Table 1 and Methods). The 11 species co-occur in forests in northern Minnesota; however, five are boreal with southern range limits in or near Minnesota and six are temperate with northern range limits not far north of the Minnesota–Canada border<sup>19</sup>. Fluctuations in soil moisture levels (volumetric water content (VWC), m<sup>3</sup> H<sub>2</sub>O per m<sup>3</sup> soil) occurred at both sites and across all years (Extended Data Fig. 1 and Extended Data Table 2), and spanned from 0.27 to 0.05 VWC, representing a range from slightly wetter than field capacity to slightly drier than the permanent wilting point (of approximately –1.5 MPa) for these sandy loam soils<sup>23,24</sup>. Leaf temperature ( $T_{leaf}$ ) and vapour pressure gradient (VPG) also varied considerably across all photosynthetic measurements (Extended Data Fig. 2).

All species responses were consistent with the hypothesis that effects of experimental warming on carbon gain would be less positive or more negative during periods of low soil moisture (Fig. 1, Table 1 and Extended Data Table 3). In moist soils, all angiosperm species (and no gymnosperms) showed higher maximum carboxylation capacity at 25 °C ( $V_{cmax-25}$ ) when grown at increased temperature compared to ambient temperatures (Extended Data Fig. 3), helping to explain the higher light-saturated net photosynthesis ( $A_{net}$ ) in warmed plants when soil water limitations were modest (Fig. 1). This higher maximum carboxylation capacity in well-watered, warmed angiosperms assessed at a standardized temperature is indicative of an acclimation response (upregulation of  $V_{cmax-25}$ ) to growth in elevated temperatures. However, every species showed marked sensitivity of  $A_{net}$  to drying soil moisture (Fig. 1). More relevant to our overarching hypothesis,  $A_{net}$  in all species declined more steeply with decreasing soil moisture in warmed than in ambient conditions (Fig. 1); therefore, when compared at a common soil moisture, plants showed the most positive (or least negative) effects of experimental warming on  $A_{net}$  when soil moisture availability was high, whereas positive effects decreased (or negative effects increased) as soil moisture availability declined (Fig. 1).

In other words, we found a significant interaction between the increased temperature treatment and VWC for  $A_{net}$  (Table 1;  $F_{1,553} = 40.9$ ,  $P < 0.0001$ ) in a model that included treatment (increased or ambient temperature), species, VWC and two other environmental drivers ( $T_{leaf}$  and VPG). Moreover, although species differed from each other in  $A_{net}$ , they did not differ in how VWC influenced their response

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**Fig. 1 | Photosynthesis is reduced by drying soils, and more so with simulated climate warming.** In situ  $A_{\text{net}}$  in relation to soil moisture (VWC) by species for ambient (blue) and experimentally warmed (red) plants. Data are from multiple days across three years ( $n = 1,991$  across species). The slope of  $A_{\text{net}}$  versus VWC was significantly steeper for warmed than for ambient plants (Table 1;  $F_{1,553} = 40.9$ ,  $P < 0.0001$ ). The arrows show the median VWC across all measurements for the ambient (up arrow) and warmed (down arrow) plants of each species. Species are arranged from top to bottom by their geographical ranges (temperate species in top two rows, boreal in bottom two rows). Sample sizes per species shown in Extended Data Table 3.

to warming (no warming  $\times$  soil moisture  $\times$  species interaction, Table 1;  $F_{10,1,797} = 1.2$ ,  $P = 0.30$ ). Thus, species for which growth was enhanced (for example, *Acer* and *Quercus*) or reduced (for example, *Abies* and *Picea*) under climate warming<sup>19</sup> were similar in terms of how their photosynthetic responses to warming were shaped by soil moisture availability. When analyses were made for every species independently, the slope of  $A_{\text{net}}$  to VWC was always steeper in warmed than in ambient plants (Fig. 1 and Extended Data Table 3), and the interaction of warming  $\times$  VWC was significant ( $P < 0.05$  in 10 species,  $P = 0.10$  in the other).

Additionally, and as expected because of greater evaporative gradients from warmed plants and soils to the atmosphere<sup>3,8,9,20</sup>, the warming treatment reduced soil moisture (Extended Data Fig. 1). Thus, on any given day, warmed plants operated at lower soil moisture levels than ambient plants, moving them to a lower VWC on the  $A_{\text{net}}$ -VWC relationship than ambient plants. This is illustrated by arrows showing the average VWC of ambient and warmed plants in Fig. 1.

Paralleling the response of  $A_{\text{net}}$ , leaf diffusive conductance ( $g_s$ ) decreased in drying soils; it was generally equal or greater in warmed than in ambient plants in moist soils, but similar or lower in warmed than in ambient plants in dry soils (Fig. 2). Moreover, the relationship between  $g_s$  and VWC had a steeper slope in the warmed than in the ambient treatment (Fig. 2 and Table 1), the same as for  $A_{\text{net}}$  (Fig. 1). Evidence suggests that the changes in  $g_s$  contributed to the shrinking positive effect of warming on  $A_{\text{net}}$  as soil water availability decreased (Fig. 1). First,  $g_s$  declined proportionally more than  $A_{\text{net}}$  with increasing soil water deficits (that is,  $A_{\text{net}}/g_s$  was greater in drier than wetter soils in

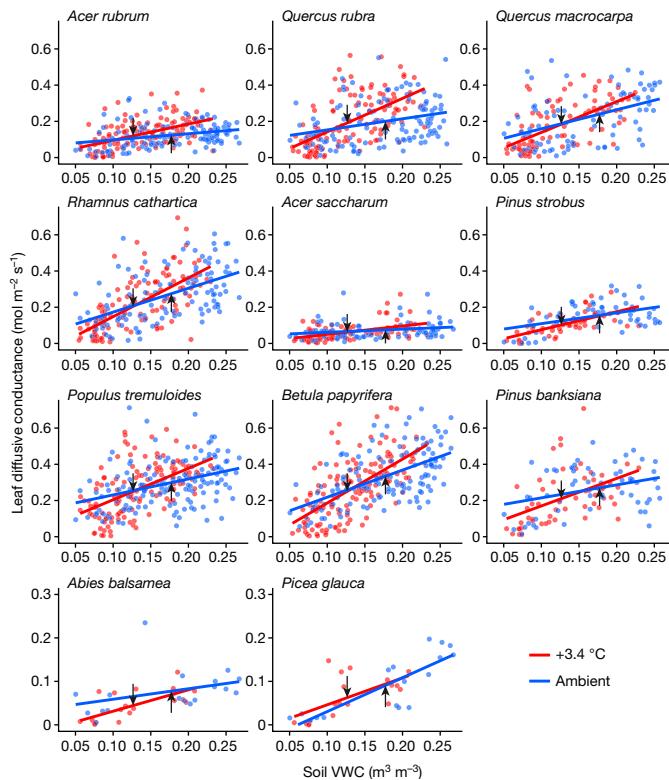
**Table 1 | Summary of models of treatment and environmental effects on leaf gas exchange**

Source of variance	$(A_{\text{net}})$		$(g_s)$	
	$F$	$P > F$	$F$	$P > F$
Species	<b>72.61</b>	<0.0001	<b>32.18</b>	<0.0001
Warm	<b>14.10</b>	<b>0.0003</b>	1.28	0.2587
Species $\times$ warm	<b>3.29</b>	<b>0.0003</b>	0.79	0.6430
Soil water	<b>215.61</b>	<0.0001	<b>147.72</b>	<0.0001
Soil water $\times$ species	<b>2.02</b>	<b>0.0278</b>	<b>6.17</b>	<0.0001
Soil water $\times$ warm	<b>40.88</b>	<0.0001	<b>6.44</b>	<b>0.0113</b>
Soil water $\times$ species $\times$ warm	1.17	0.3033	0.47	0.9130
VPG	<b>29.38</b>	<0.0001	<b>17.10</b>	<0.0001
VPG $\times$ species	<b>10.11</b>	<0.0001	<b>8.57</b>	<0.0001
VPG $\times$ warm	0.33	0.5686	0.42	0.5208
VPG $\times$ soil water	<b>5.59</b>	<b>0.0182</b>	0.30	0.5858
VPG $\times$ species $\times$ warm	1.39	0.1780	0.57	0.8427
VPG $\times$ species $\times$ soil water	<b>4.17</b>	<0.0001	1.35	0.1969
VPG $\times$ warm $\times$ soil water	<b>4.24</b>	<b>0.0396</b>	0.03	0.8629
$T_{\text{leaf}}$	<b>26.75</b>	<0.0001	3.32	0.0684
$T_{\text{leaf}} \times$ species	<b>11.77</b>	<0.0001	<b>6.65</b>	<0.0001
$T_{\text{leaf}} \times$ warm	0.05	0.8151	0.40	0.5251
$T_{\text{leaf}} \times$ soil water	<b>3.95</b>	<b>0.0469</b>	0.60	0.4382
$T_{\text{leaf}} \times$ VPG	0.69	0.4066	0.01	0.9157
$T_{\text{leaf}} \times$ species $\times$ warm	1.53	0.1225	0.55	0.8551
$T_{\text{leaf}} \times$ species $\times$ soil water	<b>3.46</b>	<b>0.0002</b>	1.59	0.1035
$T_{\text{leaf}} \times$ species $\times$ VPG	<b>2.39</b>	<b>0.0081</b>	1.70	0.0758
$T_{\text{leaf}} \times$ warm $\times$ soil water	<b>5.19</b>	<b>0.0228</b>	0.01	0.9047
$T_{\text{leaf}} \times$ warm $\times$ VPG	<b>3.46</b>	<b>0.0002</b>	0.01	0.9157
$T_{\text{leaf}} \times$ soil water $\times$ VPG	1.83	0.0502	0.19	0.6649
Full-model adjusted $R^2$	0.6342		0.6013	

Mixed models are shown for  $A_{\text{net}}$  and  $g_s$  in relation to species, +3.4 °C warming treatment (warm), volumetric water content (soil water), vapour pressure gradient (VPG), leaf temperature ( $T_{\text{leaf}}$ ) and all interactions except the five-way interaction. Plot, block and site were included as random effects in the model. Both models were significant, at  $P < 0.0001$ . Data are for 11 species ( $n = 1,991$  for  $A_{\text{net}}$ ; 1,903 for  $g_s$ ). Bold values indicate variables that are significant at  $P < 0.05$ . Four-way interactions were not significant and are not shown.  $F$  and  $P$  indicate  $F$ -statistics and  $P$  values, respectively.

every species) and the increase in  $A_{\text{net}}/g_s$  with decreasing soil moisture was larger in warmed compared to ambient plants. Such patterns are consistent with increasing stomatal limitation to  $A_{\text{net}}$  in drier soils and with greater stomatal limitation in warmed than in ambient plants in drier soils. Second, corroborating this, quantitative estimates of the percentage of limitation of  $A_{\text{net}}$  by stomatal conductance<sup>25,26</sup> (rather than by biochemical limitations), also increased more steeply with decreasing VWC in warmed than in ambient plots (Extended Data Fig. 4).

A key question is the degree to which the different responses of  $g_s$  and  $A_{\text{net}}$  to VWC for plants in the contrasting warming treatments were influenced by effects of treatments on, or by ambient variation in, other environmental factors such as  $T_{\text{leaf}}$  and VPG. VWC was very weakly positively correlated with  $T_{\text{leaf}}$  and unrelated to VPG across all measurement dates (Extended Data Fig. 2); therefore, low soil moisture effects were not confounded by high VPG or high  $T_{\text{leaf}}$  in this dataset. The differential response of  $g_s$  to VWC in warmed versus ambient plants was independent of either VPG or  $T_{\text{leaf}}$  (no three-way interactions, Table 1). The greater decline of  $A_{\text{net}}$  with decreasing VWC in warmed than in ambient plants was slightly steeper at higher levels of  $T_{\text{leaf}}$  and VPG (illustrated by three-way interactions for  $A_{\text{net}}$  with warming treatment, VWC and either  $T_{\text{leaf}}$  or VPG, Table 1), but was apparent regardless of VPG or  $T_{\text{leaf}}$  (Extended Data Fig. 5). Although the relationship of  $g_s$  (but not  $A_{\text{net}}$ ) to VPG was nonlinear, replacing VPG with  $\log(\text{VPG})$  in models in Table 1 only marginally influenced results and did not



**Fig. 2 | Leaf conductance is reduced by drying soils, and more so with simulated climate warming.** Leaf diffusive conductance in relation to soil moisture (VWC) by species for ambient (blue) and experimentally warmed (red) plants. Data are from multiple days across three years ( $n = 1,903$  across species). The slope of  $g_s$  versus VWC was significantly steeper in warmed than in ambient plants (Table 1;  $F_{1,937} = 6.4$ ,  $P = 0.0113$ ). The arrows show the median VWC across all measurements for the ambient and warmed plants.

show any interaction of treatment  $\times$  log(VPG)  $\times$  VWC, suggesting that nonlinearity of VPG effects did not mask important interactions in the mixed models.

Recent work has shown that under present and projected future climate conditions, canopy surface conductance and evapotranspiration in many biomes, including mesic forests, may be limited by both high vapour pressure deficits (closely related to VPG) and low soil water availability<sup>2</sup>. Our results are consistent with that, as low VWC and high VPG independently constrained  $A_{net}$  and  $g_s$  (Extended Data Fig. 5).

It is also useful to view these results in the context of the temperature response functions of  $A_{net}$ . For both well-hydrated detached leaves<sup>21</sup> and in situ leaves (Extended Data Fig. 2), the broad temperature optima ( $T_{opt}$ ) of  $A_{net}$  for these species was around 22–27 °C. As plants were measured across a wide range of  $T_{leaf}$  (95% fell between 13.7 and 36.8 °C, Extended Data Fig. 2), approximately one-third of ambient treatment measurements were made below  $T_{opt}$  (for example,  $T_{leaf} < 22$  °C) and another third were made above  $T_{opt}$  (for example,  $T_{leaf} > 29$  °C). Warming by +3.4 °C should have alleviated low temperature limitation for the former and exacerbated high temperature limitations for the latter. The remaining measurements were made when  $T_{leaf}$  was near  $T_{opt}$  (that is, in the range of 22–29 °C). More influential to the results was that non-optimal VWC induced stomatal closure (Fig. 2), causing a high proportion of leaves to photosynthesize below their capacity at any given  $T_{leaf}$  (Extended Data Figs. 2, 4).

Results above clearly demonstrate a more pronounced decline in  $A_{net}$  with decreasing VWC in warmed than in ambient plants—congruent with climate-warming stimulation of  $A_{net}$  in moist soils and depression of  $A_{net}$  in dry soils—and that a more pronounced increase in stomatal limitation of  $A_{net}$  of warmed plants played a part. However, this leads to the question of why the shift with declining VWC from biochemically

to stomatally limited photosynthesis was steeper in warmed than in ambient plants of all species (Extended Data Fig. 4). We suggest, from several lines of evidence, that a combination of factors drove these responses (Extended Data Fig. 6).

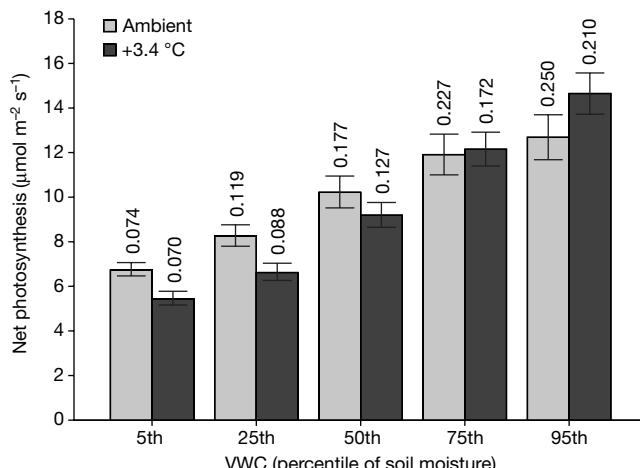
In moist soils, angiosperm species had strong increases in  $A_{net}$  and  $g_s$  in warmed conditions likely because of both higher carboxylation capacity (greater  $V_{cmax-25}$  in warmed conditions, Extended Data Fig. 3) and higher carbon demand for photosynthate<sup>28</sup>, as they grew 23% faster on average in warmed than in ambient conditions<sup>19</sup>. In drier soils, increased stomatal limitation eliminated most of the potential gain that higher  $V_{cmax-25}$  might provide (Extended Data Figs. 3, 6), and perhaps eliminated any warming-induced increase in carbon sink strength. Warmed angiosperm plants also likely had higher dark respiration in the light (as their dark respiration was 20% higher than that of ambient plants<sup>22</sup>) and higher photorespiration<sup>27</sup> at all VWC levels (Extended Data Fig. 6).

The responses of gymnosperms were similar, except that changes in  $V_{cmax-25}$  with warming were less positive even in moist soils; additionally, a negative overall growth response (−26% growth response on average<sup>19</sup>) to warming, coupled with more negative effects of warming on carbon gain when soils were dry, suggests a small warming-induced increase in carbon sink strength at best when soils were wet and a larger decrease when soils were dry (Extended Data Fig. 6). Collectively these factors are likely to have contributed to making the responses of gymnosperms to warming more negative than that of angiosperms at every level of VWC.

Overall, the likely mechanisms suggest that warmed plants did not have greater stomatal sensitivity to soil water deficits as such. Instead, under moist conditions, biochemical limitations to photosynthesis were dominant or co-dominant (Extended Data Fig. 4) and warmed plants had a photosynthetic advantage because of less biochemical limitation (that is, higher realized  $V_{cmax}$ ), whereas under drier conditions, stomatal limitations became dominant, and any advantage of warming disappeared (and in driest soils, became a hindrance).

The net effect (across the growing season) of warming on photosynthetic carbon gain would be determined by both the shifting effect of warming on  $A_{net}$  as it varied with soil water status and the effect of climate warming on soil water status itself. Figure 1 shows the response of warmed versus ambient plants across all levels of soil moisture, that is, comparing the effect of warming on photosynthetic processes at a common soil moisture (and typically not a common date). By contrast, in Fig. 3 we show  $A_{net}$  averaged across species in warmed versus ambient plants at a common time, under conditions differing in soil moisture across time and treatments, from dry to wet (representing the 5th, 25th, 50th, 75th and 95th wettest percentiles of VWC among all measurements for each treatment, Fig. 3). Although soils were typically somewhat drier in the warmed treatment, the percentiles (from dry to wet) within each treatment occurred on similar sets of days. Thus, Fig. 3 shows the estimated aggregated effect of both direct physiological effects of warming and indirect soil moisture effects of warming treatments on realized average photosynthetic rates, equally weighted across all 11 species.

The warming treatment had a markedly different effect on  $A_{net}$  when soils were dry rather than wet (Fig. 3). For the 11 species, warming under high soil moisture conditions (the 95th percentile of VWC in each treatment) increased  $A_{net}$  by 15% on average (Fig. 3). On days with drier conditions, the mean stimulation of  $A_{net}$  disappeared; this occurred at around the 65th percentile of VWC on average across the 11 species. Thus, warming increased average  $A_{net}$  of the community on only the third of days with highest soil moisture. Species (such as the temperate *Acer* and *Quercus*) with more positive average responses to warming had positive responses for a larger fraction of days and soil water conditions than species with more neutral or negative responses (such as the boreal *Abies*, *Betula*, *Picea* and *Pinus*). On average across species,  $A_{net}$  was reduced by the warming treatment by 9%, 18% and 18%, respectively, when soil moisture was at its median, 25th and 5th percentiles. Note that comparisons of  $A_{net}$  at the median VWC of



**Fig. 3 | Warming stimulates photosynthesis on average in moist soils, but not otherwise.** Mean  $A_{\text{net}}$  ( $\pm$ s.e.m.) of 11 temperate and boreal species in ambient and warmed treatments compared during periods that ranged from dry to wet. Periods represent soil moisture percentiles within treatments across all measurements, from dry to wet (that is, the 5th, 25th, 50th, 75th and 95th wettest percentiles of VWC for each treatment). The percentiles (from dry to wet) occurred on nearly identical days in both treatments. Values represent the predictions for each warming treatment averaged across all 11 species at each VWC level, based on the coefficients for VWC from within-treatment mixed models using VWC, species and their interaction ( $n = 996$  for ambient, 995 for warmed; VWC,  $P < 0.0001$  in both treatments based on  $F$ -tests). The s.e.m. is derived from the standard error of the slope of  $A_{\text{net}}$  versus VWC within each treatment. Note that the mean VWC by treatment is also shown at each soil moisture percentile above each graph.

ambient and warmed treatments can also be obtained for each species from the arrows in Fig. 1. Results restricted to the nine species measured in two or three years, or to the five species measured in all three years, were generally similar to results for all 11 species: when soil moisture was high, warming increased  $A_{\text{net}}$ , but whenever substantial soil moisture deficits occurred, warming decreased  $A_{\text{net}}$  (Extended Data Table 4).

These results provide information on how soil moisture may modulate the effects of climate warming in seasonally cold forest ecosystems, which represent approximately half of global forests<sup>29</sup>. During periods of low soil moisture, stomatal limitation of photosynthesis reduced or eliminated the potential benefit of amelioration of low temperature constraints on photosynthetic kinetics by warming (Figs. 1, 2 and Extended Data Figs. 3, 4, 6). On average, warmed plants had higher  $g_s$  and  $A_{\text{net}}$  than ambient plants when soils were moist (Figs. 1, 2). As soils dried, plants in both treatments showed reduced  $g_s$ , but warmed plants of all species had reductions in both  $g_s$  and  $A_{\text{net}}$  that were proportionally higher than in ambient plants. In a warmer future, greater increases in evapotranspiration than in precipitation during the growing season<sup>3</sup> should also reduce soil water stores<sup>9</sup>, pushing plants in the future climate further down the ' $A_{\text{net}}$ –VWC curve' and further reducing or eliminating positive effects of warming on photosynthetic carbon gain.

Across the three study years, the distribution of soil moisture on the dates of photosynthesis measurements closely matched the distribution of soil moisture across all days (Extended Data Table 2); the three study years were also similar in temperature and precipitation to the 35-year average for these sites (Extended Data Table 1). Thus, the observed responses to experimental warming (Figs. 1–3) are likely to be indicative of responses to future climate warming in northern Minnesota if rainfall patterns are similar to the recent past, and suggest, more generally, that soil water limitations may considerably constrain the realized potential benefits of warming in seasonally cold environments across high latitude forests. Moreover, our results can help to explain observations that climate change to date has had more negative effects on boreal forests in central and western North America than on those

further east<sup>5,6,9–11,16,18</sup>. Given higher precipitation and lower evapotranspiration, soils in eastern North American boreal forests are more often moist, and thus higher temperatures are more likely to enhance photosynthesis, whereas in boreal forests in central and western regions, low soil moisture and associated stomatal closure more often constrain photosynthetic carbon gains<sup>3,5,9–11</sup>.

Climate warming is likely to extend the season of active photosynthesis, and the effects of increasing  $\text{CO}_2$  concentrations on  $g_s$  may result in enhanced soil moisture<sup>5,10,15</sup>; both could help to offset the negative effects of soil drying on photosynthesis that result from higher potential evapotranspiration relative to growing season precipitation and from lower soil moisture recharge, resulting from higher rainfall intensity and more run-off<sup>1,3,9–11</sup>. However, the relative magnitude of such offsets is unknown<sup>1,3,9–11</sup>. Furthermore, although the mechanisms that underlie the observations in this experiment should apply to trees of all sizes, larger trees may differ in their sensitivity to drying soils from the juveniles used in this study, influencing the magnitude of soil moisture-related modulation of the effects of climate warming on photosynthesis.

In summary, these results have important implications for the future, arising from two independent but additive mechanisms. First, future warmer conditions will lead to increasingly strong stomatal limitation of photosynthesis in drying soils, such that soil water limitations of historically typical magnitude will eliminate some or all of the increased carbon gain possible from greater photosynthetic capacity. Second, higher evapotranspiration in a warmer world<sup>5,9–11</sup> will result in chronically lower average soil moisture, further reducing net photosynthesis via the same mechanism of decreased stomatal conductance. Thus, low soil moisture will exert a powerful braking effect on, or even reverse, potential benefits of climate warming on tree photosynthesis in mesic, seasonally cold environments.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at <https://doi.org/10.1038/s41586-018-0582-4>.

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**Author contributions** P.B.R., R.A.M., R.L.R. and S.E.H. designed the overall experiment. P.B.R., R.A.M. and A.S. designed the specific study reported herein. R.L.R. designed the warming system, R.L.R. and A.S. implemented the warming system, and A.S. and K.M.S. coordinated the day-to-day field measurements. P.B.R. coordinated the overall experiment and this specific study and analysed the data. P.B.R. wrote the first draft and jointly wrote subsequent drafts of the manuscript with the other co-authors.

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#### Additional information

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## METHODS

The experiment is located at two University of Minnesota field stations; the Cloquet Forestry Center, Cloquet MN ( $46^{\circ} 40' 46''$  N,  $92^{\circ} 31' 12''$  W, 382 m a.s.l.,  $4.8^{\circ}\text{C}$  mean annual temperature, 783 mm mean annual precipitation) and the Hubachek Wilderness Research Center, Ely, MN ( $47^{\circ} 56' 46''$  N,  $91^{\circ} 45' 29''$  W, 415 m a.s.l.,  $2.6^{\circ}\text{C}$  mean annual temperature, 726 mm mean annual precipitation)<sup>19,20</sup>. At both sites, treatments were positioned in relatively open (recently cleared) overstory conditions. The overall experimental design was a 2 (site)  $\times$  2 (treatment) factorial experiment, with six replicates of each for a total of 24 circular 3-m diameter plots; with seedlings of 11 focal species planted in every plot. Treatments included two levels of simultaneous open-air plant and soil warming (ambient,  $+3.4^{\circ}\text{C}$ ); warming was accomplished with infrared lamp heaters and soil heating cables (dummy lamps and cables in the ambient plots). Warming was implemented from early spring to late fall each year in open-air plots (that is, without chambers) via a feedback control that acts concurrently and independently at the plot scale to maintain a fixed temperature differential from ambient conditions above- and belowground. On average, we achieved 24-h per day average warming of  $+3.4^{\circ}\text{C}$  (during April–November) and midsummer midday (09:00–15:00 during June–September) aboveground warming of  $+2.9^{\circ}\text{C}$  across the 2009–2011 growing seasons<sup>19,20</sup>. Plant and soil temperature and soil moisture (0–20 cm depth) were measured continuously and recorded hourly in every plot throughout the study. Plant surface temperature was measured with infrared thermometers mounted above the plant canopy in every plot (IRR-P: Apogee Instruments Inc.). Volumetric water content from 0 to 20 cm depth was measured in each plot using a 30-cm Campbell Scientific CS-616 probe inserted at  $45^{\circ}$ . VWC ( $\text{m}^3 \text{H}_2\text{O}$  per  $\text{m}^3$  soil) was monitored hourly in all plots and corrected<sup>20</sup> for soil textural and temperature differences using a Campbell Scientific method for user-specific calibration of water reflectometers (Model CS616). Both sites have well-drained, coarse-textured upland soils<sup>19,20</sup>. In mid-continent boreal and temperate biomes, climate change will increase plant and air temperatures, and the associated increases in VPG and evapotranspiration are likely to more than offset any increase in total atmospheric water vapour or precipitation, resulting in increased soil water deficits<sup>3,7–10</sup>.

In 2008, 11 juveniles of each of 11 tree species were planted into existing low shrub, herb and fern vegetation in every plot (around 2,900 juveniles; average of approximately 3-year-old plants in 2009). The 11 species include six native broadleaf (*Acer rubrum*, *Acer saccharum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus macrocarpa* and *Quercus rubra*), one naturalized broadleaf (*Rhamnus cathartica*) and four native needle-leaved (*Abies balsamea*, *Picea glauca*, *Pinus banksiana* and *Pinus strobus*) species, all of which are present in the ecolton region. Local ecotypes (collected between  $46^{\circ} 0'$  and  $48^{\circ} 30'$  N latitude in northeastern Minnesota) of all species except *Rhamnus* were planted from material obtained from two Minnesota Department of Natural Resources nurseries in northern Minnesota. *Rhamnus* seedlings were transplants dug up from forests in north-central Minnesota.

In situ measurements of light-saturated net photosynthesis ( $A_{\text{net}}$ ) and leaf diffusive conductance ( $g_s$ ) were made using six Li-Cor 6400 portable photosynthesis systems (Li-Cor). Simultaneous leaf temperature measurements were made for most species using the internal fine-wire thermocouple located in the bottom of the  $2 \times 3\text{-cm}^2$  Li-Cor leaf chamber (6400-02B LED) and directly touching the leaf during the measurement. However, for two conifers (balsam fir and spruce), we used a conifer chamber LED light source (6400-22L) and leaf temperature was calculated based on energy balance (for details see Li-Cor 6400XT manual; Li-Cor). Leaf temperatures measured in the cuvette and canopy surface temperatures (measured independently with infrared thermometers, as described above) were strongly correlated. Cuvette leaf temperatures were usually around  $2^{\circ}\text{C}$  higher than canopy temperature. This is largely because the cuvette and the enclosed leaf warmed up from being in the sun; additionally, leaves were selected for photosynthesis from upper canopy leaves in sunlit positions, whereas part of the surface of the plant canopy sensed by the infrared thermometers was often in partial shade. Measurements were made throughout the growing seasons (June–September) of 2009–2011. A total of 2,052 measurements of  $A_{\text{net}}$  and 1,964 of  $g_s$  were made on a total of 1,338 individuals on 54 dates across species, treatments, sites and time (1,991 and 1,903 measurements, respectively were made with matching soil VWC data). Individuals were three- to five-years old at the time of measurements. Measurements were made in morning or early afternoon (that is, typically between 08:30–14:00 solar time). Not all species were measured each year owing to the time-consuming nature of the measurements (five species were measured in all three years, four in two years and two in one year). On every measurement date, any species included in that sampling was measured equally across contrasting warming treatments.

Individuals to be sampled were chosen randomly from those not previously sampled. Every measurement was made on a unique leaf. Over the three years, individual plants were usually measured once ( $n=839$ ) or twice ( $n=338$ ), but owing to low survival in some species, other individuals were measured three ( $n=121$ ), four ( $n=30$ ), five ( $n=6$ ) or six ( $n=4$ ) times. Fully expanded, healthy upper canopy leaves were sampled from individuals in both ambient and  $+3.4^{\circ}\text{C}$  treatments at both sites. Light was maintained in the leaf chamber at saturating levels using the LED light source. Airflow was set at  $500 \mu\text{mol s}^{-1}$  and  $\text{CO}_2$  reference concentrations were set at  $400 \mu\text{mol mol}^{-1}$ .

Estimates of  $V_{\text{cmax-25}}$  from the one-point method<sup>30</sup> and estimates of the percentage of stomatal limitation<sup>25,26</sup> of  $A_{\text{net}}$  were also made. For data from other years for which full  $A-C_i$  curves were measured, calculated  $V_{\text{cmax-25}}$  from the one-point method from single points of those  $A-C_i$  curves very closely matched (near 1:1 line,  $R^2=0.96$ ) the  $V_{\text{cmax-25}}$  values estimated from the entire curves, strongly supporting the appropriateness of the one-point method for our field measurements for this set of species. The percentage of stomatal limitation was taken as the percentage reduction in  $A_{\text{net}}$  from the maximal rate estimated with no stomatal limitation ( $A_{\text{gmax}}$ ).  $A_{\text{gmax}}$  was estimated (for each species in both treatments) in three ways: (1) based on calculations from  $A-C_i$  curves of nine of the eleven species made in later years of the study on a separate cohort of plants; (2) based on the 95th percentile of  $A_{\text{net}}$  measurements from the current study, and (3) based on the  $A_{\text{gmax}}$  estimates from the  $A-C_i$  curves, adjusted to reflect realized  $A_{\text{net}}$  in the current study using the correlation of values from 1 and 2. For method 1, we used the relationship between  $A-C_i$  curves and the field 95th percentile  $A_{\text{net}}$  for nine species to estimate  $A_{\text{gmax}}$  for the two species without  $A-C_i$  curves. The overall patterns shown in each panel of Extended Data Fig. 4 are nearly identical using any of the three metrics. We used metric 3, because it combined independent estimates of net photosynthetic rates from outside of this study, with maximal rates that better reflected realized rates in the study (and thus resulted in fewer values below zero for the percentage of stomatal limitation). We recognize the impossibility of negative values for the percentage of stomatal limitation, but retained them for statistical purposes.

A mixed model was used to compare  $A_{\text{net}}$  and  $g_s$  to treatment combinations, soil moisture conditions, VPG and leaf temperature. Models included the following independent variables: species, warming treatment, VWC (on the day the gas exchange measurement was made), VPG,  $T_{\text{leaf}}$  and all interactions (up to four-way) among variables. Plot, block and site were added to each model as a random effect. Models were also run separately for the subset of nine species measured in at least two years (Extended Data Table 4), for the five species measured in all three years (Extended Data Table 4) and for each species individually (Extended Data Table 3). Results were similar across these different models. Moreover, comparisons across species on common dates were made in three different ways. First, we used coefficients from mixed models for each temperature treatment to estimate  $A_{\text{net}}$  across a range of VWC percentiles (Fig. 3). Second, we ran mixed models, including species, treatments and VWC bin classes to develop LSMEANS for all species  $\times$  treatment  $\times$  VWC bin combinations. Third, we averaged raw species means for VWC bin classes across treatments. All three approaches resulted in similar outputs.

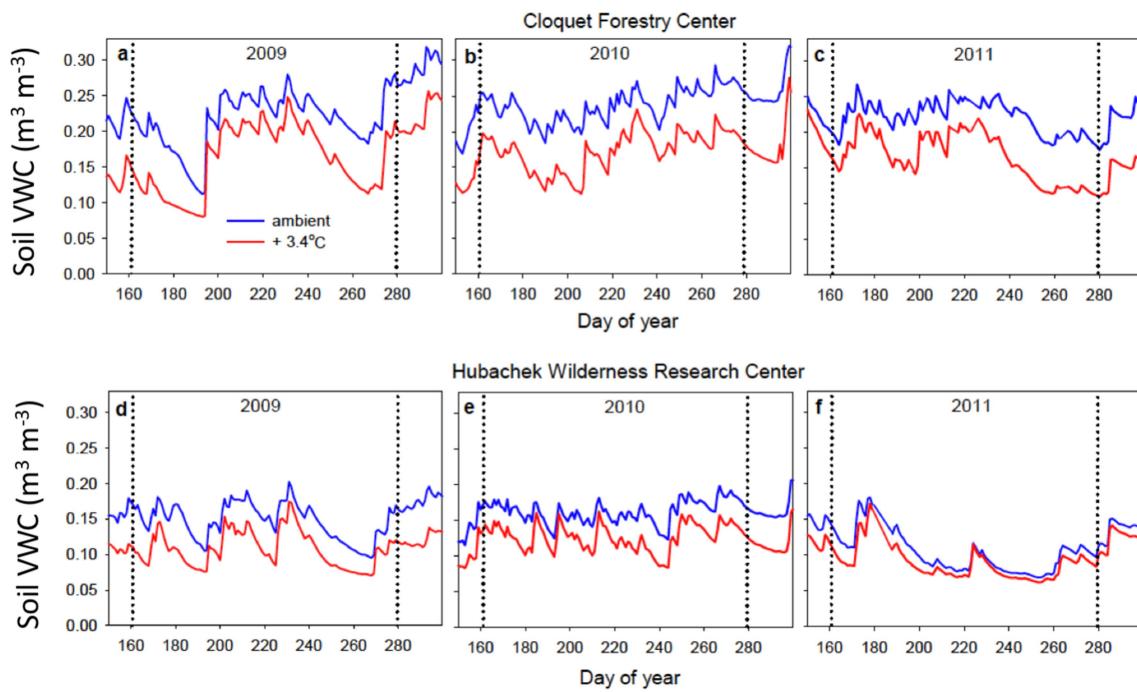
The three experimental years were typical of long-term climate (Extended Data Table 1); moreover, over the three years, the dates when leaf physiological measurements were made were well-distributed from early June to late September (between day of year 162 and 269), and represented a similar range of frost-free temperatures and soil moisture as occurred across that growing season period in 2009–2011 (Extended Data Table 2). There was no evidence that mid-summer, which is warmer, was on average drier during these three particular years, nor did periods of low VWC occur in times of high VPG. As a result, there was no confounding of soil moisture deficits with leaf or air temperatures or VPG during our study; thus, physiological effects related to low soil moisture should have been largely independent of effects of air temperature (or VPG).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

## Data availability

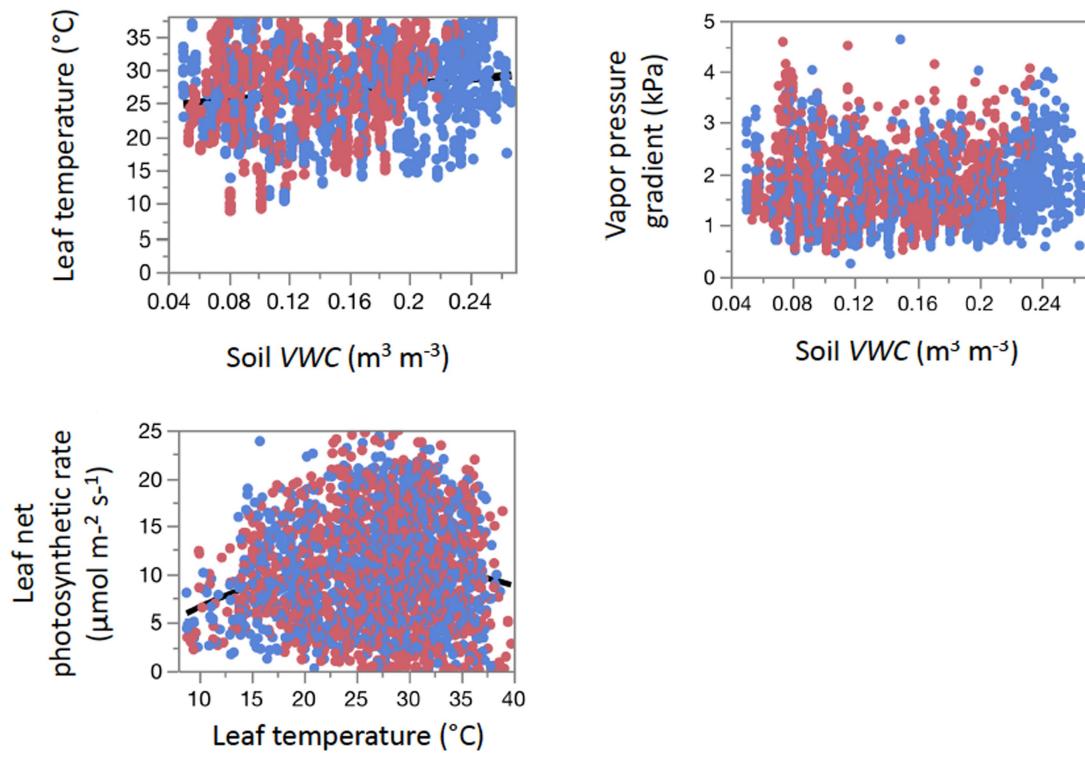
The data reported in this paper are available from the Environmental Data Initiative (EDI) at <https://doi.org/10.6073/pasta/258239f68244c959de0f97c922ac313f>.

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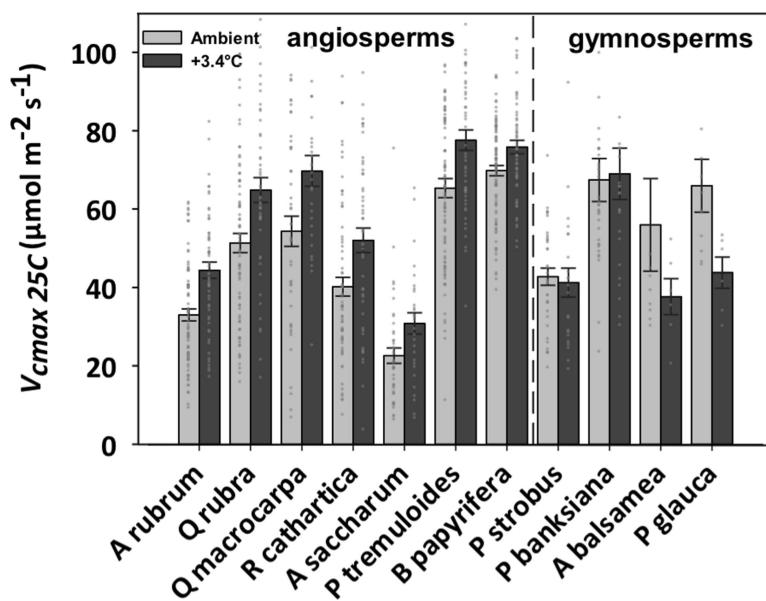
**Extended Data Fig. 1 | Soil water (VWC) in relation to day of year.**  
a–f, VWC ( $\text{m}^3 \text{m}^{-3}$ ; 0–20 cm depth) was averaged by day, variation shown daily across the season among treatments, sites and years. Daily values represent means among all plots within a treatment at each site.

Measurements were logged continuously, recorded hourly, thus a total of approximately 3,600 measurements for each of the 24 plots in each year for the time period are shown. Vertical dashed lines show the range of dates during which photosynthetic measurements were made.



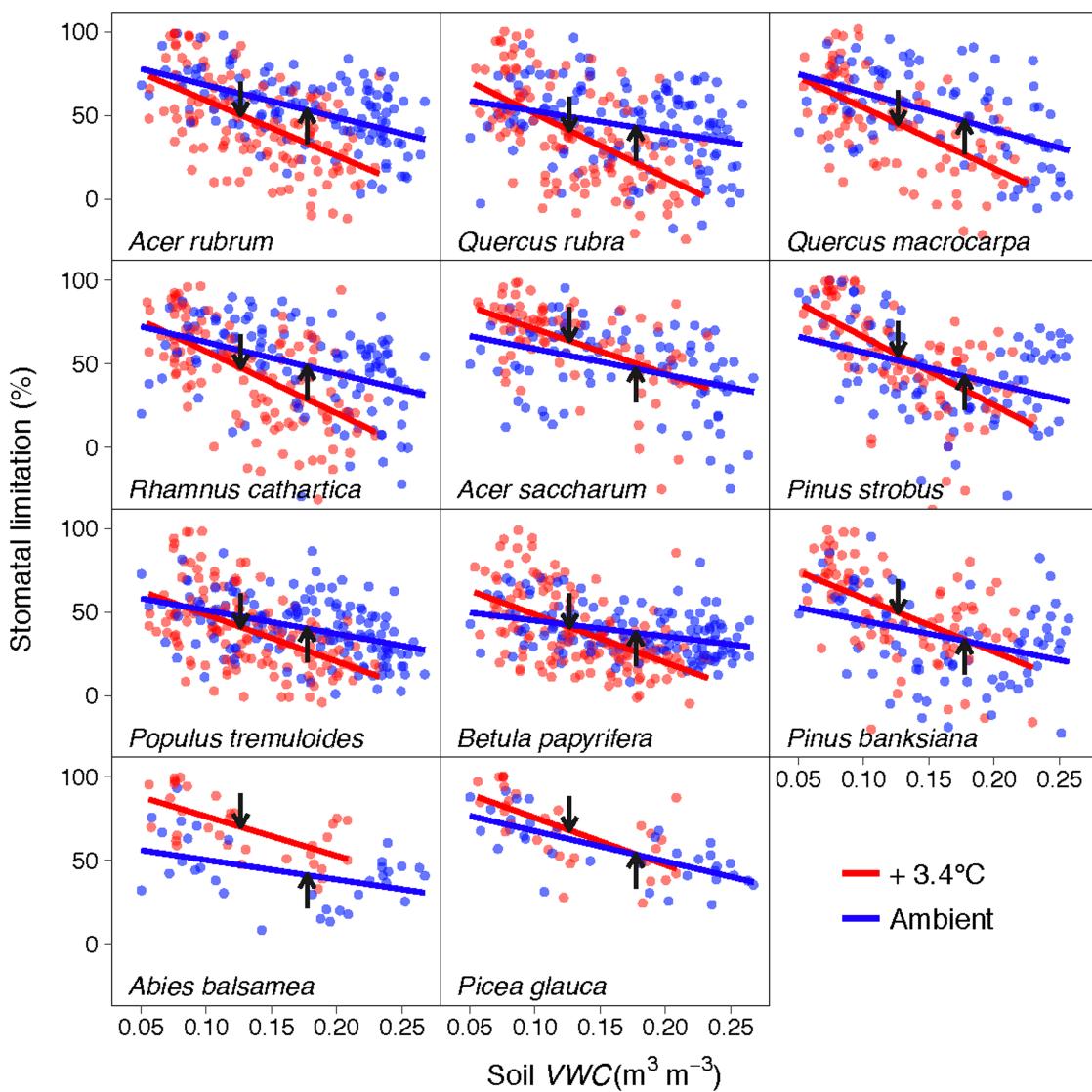
**Extended Data Fig. 2 | Range of temperature, evaporative demand (VPG) and soil moisture across the three growing seasons during gas exchange measurements.** Top, average leaf temperature and VPG for all gas exchange measurements across the three years in relation to soil water (VWC). There was no significant correlation between VPG and VWC over the three-year period ( $P > 0.30$ ); there was a significant correlation

( $R^2 = 0.03, P < 0.001$ ) between leaf temperature and VWC across warming treatments. Bottom, net photosynthetic rate in relation to leaf temperature (polynomial fit all data pooled,  $R^2 = 0.02, P < 0.001$ ). Blue, ambient; red, +3.4  $^{\circ}\text{C}$ . Sample sizes, approximately 1,989–2,050, around half in each warming treatment. A few data points are out of the  $y$ -axis range and therefore not visible.



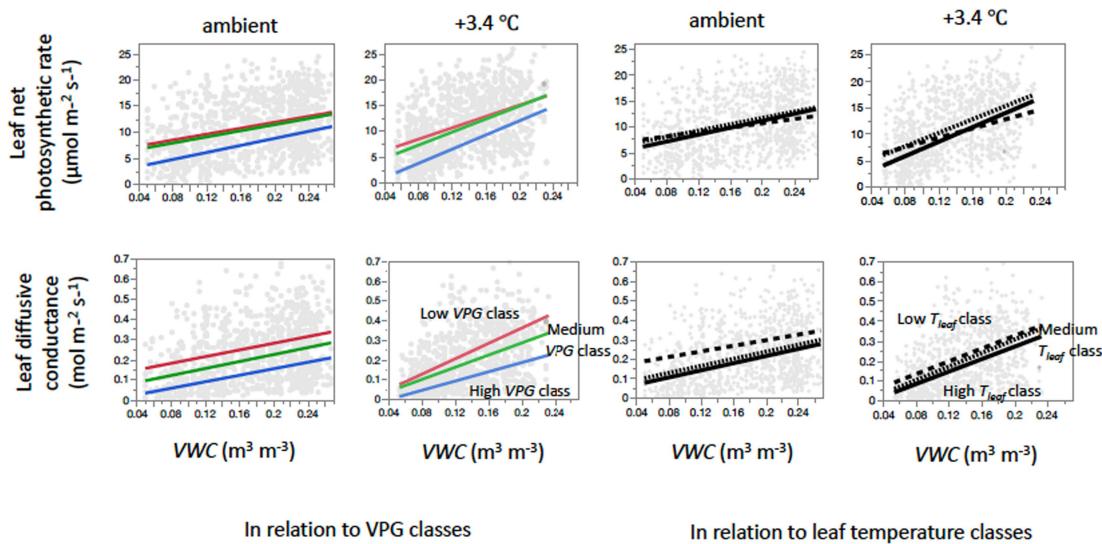
**Extended Data Fig. 3 | Maximum biochemical photosynthetic capacity in moist soils.** Mean ( $\pm$ s.e.m.) maximum carboxylation capacity ( $V_{c\max-25}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 25 °C of 11 gymnosperm and angiosperm trees species in ambient (grey) and +3.4 °C experimentally warmed (black) treatments for days with moist soils (data are shown for the highest half of VWC observations, those with VWC > 0.148). Species within groups are arranged from left to right from most temperate to most boreal distribution (as in Fig. 1). Data are from multiple days across three years

and otherwise averaged across the spectrum of moist soil water availability. Individual measurements are shown as small grey dots. Sample sizes by species for ambient, +3.4 °C: *A. rubrum*, 78, 55; *Q. rubra*, 75, 47; *Q. macrocarpa*, 43, 28; *R. cathartica*, 69, 48; *A. saccharum*, 44, 29; *P. tremuloides*, 92, 50; *B. papyrifera*, 91, 56; *P. strobus*, 36, 22; *P. banksiana*, 36, 24; *A. balsamea*, 10, 6; *P. glauca*, 11, 6. A few data points are out of the y-axis range and are therefore not visible.



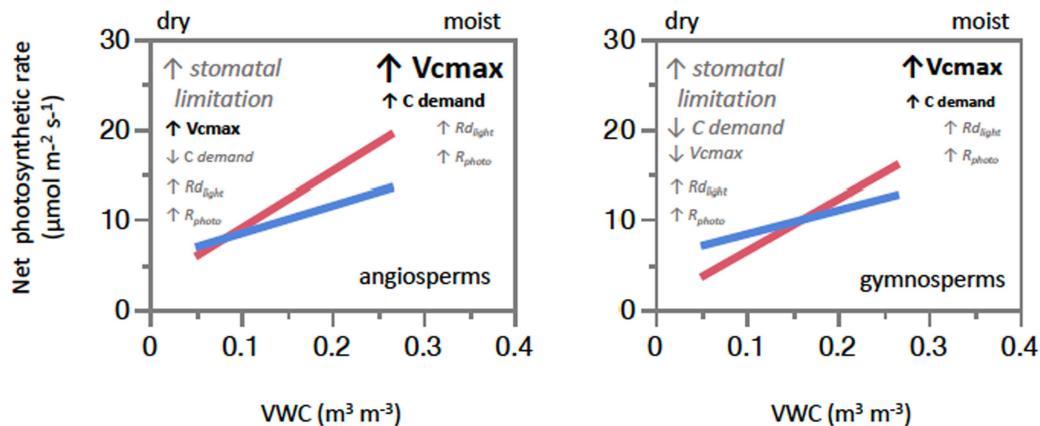
**Extended Data Fig. 4 | Percentage of stomatal limitation of net photosynthesis in relation to soil moisture (VWC) by species for ambient and experimentally warmed plants.** The percentage of stomatal limitation was calculated according to previous studies<sup>25,26</sup>. Data are from multiple days across three years ( $n = 1,991$  across species). In a full model analogous to those used in Table 1, the slope of the percentage of

stomatal limitation versus VWC was significantly steeper in warmed (red) than ambient (blue) plants (interaction of VWC  $\times$  warming treatment,  $F_{1,593} = 38.1, P < 0.0001$ ). The arrows show the median VWC across all measurements for the ambient and warmed plants of each species. Species are arranged from top to bottom by their geographical ranges (temperate species in top two rows, boreal in bottom two rows).



**Extended Data Fig. 5 | Relationships of net photosynthesis and leaf conductance to soil water content for different VPG classes and leaf temperatures.** Relationships are shown for two temperature treatments, for three VPG classes (left four panels) and three leaf temperature classes (right four panels). Data are pooled across all species and show the regression line for  $A_{net}$  and  $g_s$  in relation to VWC in three VPG classes

(0.4–1.6, 1.6–2.8, 2.8–4.0 kPa; red, green and blue lines, respectively) and for ambient and warmed (+3.4 °C) treatment plants; and in relation to VWC in three  $T_{leaf}$  classes (8–20, 20–32, 32–38 °C; dashed, dotted, and solid black lines, respectively) for ambient and warmed (+3.4 °C) treatment plants. Sample sizes in each panel, around 950–995. A few data points are out of the y-axis range and therefore not visible.



**Extended Data Fig. 6 | Conceptual illustration of mechanisms that influence the effect of climate warming on the response of realized  $A_{\text{net}}$  and soil water content (VWC).** Schematics are shown for angiosperms (left) and gymnosperms (right). Red lines indicate warmed treatment plants, blue lines ambient plants. The regression lines are pooled for all seven angiosperms and all four gymnosperms, at each warming treatment. The arrows show the direction of the effect of warming treatment on specific factors and the size of the letters indicates the relative magnitude of those effects on  $A_{\text{net}}$ . Bold fonts in black indicate changes that increase  $A_{\text{net}}$  in warmed plants relative to ambient plants, italic fonts in grey indicate changes that decrease  $A_{\text{net}}$  in warmed plants relative to ambient plants. For angiosperms in moist soils, warmed plants exhibit large increases in  $V_{\text{cmax}}$  and in carbon demand (from 23% higher growth<sup>19</sup>) that far outweigh the likely modest increases in dark respiration in the light ( $R_{\text{light}}$ )<sup>22</sup> and in photorespiration ( $R_{\text{photo}}$ )<sup>27</sup>, to result in large increases in  $A_{\text{net}}$ . For angiosperms in dry soils, however, experimental warming results in lower water availability that slows growth, reducing carbon demand in warmed (compared to ambient) plants. In dry soils, warming also increases stomatal limitation of photosynthesis (perhaps due in part

to slightly higher VPG in warmed plots), and constrains the magnitude of positive effects of  $V_{\text{cmax}}$  on  $A_{\text{net}}$ . The combination of increased  $R_{\text{light}}$  and  $R_{\text{photo}}$  and reduced carbon demand, slightly outweigh increased  $V_{\text{cmax}}$  and result in slightly reduced  $A_{\text{net}}$  in warmed compared to ambient angiosperms. The responses of gymnosperms are similar, except that changes in  $V_{\text{cmax}}$  with warming are less positive (than in angiosperms) in moist soils and negative in dry soils; additionally, the negative overall growth response (−26% growth response on average<sup>19</sup>) to warming suggests at most a small warming-induced increase in carbon sink strength when soils are wet and a larger decline when soils are dry. Collectively these factors make the  $A_{\text{net}}$  response of gymnosperms to warming more negative than that of angiosperms at every VWC level. Additionally (not shown in this conceptual figure, see Fig. 1), climate warming leads to higher evapotranspiration and thus more pronounced soil drying, therefore warmed plants operate at lower levels of VWC on average (Fig. 1) and at the vast majority of points in time (Extended Data Fig. 1), promoting the tendency of warmed plants to have lower  $A_{\text{net}}$  on average than ambient plants (Fig. 3).

**Extended Data Table 1 | Annual climate means for the two sites before and during the experiment**

		Prior to experiment (1973-2008)		During experiment (2009-2011)	
Weather Station	Mean annual precipitation (mm) (SD)	Mean annual temperature (°C) (SD)	Mean annual precipitation (mm) (SD)	Mean annual temperature (°C) (SD)	
Cloquet	783.4 (138.5)	4.8 (1.0)	776.1 (117.8)	5.1 (0.8)	
Tower (Ely)	725.9 (135.5)	2.6 (1.0)	615.7 (123.6)	3.6 (0.5)	

The Tower (Ely) weather station is 43 km from the research site. The Cloquet weather station is 3 km from the research site. Data are mean  $\pm$  s.d. among years.

**Extended Data Table 2 | VWC percentiles for measurement dates and all dates**

Dataset	Treatment	5%	25%	mean	50%	75%	95%
Measurement days	ambient	0.080	0.131	0.172	0.181	0.227	0.247
All days	ambient	0.082	0.146	0.182	0.182	0.229	0.259
Measurement days	+3.4 °C	0.071	0.089	0.132	0.124	0.171	0.203
All days	+3.4 °C	0.072	0.103	0.137	0.132	0.173	0.211

VWC (0–20 cm depth) values are recorded hourly in every plot across the shown time periods, averaged by day by treatment and then assessed by percentiles for measurement days and all days in both treatments. VWC percentiles are shown for days when leaf gas exchange measurements were made (measurement days) versus all days between (and including) day of year 162 (11 June) and 269 (26 September) from 2009 to 2011 across sites (all days). Results show that for both warming treatments, soil moisture conditions across the measurement days were well-matched to the average conditions across the three growing seasons.

Extended Data Table 3 | Species-specific models of photosynthesis in relation to warming treatment and soil moisture

Species	n	VWC	Warm	Warm x VWC	R <sup>2</sup>	a amb/ +3.4	b amb/ +3.4
<i>Abies balsamea</i>	62	<0.0001	<b>0.0241</b>	<b>0.0227</b>	0.42	4.8/0.1	12.6/35.2
<i>Acer rubrum</i>	245	<0.0001	<0.0001	<b>0.0080</b>	0.30	2.0/1.4	26.9/47.3
<i>Acer saccharum</i>	166	<0.0001	<b>0.0123</b>	<b>0.0031</b>	0.23	2.3/0.3	12.6/32.7
<i>Betula</i> <i>papyrifera</i>	271	<0.0001	0.1199	<0.0001	0.31	11.5/4.9	24.7/72.4
<i>Picea glauca</i>	59	<0.0001	0.5125	0.1008	0.62	2.0/-1.2	38.7/56.9
<i>Pinus banksiana</i>	136	<0.0001	0.9653	<b>0.0108</b>	0.25	9.6/2.8	25.8/68.9
<i>Pinus strobus</i>	132	<0.0001	0.4752	<b>0.0326</b>	0.38	2.9/0.0	32.2/53.8
<i>Populus</i> <i>tremuloides</i>	271	<0.0001	<b>0.0001</b>	<b>0.0047</b>	0.24	8.4/5.9	34.2/64.9
<i>Quercus</i> <i>macrocarpa</i>	175	<0.0001	<b>0.0001</b>	<b>0.0394</b>	0.33	3.0/2.1	46.5/73.8
<i>Quercus rubra</i>	242	<0.0001	<0.0001	<0.0001	0.27	6.5/1.9	22.6/73.1
<i>Rhamnus</i> <i>cathartica</i>	231	<0.0001	<0.0001	<b>0.0014</b>	0.29	3.5/1.1	32.5/67.6

Species-specific tests (*P* values) of  $A_{\text{net}}$  versus VWC, warming treatment and their interaction are shown, including the sample size (*n*) and  $R^2$  of the full model. Intercepts (*a*) and slopes (*b*) for  $A_{\text{net}}$  versus VWC in ambient (amb) and warmed (+3.4 °C; warm) treatments were examined separately. Relationships are shown in Fig. 1. For all species, the relationship of  $A_{\text{net}}$  to VWC was positive. For species for which the relationship of  $A_{\text{net}}$  to warming was significant, it was positive, except it was negative for *A. balsamea*. All full models were significant ( $P < 0.0001$ ). Sample sizes for the percentage of stomatal limitation (Extended Data Fig. 4) were nearly identical to those shown here for  $A_{\text{net}}$  versus VWC (within 1% for each species). Sample sizes for  $g_s \times \text{VWC}$  shown in Fig. 2 are identical, except for *A. balsamea* (*n* = 38), *P. glauca* (*n* = 36), *P. banksiana* (*n* = 117), *P. strobus* (*n* = 112) and *R. cathartica* (*n* = 228). For Figs. 1, 2 and Extended Data Fig. 4 roughly half of the measurements were in each warming treatment.

## Extended Data Table 4 | Summaries of mixed model analyses for species examined across two or three years

## A. Nine species measured in two or three years

Source of variance	$(A_{\text{net}})$		$(g_s)$	
	F	P>F	F	P>F
Species	158.03	<b>&lt;0.0001</b>	103.17	<b>&lt;0.0001</b>
Warm	47.65	<b>&lt;0.0001</b>	11.26	<b>0.0035</b>
Species*Warm	4.65	<b>&lt;0.0001</b>	1.40	0.1923
Soil water	492.98	<b>&lt;0.0001</b>	527.41	<b>&lt;0.0001</b>
Soil water *Species	3.64	<b>0.0003</b>	14.24	<b>&lt;0.0001</b>
Soil water *Warm	59.18	<b>&lt;0.0001</b>	18.90	<b>&lt;0.0001</b>
Soil water*Species*Warm	1.23	0.2755	1.11	0.3504
Full model R <sup>2</sup>	0.56		0.52	

## B. Five species measured in three years

Source of variance	$(A_{\text{net}})$		$(g_s)$	
	F	P>F	F	P>F
Species	177.60	<b>&lt;0.0001</b>	100.91	<b>&lt;0.0001</b>
Warm	55.31	<b>&lt;0.0001</b>	19.46	<b>0.0004</b>
Species*Warm	3.72	<b>0.0051</b>	0.92	0.4529
Soil water	341.67	<b>&lt;0.0001</b>	463.80	<b>&lt;0.0001</b>
Soil water *Species	1.20	0.3098	14.63	<b>&lt;0.0001</b>
Soil water *Warm	53.15	<b>&lt;0.0001</b>	22.14	<b>&lt;0.0001</b>
Soil water*Species*Warm	1.62	0.1675	0.99	0.4106
Full model R <sup>2</sup>	0.53		0.49	

**a, b.** Analyses for  $A_{\text{net}}$  and  $g_s$  in relation to +3.4 °C warming treatment (Warm), species, soil water (VWC) and their interactions for nine species measured in at least two years (**a**) and five species measured in all three years (**b**). Plot, block and site were included as random effects in the models. Data for **a** are a subset of nine species (all species except *A. balsamea* and *P. glauca*) measured in at least two years ( $n = 1,870$  for  $A_{\text{net}}$ ; 1,829 for  $g_s$ ). Data for **b** are a subset of five species (*A. rubrum*, *B. papyrifera*, *P. tremuloides*, *Q. rubra* and *R. cathartica*) measured in all three years ( $n = 1,260$  for  $A_{\text{net}}$ ; 1,259 for  $g_s$ ). Both models were significant at  $P < 0.0001$ .

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
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- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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*Give P values as exact values whenever suitable.*
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*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*

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# Field-specific reporting

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Life sciences       Behavioural & social sciences       Ecological, evolutionary & environmental sciences

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## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Field experiment in forest with experimental warming
Research sample	A total of 24 plots, with approximately 2,500 individual tree saplings.
Sampling strategy	Each plot originally had 11 individuals of each species. At times of gas exchange measurements (made 54 times over three years) all species to be included in that sampling campaign were measured in roughly equal numbers across blocks, treatments, and sites
Data collection	Several researchers used standard field gas exchange equipment (Li-COR) to measure light saturated net photosynthesis in the field under standard protocols.
Timing and spatial scale	Measures were made from early to late in the growing season across three years, across all plots in the two sites.
Data exclusions	Data with equipment failure or identified as statistical outliers were removed. These represented less than half of one percent of the entire sample.
Reproducibility	Statistical analyses showed consistent results across sites and species.
Randomization	Original planting of seedlings into each plot was random with respect to which of 141 locations each would go into.
Blinding	<i>Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.</i>

Did the study involve field work?  Yes  No

## Field work, collection and transport

Field conditions	Ambient conditions across hours, days, seasons and years in sub-boreal forest
Location	northern Minnesota, USA (all details given in paper)
Access and import/export	n/a
Disturbance	n/a

## Reporting for specific materials, systems and methods

### Materials & experimental systems

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
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### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
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<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging