Climate warming effects 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[1-11]. 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[4-6, 9-11]. Here we show that 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, +3.4 °C warming increased light-saturated net photosynthesis (A_{net}) and leaf diffusive conductance (g_s) on average on the one-third of days with the wettest soils. In all 11 species g_s, and as a result A_{net}, decreased during dry spells, and did so more sharply in warmed than ambient plants. Consequently, across the 11 species, warming reduced A_{net} on the two-thirds of days with driest soils. Thus, low soil moisture may reduce, or even reverse, potential benefits of climate warming on photosynthesis in mesic, seasonally cold environments, both during drought and in regularly occurring, modestly dry portions of the growing season.

A changing climate will influence plants by altering temperature, precipitation, and soil moisture, as well as their variability and seasonality[1-11]. In temperate and boreal climates, temperatures swing seasonally from cold (and limiting to biological processes) to warm and periodically dry when moisture can be limiting[2-6, 9-11]. Both the ‘law of the minimum’ and multiple limitation theory[12-14] provide a conceptual basis for predicting climate warming interactions with soil moisture. While higher temperatures may alleviate enzymatic limits to the biochemistry of photosynthesis, realized rates of CO_2 assimilation may decrease if and when low soil water causes stomatal closure and limits the CO_2 substrate for photosynthesis. As growing season conditions in temperate and boreal forests are likely to become effectively drier than in the past[3, 8-9], because climate warming will increase evapotranspiration more than precipitation[3,9] and increase precipitation variability[1, 9], the importance of water availability to climate responses may grow larger in the future[3-6, 9-11, 15-18].

Mid- and high-latitude plants will therefore likely experience both positive and negative effects of climate warming on photosynthesis within and across years—positive when soil moisture is ample but negative when soils are drier [4-6, 9-11, 15-17]. Whether such effects are in aggregate positive or negative likely depends on the balance of time that warming alleviates low temperature limitations to plant function *versus* causes 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 soil moisture effects[4-6, 9-11, 16-18].

Herein 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 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[19-22] where juveniles (three-to-five years old at 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, see Methods). The 11 species co-occur in forests in northern Minnesota, but 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[19]. Fluctuations in soil moisture levels (volumetric water content, cm^3 H_2O/cm^3 soil, *VWC*) occurred at both sites and in all years (Extended Data Fig. 1, 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 (≈ -1.5 MPa) for these sandy loam soils [23, 24].

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, Extended Data Table 3). In moist soils, all angiosperm species (and no gymnosperms) showed higher maximum carboxylation capacity at 25 °C ($V_{cmax-25}$) in warmed than ambient conditions (Extended Data Fig. 3), helping to explain their higher A_{net} in warmed plants when soil water limitations were modest (Fig. 1). Every species showed marked sensitivity of A_{net} to drying soil moisture (Fig. 1). More germane to our overarching hypothesis, A_{net} in all species declined more steeply with decreasing soil moisture in warmed than ambient conditions (Fig. 1); hence, 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 declined (or negative effects grew) as soil moisture availability declined (Fig. 1).

In other words, we found a significant interaction between the warming treatment and *VWC* for A_{net} (Table 1; $F_{1,553} = 40.9$, $P<0.0001$) in a model that included warming treatment, species, *VWC*, and two other environmental drivers (leaf temperature, T_{leaf}, and vapor pressure gradient, *VPG*). Moreover, although species differed from each other in A_{net}, they did not differ in how *VWC* influenced their response to warming (no warming x soil moisture x species interaction, Table 1; $F_{10,1797} = 1.2$, $P=0.30$). Hence, species whose growth was enhanced (e.g., *Acer*, *Quercus*) or reduced (e.g., *Abies*, *Picea*) under climate warming[19] 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_{net} to *VWC* was always steeper in warmed than in ambient plants (Fig. 1, Extended Data Table 3), and the interaction of warming x *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[3, 8, 9, 20], 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_{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_{net}, leaf diffusive conductance (g_s) declined in drying soils; and was generally equal or greater in warmed than ambient plants in moist soils, but similar or lower in warmed than ambient plants in dry soils (Fig. 2). Moreover, the relationship of g_s to *VWC* had a steeper slope in the warmed than ambient treatment (Fig. 2, Table 1), just as for A_{net} (Fig. 1). Evidence suggests that the changes in g_s contributed to the shrinking positive effect of warming on A_{net} as soil water availability declined (Fig. 1). First, g_s declined proportionally more than A_{net} with increasing soil water deficits (i.e., A_{net}/g_s was greater in drier than wetter soils in every species) and the increase in A_{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_{net} in drier soils and with greater stomatal limitation in warmed than ambient plants in drier soils. Second, corroborating this, quantitative estimates of the percent limitation of A_{net} by stomatal conductance[25, 26] (rather than by biochemical limitations), also increased more steeply with declining *VWC* in warmed than ambient plots (Extended Data Figure 4).

A key question is the degree to which the different responses of g_s and A_{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_{leaf} and *VPG*. *VWC* was very weakly positively correlated with leaf temperature (T_{leaf}) and unrelated to *VPG* across all measurement dates (Extended Data Figure 2); thus low soil moisture effects were not confounded by high *VPG* or high T_{leaf} in this data set. The differential response of g_s to *VWC* in warmed versus ambient plants was independent of either *VPG* or T_{leaf} (no three-way interactions, Table 1). The greater decline of A_{net} with decreasing *VWC* in warmed than ambient plants was slightly steeper at higher levels of T_{leaf} and *VPG* (illustrated by three-way interactions for A_{net} of warming treatment, VWC and either T_{leaf} or *VPG*, Table 1), but was apparent regardless of *VPG* or T_{leaf} (Extended Data Figure 5). Although the relationship of g_s (but not A_{net}) to *VPG* was non-linear, replacing *VPG* with log(*VPG*) in models in Table 1 only marginally influenced results and did not show any interaction of treatment x log(*VPG*) x *VWC*, suggesting that non-linearity 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 vapor pressure deficit (closely related to *VPG*) and low soil water availability[2]. Our results are consistent with that, as low *VWC* and high *VPG* independently constrained A_{net} and g_{s} (Extended Data Figure 5).

It is also useful to view these results in the context of the temperature response functions of A_{net}. For both well-hydrated[21] and *in situ* (Extended Data Figure 2) leaves, the broad temperature optima (T_{opt}) of A_{net} for these species was ≈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), roughly one-third of ambient treatment measurements were made below T_{opt} (e.g. T_{leaf} <22 °C) and another third were made above T_{opt} (e.g. >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} (i.e. ≈22-29 °C range). 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 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 role. But why was the shift with declining *VWC* from biochemically to stomatally limited photosynthesis steeper in warmed than ambient plants of all species (Extended Data Figure 4)? We posit, from several lines of evidence, that a combination of factors drove these responses (Extended Data Figure 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 $V_{cmax\text{-}25}$ in warmed conditions, Extended Data Figure 3) and higher carbon demand for photosynthate[28], as they grew 23% faster on average in warmed than ambient conditions[19]. In drier soils, increased stomatal limitation eliminated most of the potential gain that higher $V_{cmax\text{-}25}$ might deliver (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[22]) and higher photorespiration[27] at all *VWC* levels (Extended Data Figure 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[19]) to warming, coupled with more negative warming effects on carbon gain when soils were dry, suggests a small warming-induced increase in C sink strength at best when soils were wet and a larger decline when soils were dry (Extended Data Figure 6). Collectively these factors likely contributed to making the responses of gymnosperms to warming more negative than that of angiosperms at every *VWC* level.

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

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 vs. ambient plants across all levels of soil moisture, i.e., comparing the effect of warming on photosynthetic processes at a common soil moisture (and typically not a common date). In contrast, in Fig. 3 we show A_{net} averaged across species in warmed vs. 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 usually 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 net effect of both direct physiological warming impacts and indirect soil moisture impacts of warming treatments on realized average photosynthetic rates, equally weighting all 11 species.

The warming treatment had a markedly different impact 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). Under days with drier conditions, the mean stimulation of A_{net} disappeared; this occurred at ≈the 65th percentile of *VWC* on average across the 11 species. Hence, warming increased average A_{net} of the community on only the third of days with highest soil moisture. Species (like 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 (like 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 ambient and warmed treatments can also be gleaned for each species from the arrows in Fig. 1. Results restricted to the 9 species measured in 2 or 3 years, or to the 5 species measured in all 3 years, were generally similar to results for all 11 species: when soil moisture was high, warming increased A_{net}, but whenever substantial soil moisture deficits occurred, warming decreased A_{net} (Extended Data Table 4).

These results provide novel information about how soil moisture may modulate the effects of climate warming in seasonally cold forest ecosystems, which represent roughly half of global forests[29]. 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, Extended data Figs 3,4, 6). On average, warmed plants had higher g_s and A_{net} than ambient plants when soils were moist (Figs 1-2). As soils dried, plants in both treatments reduced g_s, but warmed plants of all species reduced both g_s and A_{net} proportionally more than did ambient plants. In a warmer future, greater increases in evapotranspiration than precipitation during the growing season[3] should also reduce soil water stores[9], pushing plants in the future climate further down the "A_{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, which were also similar to the 20-year average for these sites (Extended Data Table 1). Thus, the observed responses to warming (Figs. 1-3) are likely indicative of future conditions in northern Minnesota if rainfall patterns are roughly 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. Moreover, our results can help 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[5,6,9-11,16,18]. Given higher precipitation and lower evapotranspiration, soils in eastern North American boreal forests are more often moist, and thus higher temperatures arer 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 gain[3,5,9-11].

Climate warming is likely to extend the season of active photosynthesis, and the effects of rising CO_2 concentrations on g_s may result in enhanced soil moisture[5,10,15]; both could help offset the negative effects of soil drying on photosynthesis resulting from higher potential evapotranspiration relative to growing season precipitation and from lower soil moisture recharge resulting from higher rainfall intensity and more run-off[1,3,9-11]. However, the relative magnitude of such offsets is unknown[1,3,9-11]. Furthermore, although the mechanisms underlying 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 climate warming effects 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. Additionally, higher evapotranspiration in a warmer world[5,9-11] 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.

References

1. Kao, S. C. & Ganguly, A. R. Intensity, duration, and frequency of precipitation extremes under 21st-century warming scenarios. *J. Geophys. Res.*, **116** (D16), D16119 (2011). DOI: 10.1029/2010JD015529

2. Novick KA *et al.* The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. *Nature Climate Change*. *6*, 1023-1027. DOI: 10.1038/nclimate3114 (2016)

3. Seager, R., *et al.* Dynamical and thermodynamical causes of large-scale changes in the hydrological cycle over North America in response to global warming. *J of Climate* **27**, 7921-7948 (2014).

4. Moyes, A. B., Castanha, C., Germino, M. & Kueppers, L. M. Warming and the dependence of limber pine (*Pinus flexilis*) establishment on summer soil moisture within and above its current elevation range. *Oecologia* **171**, 271-282 (2013).

5. Price, D.T., et al. Anticipating the consequences of climate change for Canada’s boreal forest ecosystems. *Environ Rev* **21**, 322–365 (2013).

6. Hogg, E.H., Michaelian, M., Hook, T.I., & Undershultz M.E. Recent climatic drying leads to age-independent growth reductions of white spruce stands in western Canada. *Glob.Change Biol.*, **23**, 5297–5308. (2017).

7. *IPCC, Climate Change 2013: The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, 2013); www.ipcc.ch/report/ar5/wg1/.

8. Sherwood, S. & Fu, Q. A Drier Future. *Science* **343**, 737-739 (2014).

9. Wang, Y., Hogg, E. H., Price, D. T., Edward, J. & Williamson, T. Past and projected future changes in moisture conditions in the Canadian boreal forest. *Forestry Chronicle* **90**, 678-691 (2014).

10. Girardin, M. *et al.* No growth stimulation of Canada's boreal forest under half-century of combined warming and CO_2 fertilization. *Proc. Natl Acad. Sci. USA* **113**, E8406–E8414. (2016)

11. D'Orangeville, L., L Duchesne, D Houle, D Kneeshaw, B Côté, N Pederson. Northeastern North America as a potential refugium for boreal forests in a warming climate. *Science* **352**, 1452-1455 (2016).

12. Wong, S. C., Cowan, I. R. & Farquhar, G. D. Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**, 424–426 (1979).

13. Bloom, A. J., Chapin I, F. S. & Mooney, H. A. Resource limitation in plants - an economic analogy. *Ann. Rev. Ecol. Systematics* **16**, 363–392 (1985).

14. Rastetter, E. B. & Shaver, G. R. A model of multiple element limitation for acclimating vegetation. *Ecology* **73**, 1157-1174 (1992).

15. Buermann, W., Bikash, P. R., Jung, M., Burn, D. H. & Reichstein, M. Earlier springs decrease peak summer productivity in North American boreal forests. *Environ. Res. Lett*. **8**, 024027 (2013). doi:10.1088/1748-9326/8/2/024027

16. Ma, Z. *et al.* Regional drought-induced reduction in the biomass carbon sink of Canada's boreal forests. *Proc. Natl Acad. Sci. USA* **109**, 2423–2427 (2012).

17. Moyes, A. B., Germino, M. J. & Kueppers, L. M. Moisture rivals temperature in limiting photosynthesis by trees establishing beyond their cold-edge range limit under ambient and warmed conditions. *New Phytologist* **207**, 1005-1014 (2015).

18. Peng, C. *et al.* A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature Climate Change* **1,** 467-471 (2011).

19. Reich, P. B. *et al*. Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nature Climate Change* **5**, 148-152 (2015).

20. Rich, R.L. *et al*. Design and performance of combined infrared canopy and belowground warming in the B4WarmED (Boreal Forest Warming at an Ecotone in Danger) experiment. *Glob. Change Biol.* **21**, 2334–2348 (2015).

21. Sendall, K. M. *et al*. Effects of experimental forest warming on photosynthetic temperature optima of temperate and boreal tree species. *Glob. Change Biol.* **21**,1342-1357 (2015).

22. Reich *et al*. Boreal and temperate trees show strong acclimation of respiration to warming. *Nature* 531, 633-636 (2016).

23. Rawls, W. J., Brakensiek, D. L. & Sexton, K. E. Estimation of soil water properties, *Transactions of the ASAE* **25**: 1316-1320 & 1328 (1982).

24. Campbell, G. S. & Norman, J. M. *An Introduction to Environmental Biophysics*, Springer-Verlag, New York, NY (1998).

25. Sharkey, T. D. Photosynthesis in intact leaves of C3 plants: physics, physiology and rate limitations. *The Botanical Review*, **51**, 53-105. (1985).

26. Jones, H. G. (1985). Partitioning stomatal and non-stomatal limitations to photosynthesis. *Plant, Cell & Environment*, *8*(2), 95-104.

27 Sage, R.F., D.S. Kubien, The temperature response of C_3 and C_4 photosynthesis. *Plant, Cell & Environment*, **30**, 1086–1106 (2007).

28. Körner C. Paradigm shift in plant growth control. *Curr Opin Plan Biol*. **25**,107-114 (2015).

29. Food and Agriculture Organization of the United Nations, *State of the World's Forests*, Rome, 179 pp. (2011). http://www.fao.org/docrep/013/i2000e/i2000e00.htm

30. De Kauwe, M. G., *et al.* A test of the 'one-point method' for estimating maximum carboxylation capacity from field-measured, light-saturated photosynthesis. *New Phytologist*, *210*, 1130-1144 (2016)

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author contributions P. B. R., R. A. M. and R. L. R. designed the study. 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. analyzed the data. P. B. R. wrote the first draft and along with the other co-authors jointly wrote the manuscript.

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Fig. 1. Photosynthesis is reduced by drying soils, and more so with simulated climate warming. *In situ* light-saturated net photosynthesis (A_{net}) in relation to soil moisture (volumetric water content, *VWC*) by species for ambient (blue) and experimentally warmed (red) plants. Data shown are from multiple days across three years (n=1991 across species). The slope of A_{net} vs. *VWC* was significantly steeper in warmed than ambient plants (Table 1; $F_{1,553} = 40.9$, $P<0.0001$). 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 geographic ranges (temperate species in top two rows, boreal in bottom two rows). Sample sizes per species shown in ED Table 3.

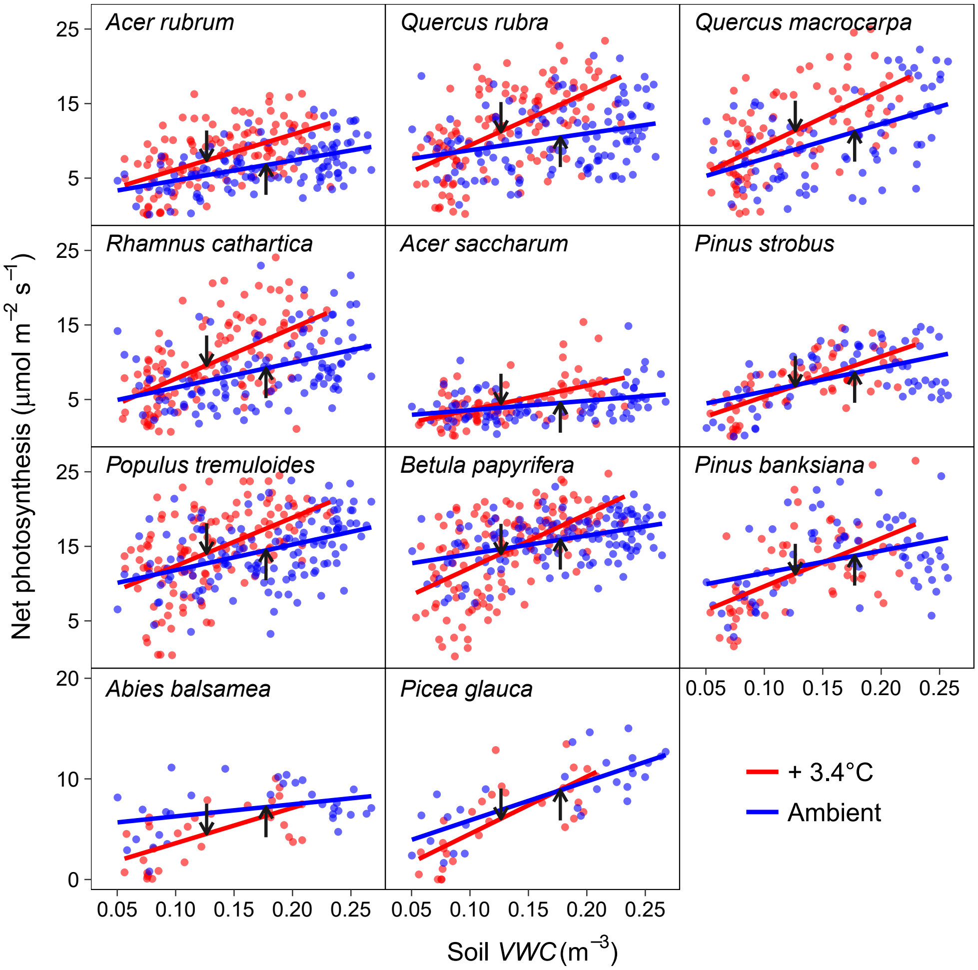

Fig. 2. Leaf conductance is reduced by drying soils, and more so with simulated climate warming. Leaf diffusive conductance in relation to soil moisture (volumetric water content, *VWC*) by species for ambient (blue) and experimentally warmed (red) plants. Data shown are from multiple days across three years (n=1903 across species). The slope of g_s vs *VWC* was significantly steeper in warmed than 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.

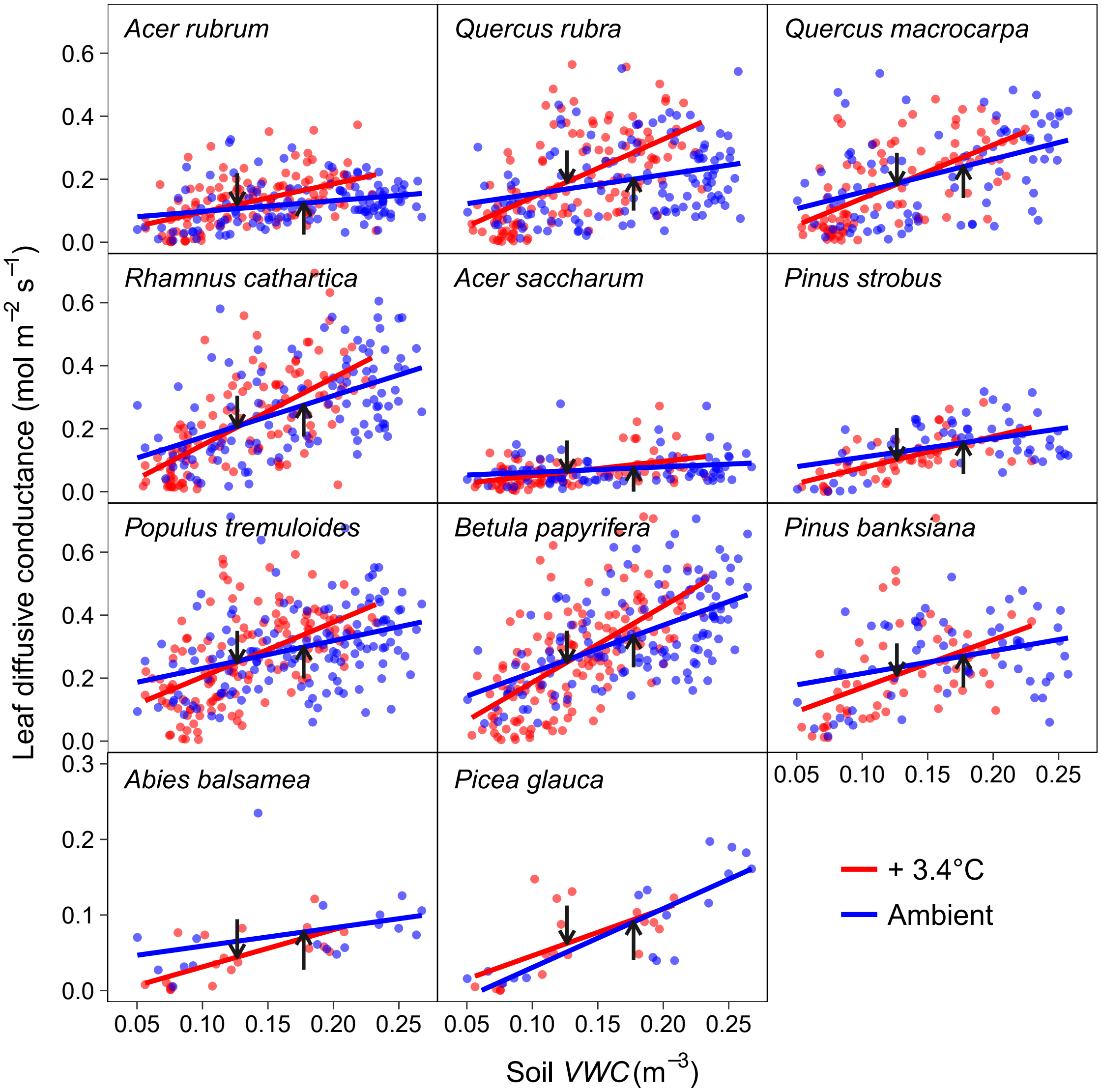

Fig. 3. Warming stimulates photosynthesis on average in moist soils, but not otherwise. Mean A_{net} (± one standard error) of 11 temperate and boreal species in ambient and warmed treatments compared during periods that ranged from dry to wet. Periods represent soil moisture percentiles within treatments across all measurements, from dry to wet (i.e, the 5^{th}, 25^{th}, 50^{th}, 75^{th}, and 95^{th} 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 standard error is derived from the standard error of the slope of A_{net} vs. *VWC* within each treatment. Note that the mean *VWC* by treatment is also shown at each soil moisture percentile (see insert values).

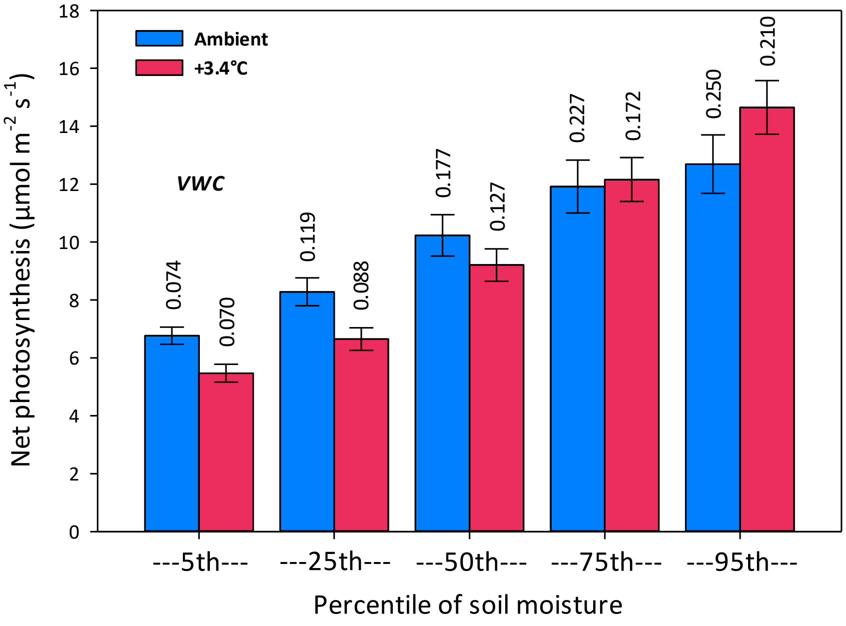

Table 1. Summary of mixed models for light-saturated net photosynthetic rate (A_{net}) and leaf diffusive conductance (g_s) in relation to species, +3.4 °C warming treatment (Warm), volumetric water content (Soil water), vapor pressure gradient (*VPG*), leaf temperature (T_{leaf}), and all interactions 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 = 1991 for A_{net}, 1903 for g_s). Four-way interactions were not significant and are not shown.

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

Methods

The experiment is located at two University of Minnesota field stations; the Cloquet Forestry Center, Cloquet MN (46°40’46” N, 92°31’12” W, 382 m a.s.l., MAT, 4.8°C mean annual temperature, 783 mm mean annual precipitation) and the Hubachek Wilderness Research Center, Ely, MN, (47°56’46” N, 91°45’29” W, 415 m a.s.l., MAT, 2.6° C mean annual temperature, 726 mm mean annual precipitation)[19, 20]. At both sites, treatments were positioned in relatively open (recently cleared) overstory conditions. The overall experimental design was a 2 (site) x 2 (treatment) factorial, with six replicates of each for a total of 24 circular 3-meter 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 °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 (i.e. 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-hour per day warming of +3.4 °C (≈April-November) and midsummer midday (0900-1500 h during June- Sept) aboveground warming of +2.9 °C across the 2009 to 2011 growing seasons[19, 20]. 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., Logan, UT, USA). 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°. Volumetric water content (cm^3 H_2O/cm^3 soil, *VWC*) was monitored hourly in all plots and corrected[20] 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[19, 20]. In mid-continental boreal and temperate biomes, climate change will increase plant and air temperatures, and the associated increases in vapor pressure gradients (*VPG*) and evapotranspiration are likely to more than offset any increase in total atmospheric water vapor or precipitation, resulting in increased soil water deficits[3, 7-10].

Eleven juveniles of each of 11 tree species were planted in 2008 into existing low shrub, herb, and fern vegetation in every plot (≈2,900 juveniles; average of ≈3 years-old in 2009). The 11 species include six native broadleaf (*Acer rubrum*, *A. saccharum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus macrocarpa* and *Q. rubrum*), 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 ecotonal region. Local ecotypes (collected between 46°0’ and 48°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 measures of light-saturated net photosynthesis and leaf diffusive conductance were made using six Li-Cor 6400 portable photosynthesis systems (Li-Cor, Lincoln, NE). Simultaneous leaf temperature measurements were made for most species using the internal fine wire thermocouple located in the bottom of the 2x3cm 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, Lincoln, NE, USA). Leaf temperature measured in the cuvette and canopy surface temperature (measured independently with infrared thermometers, as described above) were strongly correlated. Cuvette leaf temperature was usually ≈2°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 plant canopy surface sensed by the infrared thermometers were often in partial shade. Measurements were made throughout the growing seasons (June to September) of 2009 through 2011. A total of ≈1,900 measurements on a total of 54 dates were made across species, treatments, sites, and time. Individuals were three- to five-years old at the time of measurements. Measurements were made in morning or early afternoon (i.e. typically between 0830-1400 solar time). Not all species were measured each year due 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. Fully expanded, healthy upper canopy leaves were sampled from individuals planted in a combination of ambient and +3.4 °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 μmol s^{-1} and CO_2 reference concentrations were set at 400 μmol mol^{-1}.

Estimates of V_{cmax} from the one-point method [30] and estimates of the percent stomatal limitation[25,26] of A_{net} were also made. For data from other years where full A-C_i curves were measured, calculated V_{cmax} 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_{cmax} values estimated from the entire curves, strongly supporting the appropriateness of the one-point method for our field measurements for this set of species. Percent stomatal limitation was taken as the percent reduction in A_{net} from the maximal rate estimated with no stomatal limitation (A_{gmax}). A_{gmax} was estimated (for each species in both treatments) in three ways; (i) 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, (ii) based on the 95th percentile of A_{net} measurements from the current study, and (iii) based on the A_{gmax} estimates from the A-$\mathrm{C_i}$ curves, adjusted to reflect realized A_{net} in the current study using the correlation of values from (i) and (ii). For method (i) we used the relationship between A-C_i curves and field 95th percentile A_{net} for 9 species to estimate A_{gmax} for the two species without A-C_i curves. The overall patterns shown in each panel of the Extended Data Figure 4 are nearly identical using any of the three metrics. We used metric (iii) 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 percent stomatal limitation). We recognize the impossibility of negative values for percent stomatal limitation, but retained them for statistical purposes.

A mixed model was used to compare light saturated net photosynthetic rates (A_{net}) and leaf diffusive conductance (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_{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_{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 x treatment x *VWC* bin combinations, and third we averaged raw species means for *VWC* bin classes across treatments. All three approaches resulted in similar output.

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 [DOY] 162 to 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*).

Data availability The data reported in this paper will be made available as a *Supplementary Information Data Table* (online) and deposited in an open-source community archive.