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Biodiversity bottleneck: seedling establishment under changing climatic conditions at the boreal–temperate ecotone

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Abstract Climate change is increasing global temperatures, severe rainfall events, and the occurrence and severity of drought. Changes in global climate may have negative consequences for particular plant species and for biodiversity overall. In the short term, altered temperature and precipitation regimes may have the most severe effects on plant species near their range limits and in the earliest stages of plant development. To address these issues, we assessed seedling emergence, early survival, and growth of 18 boreal, temperate, and exotic woody species at the boreal–temperate forest ecotone in central Minnesota. We experimentally warmed forest plots to mimic projected warming by the end of the twenty-first century (+ 1.7 °C and + 3.4 °C). We also experimentally removed summer rainfall (∼ 42% reduction) to simulate drought conditions in this region. We found that emergence and survival of boreal and exotic species was lower in experimentally warmed plots. This was exacerbated by drought. Temperate species emergence and survival was largely unaffected by climate manipulations (on average). Conversely, temperate seedling growth was greater in warmer conditions, but only when paired with drought. We found that overall seedling species richness was reduced by warming, mostly due to lower boreal and exotic species emergence and survival (conifers were also strongly negatively affected across species-range groups). If temperate seedling emergence and survival does not compensate for loss of boreal species, these forests may experience loss of biodiversity (and associated ecosystem functions) in the future.

Keywords Temperate · Boreal · Exotic · Invasive · Drought · Warming · Ecotone · Seedling · Ontogeny

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Introduction

Current global climate models project that average global temperatures will increase by 2–4 °C by the end of this century (Stocker et al. 2013). This change in temperature will likely be paired with changes in precipitation: severe rainfall events interspersed with periods of drought. Past work indicates that increasing global temperatures and altered rainfall regimes will have widespread implications for species distributions (Williams et al. 2002; Pucko et al. 2011). In many locations, some species will be pushed out of their climate niche (into warmer and drier conditions) and thus may be forced to migrate poleward or upward in elevation (Walther 2010). In the absence of migrational pathways (landscape corridors) or dispersal capability (Parmesan and Yohe 2003), some species may be locally extirpated, possibly resulting in reduced biodiversity at local scales (Pereira et al. 2010; Isbell et al. 2015). Understanding how individual species (and species groups) will respond to a changing climate will be essential to designing effective climate adaptation strategies.

In the short term, the ecological consequences of rapid changes in global climate conditions may be most pronounced at the geographical boundaries between ecosystems (ecotones), where many species are at or near their range limits (Parmesan et al. 2005; Fisichelli et al. 2013). For example, at the boreal–temperate forest ecotone, boreal tree species will likely be negatively affected by warmer/drier conditions (Reich et al. 2015), and these species may be replaced by warmer-adapted temperate species. Indeed, past work has shown that broadleaf temperate saplings at the dry end of the boreal–temperate ecotone in central North America tend to establish at higher densities and grow faster than boreal conifer saplings (Beckage et al. 2008; Fisichelli et al. 2012, 2014b). Furthermore, rapidly changing climate conditions could favor a small group of highly plastic, fast-growing, invasive species (Bellard et al. 2013). Past work has shown that woody invasive species in temperate forests may already be capitalizing on rapidly changing autumn conditions (Fridley 2016).

Due to unique species and functional group responses to warming and drought, there may be a change in species composition at the boreal–temperate forest ecotone (and elsewhere though we focus on the boreal–temperate ecotone here). Furthermore, if climate change is severe enough to drive local extirpation of species, and new species arrival (due to range modification) is dispersal limited, climate change may also cause a loss of biodiversity—at least in the short term (Thomas et al. 2004). In some cases, exotic invasive species may be introduced and become a substantial portion of local biodiversity, perhaps compensating for loss of native diversity, although such ideas are controversial (Buckley and Catford 2015; Miller and Bestelmeyer 2016).

For long-lived forest tree species, the early establishment stages (germination, emergence, survival, and early growth) are the most vulnerable to temperature and precipitation extremes (Walck et al. 2011). Longer-lived adults are likely more tolerant of changes in climate (Niinemets 2010), and migrating populations (i.e., range shifts) will eventually be the result of seed dispersal and subsequent establishment in the seedling layer. Even if adult trees have the capacity to persist in a changing climate, long-term species composition and diversity will depend on seed germination requirements (often specific and narrow, Walck et al. 2011), and early survival rates.

To assess how changing temperature and precipitation will affect physiology, ecology, and biogeochemistry at the boreal–temperate forest ecotone, we established a warming experiment in northern Minnesota (Rich et al. 2015). Herein, we focus on work addressing the question of whether these changes will affect species regeneration from seed (and overall forest diversity). In three successive years, we planted seeds of 18 temperate, boreal, and exotic woody species into ambient plots and plots warmed by $+ 1.7$ °C and $+ 3.4$ °C. We then used moveable rainout shelters to experimentally remove $\sim 42\%$ of the naturally occurring rainfall during the growing season in half of these plots. We tracked four metrics of seedling performance over 3 years (seedling emergence, survival, height, and number of leaves, hereafter referred to as "performance") and assessed the following five hypotheses: (1) temperate species will respond positively to higher temperatures (in all four measures of performance); (2) boreal species will respond negatively to higher temperatures (in all four measures of performance); (3) any negative species responses to warming, for temperate or boreal species, will be exacerbated by drought conditions (positive responses to warming will be negated by drought); (4) invasive species will perform well (in all four metrics)

and be unaffected by temperature or precipitation; and (5) due to constraints on boreal species (in particular), species diversity will be reduced in warmer plots at the boreal–temperate forest ecotone. These biodiversity losses will be exacerbated under drought conditions.

Methods

The field site

The experiment was conducted at the Boreal Forest Warming at an Ecotone in Danger (B4WarmED) experiment in northern Minnesota (USA) (Rich et al. 2015). The experiment consists of two sites within the boreal–temperate forest ecotone in Minnesota: Ely MN, 47°56′N, 91°45′W and Cloquet MN, 46°40′N, 92°31′W. Both sites are dominated by a 60- to 80-year-old aspen (*Populus tremuloides*) overstory.

The experiment was established by clearing approximately 0.4 ha of overstory vegetation in 2006 (Cloquet) and 2007–2008 (Ely). This resulted in approximately 40–60% full light conditions in all plots. Removal of the overstory, via clearcutting, is the most common silvicultural prescription used across aspen-dominated forests in Minnesota. As aspen-dominated forests are common in Minnesota, this study examines one of the most common conditions experienced by seedlings of the next generation of forests in this region.

We established three blocks (with six plots within each block) in the open canopy at both sites to account for (1) a slight southwestern facing slope at the Ely site and (2) the need for warming treatments to be closely located to a large power supply. In order to reduce confounding effects of slope and spatial proximity to electrical supply, we blocked the entire experiment. In 2008, an aboveground and belowground warming manipulation was established to warm plots to + 1.7 °C and + 3.4 °C (Rich et al. 2015). Aboveground warming was conducted using infrared heaters and an automated feedback system (proportional-integral-derivative controller) to maintain consistent levels of warming over dynamic ambient daily conditions. Soil warming was conducted using buried heat resistance cables in each of the plots. Both aboveground warming and belowground warming were maintained from mid-April to mid-November (see (Rich et al. 2015) for a detailed description of

methodology). We established two replicates within each block for each of the three warming levels (2 sites × 3 blocks × 3 warming treatments × 2 replicates = 36 total plots).

Beginning in 2012, we established event-based rainout shelters from June to September (a drought treatment) within each of the blocks at each site. Thus, within each block there is a replicate of each combination of drought × warming to assess the interactive effects of both warming and drought on seedling performance. From 2012 to 2014, we removed 40–45% of rainfall from the drought-treatment plots over the course of the growing season (June–September). Over the course of the experiment, this reduced average soil volumetric water content (cm^3 $H2O/cm^3$ soil) in drought plots by 0.013 or 8% of ambient conditions. Because soil moisture is averaged over the growing season and only 40–45% of rain events are excluded, seasonal average differences in soil moisture are not particularly strong.

Over the course of the experiment, average weather conditions were not statistically different from longer term (20 year) averages for the growing season (May 1–September 30) at either site (Table 1). While rainfall was slightly higher than average in 2012 at the Cloquet site, this difference was not maintained over the course of the experiment and thus probably was not the primary driver of the results we present here.

Tree seeds/seedlings

In May 2012, May 2013, and May 2014, we planted seeds of 18 boreal tree, temperate tree, and exotic shrub species into each of the plots at the two sites (Table 2). All seeds were collected locally and cold moist stratified or otherwise prepped prior to planting according to published standards (Bonner and Karrfalt 2008) as well as preliminary results from a growth chamber study that we conducted with the same seed set (Fisichelli et al. 2014b, Table 2). Most of the study species are found growing naturally (native and exotic species) within the boreal–temperate ecotone in Minnesota, with temperate and exotic species at or near northern range limits and boreal species at or near southern range limits. For example, boreal *Picea glauca* and *Abies balsamea* reach their southern range limits near the study sites but extend north > 1000 km to the arctic treeline. Conversely, range limits for the

Table 1 Temperature (°C) during the growing season (May 1–September 30) for each of the 3 years of the study

	Cloquet	Ely
Temperature 2012	15.6 ± 8.3	16.6 ± 9.0
Temperature 2013	15.1 ± 8.4	15.8 ± 8.2
Temperature 2014	14.8 ± 8.1	15.4 ± 7.9
Long-term average temperature	16.2 ± 5.0	14.2 ± 5.3
Rainfall 2012	980.2	661.7
Rainfall 2013	852.4	717.3
Rainfall 2014	900.9	674.4
Long-term average rainfall	805.8 ± 124.2	663.6 ± 156.9

We compare this to the long-term average between 1992 and 2012. Total precipitation during the growing season is also displayed as cumulative rainfall (mm) and compared to the long-term average for the same period (1992–2012)

temperate species in the study typically extend from the boreal–temperate ecotone south for > 1000 km. It is only within the relatively narrow ecotone that these temperate and boreal tree ranges overlap or approach one another. Exotic species are defined as non-native to North America [*Berberis* spp. (primarily *B. thunbergii*, includes *B. communis*), *Lonicera* spp. (includes *L. morrowii*, *L. tatarica*, and hybrids between these two species), and *Rhamnus cathartica*]. *Fagus grandifolia* is the only species not found in Minnesota, reaching its western limit ∼ 100 km to the east of the Cloquet site in Wisconsin. *Tsuga canadensis* has a similar western range margin, though disjunct populations exist in Minnesota. *Robinia pseudoacacia* is a temperate tree species, native to the southcentral U.S. It has rapidly expanded its range throughout the eastern U.S. through intentional and unintentional human introductions. Temperate *Quercus alba* and *Carya cordiformis* reach their northern range limits at the southern edge of the boreal–temperate ecotone in Minnesota.

Seeds were planted into randomly selected species-specific grid cells within larger seedling subplots to allow us to map and track seedlings over time. Larger seeds (*Quercus* sp. and *C. cordiformis*) were buried to ∼ 3-cm depth while all smaller seeds were sprinkled on the surface. Seedling subplots were cleared of aboveground vegetation at the time of plot establishment in May 2012. During each subsequent seedling census, these beds were cleared of loose brush but otherwise left unweeded. As mentioned above, at the time of planting, seedling plots were in approximately 40–60% full sunlight conditions. Research sites were

fenced to exclude large herbivores [moose (*Alces alces*) and white-tailed deer (*Odocoileus virginianus*)], but not small seed predators (e.g., rodents and birds). We used seed size, germination rates from a previous experiment (Fisichelli et al. 2014b), and seed availability to determine how many seeds to plant per species. To account for differences in the amount of seed planted, all emergence rates were recorded as a percentage of the number of seeds planted and survival rates were recorded as percentage of emerged seedlings (Table 1). Seeds were primarily collected from central Minnesota and neighboring Great Lakes states, with *Prunus serotina* and *F. grandifolia* collected in Iowa and *R. pseudoacacia* in Kentucky.

We recorded the number of newly emerged seedlings and surviving seedlings during 14 separate censuses: in June 2012, July 2012, August 2012, September 2012, May 2013, June 2013, July 2013, August 2013, September 2013, May 2014, June 2014, July 2014, August 2014, and September 2014. We tagged emergent seedlings at each survey to avoid confusing one, for example, observed in June 2012 that died before the July 2012 survey, with one that was first observed in July 2012.

Due to herbivory and high rates of mortality, it was impossible to track and measure the same seedlings for growth measurements over time. Instead, we measured seedling height and number of leaves for a random subset of three seedlings per species per plot for seven separate censuses: in August 2012, September 2012, July 2013, September 2013, June 2014, July 2014, and August 2014. These measurements are a

Table 2 This study included planted seeds from 18 species of boreal trees, exotic shrubs, and temperate trees

Latin name	Common name	Range	Seeds per plot 2012	Seeds per plot 2013	Seeds per plot 2014	Ave emergence (2012–2014) (%)	Seed prep	Germination from (Fisichelli et al. 2014b)
*Abies balsamea**	Balsam fir	Boreal	39	57	114	6.1	Moist strat. + scar.	4.2%
Betula papyrifera	Paper birch	Boreal	45	63	126	0.2	Moist strat.	5.0%
Picea glauca	White spruce	Boreal	45	63	126	0.6	Moist strat.	27.5%
*Pinus banksiana**	Jack pine	Boreal	42	60	120	1.6	Not needed	67.5%
*Berberis**	Barberry	Exotic	42	54	0	5.4	Moist strat.	NA
Lonicera	Honey-suckle	Exotic	15	0	0	0.0	Moist strat.	NA
*Rhamnus cathartica**	Buckthorn	Exotic	42	54	60	7.1	Moist strat. + scar.	45%
Acer saccharum	Sugar maple	Temperate	40	60	90	0.9	Moist strat.	27.5%
Carya cordiformis	Bitternut Hickory	Temperate	25	25	75	0.7	Moist strat.	20.8%
Fagus grandifolia	American beech	Temperate	30	45	0	0.0	Not needed	53.1%
*Pinus resinosa**	Red pine	Temperate	42	60	120	1.7	Not needed	65%
*Pinus strobus**	White pine	Temperate	42	60	120	1.9	Moist strat.	27.5%
Prunus serotina	Black cherry	Temperate	45	63	126	0.2	Moist strat. + scar.	2.5%
*Quercus macrocarpa**	Bur oak	Temperate	25	15	30	1.3	Moist strat.	68.8%
Quercus rubra	Red oak	Temperate	25	15	30	0.9	Moist strat.	62.5%
Querus alba	White oak	Temperate	25	15	0	0.2	Not needed	45.8%
*Robinia pseudoacacia**	Black locust	Temperate	39	54	108	1.5	Moist strat. + scar.	7.5%
Tsuga canadensis	Eastern hemlock	Temperate	45	54	108	0.5	Moist strat.	22.5%

We selected species based on native range proximity to the boreal–temperate ecotone in Minnesota, USA and planted seeds according to expected germination rates and seed availability for each species. We prepped seeds prior to planting by either cold moist stratification (Moist strat.) and/or scarification (scar.). We also show germination rates using the same seed set from a preliminary growth chamber study (Fisichelli et al. 2014a, b). Those species with higher germination rates included in the supplementary analyses are indicated with an asterisk

snapshot of seedling size per species per plot, but are not used to track growth of individuals over time.

Statistical analyses

We analyzed the effects of three temperature treatments (categorical), drought (categorical), species range (categorical: boreal, temperate, exotic), and all first- and second-order interactions on seedling percent emergence, post-emergence percent survival, seedling height, and number of leaves using mixed-effects models. Each model was designed to account for autocorrelation of variables in space (due to the blocked design) and autocorrelation of variables in time (we re-measured these plots up to 14 times) using random effects in the mixed-effects model framework.

Additional species-specific explanatory variables, e.g., distance to range edge, were not possible due to small sample sizes of emerged stems. For most analyses, species were grouped by range to test for interactive treatment effects (e.g., three-way warming × drought × range interaction).

To assess species-specific total seedling percent emergence at the end of the study, we summed emergence for each species in each grid cell in each plot for each cohort (2012, 2013, 2014) separately. We therefore included a nested random effect for site (Ely and Cloquet), block (three blocks per site), and plot (three plot measurements per species per cohort based on the grid cell layout). Based on the physical spatial hierarchy of these factors, plot was nested in block, which was nested in site. We also included a separate time-based random effect for cohort, as we planted three separate cohorts of most of the 18 species for each of the 3 years of this study. Thus, we ran a mixed-effects model using seedling percent emergence as the response factor. We included fixed effects for temperature, drought, and species identity (ID) and random effects for plot nested in block nested in site and time-based cohort.

Due to low overall rates of emergence, none of the other response variables (survival or growth) could be assessed in terms of species-specific responses. We instead assessed emergence, post-emergence survival, and growth (seedling height and leaf number) in terms of larger range group responses (temperate, boreal, and exotic), and included a random effect for cohort nested in species ID. In this framework, each individual species accounts for random variation in average responses of each range-based group. Further, because there were three cohorts of each species planted, this time-based information also accounts for random variation in species responses between years. By including random factors for this nested variation, we can focus on the average responses of groups. Since survival (13 measures), height (7 measures), and leaf number (7 measures) were measured multiple times, we further include a random effect for sampling date in these models.

All statistical models followed the following general form: Y = Temp × Drought × SpRange, random = site/block/plot, random = SpID/cohort. Where sample sizes were large enough (emergence) to assess individual species effects, SpRange was replaced as a fixed effect in the model with SpID, and

SpID was removed from the second random effect term in the model.

Due to unequal representation of conifer and broadleaf species per species-range group (temperate vs. boreal), we also ran an identical model assessing the separate effects of temperate conifer, temperate broadleaf, boreal conifer, boreal broadleaf, and exotic species on seedling emergence and survival. We did not have large enough sample sizes to analyze this model in terms of seedling height or number of leaves.

Due to low emergence and early survival rates overall, we also ran identical analyses for a subset of those eight species that emerged at > 10% across the entire experiment (Figs. S1–S2). These results were not substantively different from the full dataset we present here. Further, because lower emergence and early survival of many species in these temperate forests will likely affect longer term trends in species composition, we focus on the main results of the complete dataset.

In order to assess changes in biodiversity (i.e., species richness) of each plot over time, we counted the number of species surviving in each plot at the end of each year. We then used a mixed-effects model to assess how the fixed effects of temperature, drought, and seedling age affected species richness of plots. We included random effects for plot nested in block nested in site (as above) and cohort (to account for random interannual variation at the site).

Results

Emergence

Seedling emergence was generally low, with most species showing < 1.5% overall emergence averaged over 3 years (Fig. 1). Species with higher average emergence included boreal *A. balsamea* (6%) and *Pinus banksiana* (1.5%), non-native *Berberis* spp. (5.5%) and *R. cathartica* (7%), and temperate *Pinus resinosa* (1.5%) and *Pinus strobus* (2%). Warming had a generally negative effect on emergence ($F_{2,32} = 8.15$, $P < 0.0001$), but a particularly negative effect on the high emergence species mentioned above (warming × species interaction, $F_{34,5350} = 3.76$, $P < 0.0001$, Table 3). Drought only reduced emergence for some species (drought × species interaction, $F_{17,5350} = 1.9$, $P = 0.02$). For example, *A.*

balsamea was further reduced by $\sim 40\%$ in drought plots (Fig. S1). Other species showed low emergence overall and thus responses to treatments were not detected at the species level.

At the species-range level (boreal, exotic, and temperate), we found that warming reduced boreal seedling emergence, especially when paired with drought conditions (warming × drought × range interaction, Fig. 2). Boreal seedling emergence was more than 60% lower in warmed plots that also experienced drought conditions than in ambient temperature and precipitation plots. This was mostly driven by strong reductions in boreal conifer seedling emergence (Fig. S2). Exotic species emerged almost 50% less in the warmer plots (+ 1.7 °C), and this was further reduced by 50% in the warmest plots (+ 3.4 °C) that also experienced drought conditions (Fig. 2). Temperate broadleaf seedling emergence was

variable and unaffected by temperature or drought, whereas temperate conifers emerged less in warmer plots (Fig. S2, Table S3). Temperate seedling emergence overall was unaffected by drought or temperature when lumped into a single range group (Fig. 2).

Survival

Seedling survival varied by the main effects of species-range group and warming treatment, but not through drought or interactions (Table 4, Fig. 2). Warming significantly reduced survival of all groups by an average of 15% per census period ($F_{2,26} = 5.51$, $P = 0.01$). This was driven mostly by increased conifer mortality (both boreal and temperate) in response to warming (Fig. S2). Survival of exotic seedlings was highest (47%), followed by temperate

Fig. 1 Species-specific seedling emergence (proportion of seeds planted), based on three cohorts (2012, 2013, and 2014). There was a significant effect of warming on individual species emergence rates (Table 2). Error bars represent the standard error of the mean (averaged across years and plots as calculated using our mixed-effects model). Significant difference between groups (within a single panel), based on post hoc Student's *t* tests, are shown with an asterisk. Species are in alphabetical order by range groups (boreal, exotic, temperate)
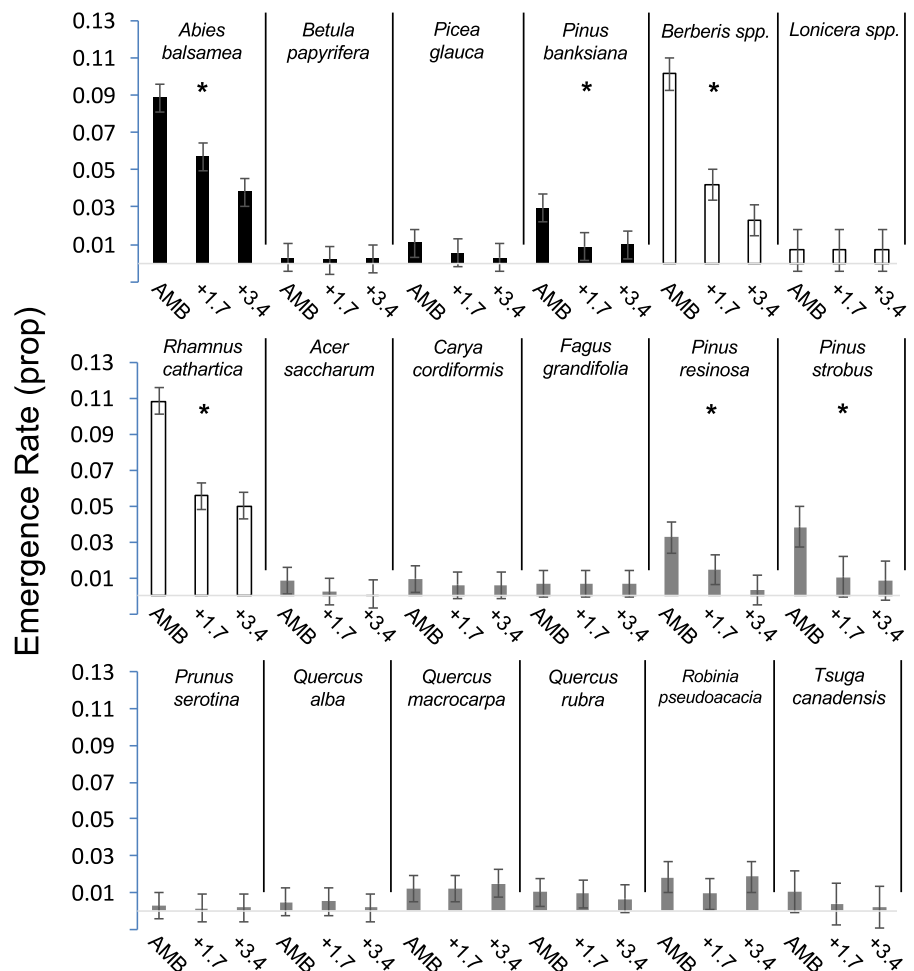
Table 3 Seedling emergence response to warming treatment, drought treatment, species identity, and all first- and second-order interactions ($r^2 = 0.17$)

Fixed effect	d.f.	F ratio	P value
Warming	2, 32	8.15	**< 0.0001***
Drought	1, 32	3.14	0.09
Species ID	17, 5350	36.6	**< 0.0001***
Warming × drought	2, 32	0.66	0.52
Warming × species	34, 5350	3.76	**< 0.0001***
Drought × species	17, 5350	1.9	**0.02***
Warming × drought × species	34, 5350	1.34	0.09

Analyses use three cohorts (2012, 2013, and 2014) and ANOVA tables of fixed effects are from linear mixed-effects models. This model also included a random effect for block and plot ID nested within site (Ely vs. Cloquet), as well as a random effect for cohort (2012, 2013, 2014)

Significant differences are indicated in bold and with an asterisk
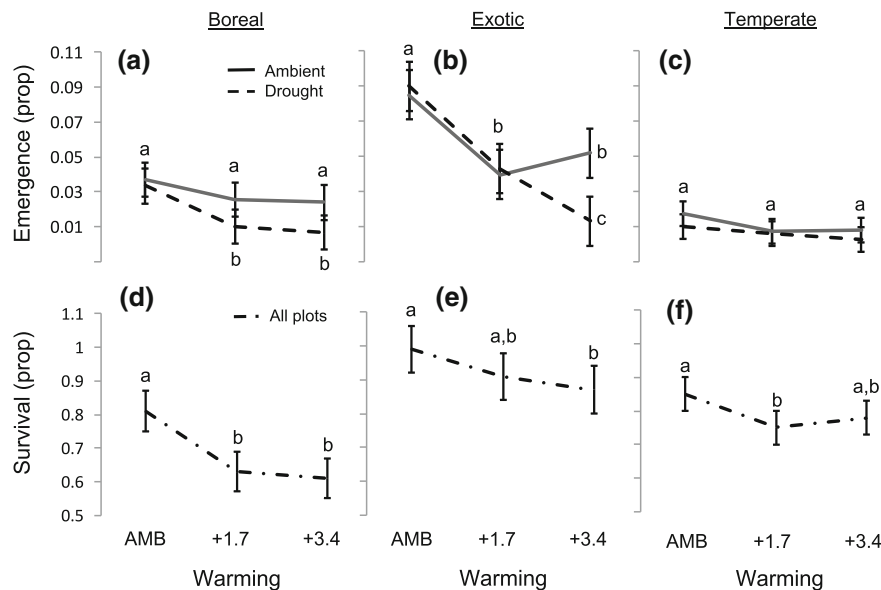
Fig. 2 Species-range group emergence and survival. Emergence rates for three cohorts (2012, 2013, and 2014) were measured for **a** boreal species, **b** exotic species, and **c** temperate species. Seedling emergence in ambient precipitation conditions is shown with a solid line, while emergence in drought conditions is shown with a dashed line. Seedling survival was not affected by drought and thus only one line, averaged across precipitation treatments, is shown for seedling survival panels (**d**–**f**). Error bars represent standard error of the mean (averaged over time and plot according to our statistical model). Post hoc Student's *t* tests were also performed. Significant differences between groups (within a single panel) are shown with lower case letters

seedlings (35%), and boreal seedling survival was lowest (20%, Fig. 2).

Growth

Warming significantly increased average temperate seedling height and average leaf production in drought conditions (Fig. 3), but had no effect on boreal and exotic species height or leaf production under any conditions (warming × drought × range interactions, Table 4).

Table 4 Seedling emergence ($r^2 = 0.27$), survival ($r^2 = 0.37$), leaf number ($r^2 = 0.29$), and height responses ($r^2 = 0.19$) to warming treatment, drought treatment, range of seedling species, and all first- and second-order interactions, based on three cohorts (2012, 2013, and 2014) and up to 3 years of data

Fixed effect	Emergence			Survival			Num leaves			Seedling height		
	d.f.	F ratio	P value	d.f.	F ratio	P value	d.f.	F ratio	P value	d.f.	F ratio	P value
Warming	2, 37	21.2	**< 0.0001***	2, 26	5.51	**0.01***	2, 32	0.23	0.79	2, 30	1.29	0.29
Drought	1, 37	5.41	**0.03***	1, 26	3.03	0.09	1, 33	0.20	0.66	1, 30	0.28	0.60
Species range	2, 46	6.02	**0.005***	2, 41	5.74	**0.006***	2, 35	0.04	0.96	2, 46	0.54	0.59
Warming × drought	2, 37	2.70	0.08	2, 26	1.66	0.21	2, 32	1.73	0.19	2, 30	0.19	0.83
Warming × range	4, 5397	18.0	**< 0.0001***	4, 4876	1.90	0.11	4, 1588	0.49	0.74	4, 2223	3.70	**0.005***
Drought × range	2, 5397	1.63	0.20	2, 4898	0.19	0.83	2, 1566	0.50	0.60	2, 2223	0.81	0.44
Warming × drought × range	4, 5397	4.76	**0.0008***	4, 4896	1.53	0.19	4, 1560	5.80	**0.0001***	4, 2223	3.66	**0.006***

ANOVA tables of fixed effects are from linear mixed-effects models. These models also included a random effect for block and plot ID nested within site (Ely vs. Cloquet), as well as a random effect for cohort (2012, 2013, 2014) nested within species identity (to allow for generalized results according to species range)

Significant differences are indicated in bold and with an asterisk

Species richness

Plot-level species richness was ≤ 8 species. Plot-level species richness was reduced by warming ($F_{2,41} = 15.6$, $P < 0.0001$) and species richness decreased over time ($F_{2,160} = 9.51$, $P = 0.0001$, Fig. 4). Species richness patterns held across cohorts and seedling ages (Fig. S3). No other factor had a significant effect on species richness of the plots. We saw an average of three species lost from the seedling layer of the plots that had been experimentally warmed in the first year. Most of this reduction in species richness occurred with just 1.7 °C warming. These effects were compounded over time, resulting in an average species richness of just two species in the seedling layer of the warmest plots after 3 years (whereas un-warmed plots still had an average of five species).

Discussion

Here, we show that richness of seedlings in open-canopy forest plots is reduced by warming. This loss of richness follows just + 1.7 °C warming—a conservative level of warming predicted by the end of the twenty-first century (Stocker et al. 2013). Contrary to our first hypothesis, we found that this trend was due to the loss of boreal species at the emergence and early survival life history stages, but this was paired with a lack of compensatory emergence or survival by temperate or exotic species. In fact, the only temperate species that germinated at relatively high rates (*P. resinosa, P. strobus, Quercus macrocarpa, R. pseudoacacia,* and *T. canadensis*) were either negatively or neutrally affected by warming (in terms of emergence).

In central and northern Minnesota, boreal species are at or near the southern edge of their range, and thus even + 1.7 °C degrees of warming may have pushed these species beyond their environmental tolerances. Negative responses of exotic species to warming were less expected, failing to support hypothesis (2). The two species that drove these negative exotic responses, *R. cathartica* and *Berberis* spp., are considered invasive in the study region and are near their northern range limits, thus positive responses were expected, but were not found. Drought did exacerbate negative emergence responses for boreal and exotic species but

enhanced temperate species growth, potentially due to increased pathogenic infection in higher rainfall environments (Ibañez et al. 2007) and thus providing mixed support for hypothesis (3). Further, temperate species growth responses to warm and dry conditions happened after the initial negative effects of warming on temperate seedling emergence. Thus, even if warming encourages increased growth of warm-adapted temperate species (Reich et al. 2015) or allows for rapid acclimatization of larger individuals (Reich et al. 2016), this may be outweighed by the negative effects of warming (and drought) on the sensitive germination, emergence, and early survival stages of seeds and seedlings in their first 3 years of establishment.

Emergence and survival

Warming had negative or neutral effects on seedling emergence and survival for all species and groups growing in our experiment. This was particularly true for boreal and exotic species and for conifers from all groups. Past observational field studies and designed experiments have shown that boreal saplings tend to

grow poorly in warmer plots at the temperate–boreal ecotone due to reduced photosynthetic rates (Fisichelli et al. 2012; Reich et al. 2015). Warmer temperatures increased photosynthetic rates of temperate species in this past work (Reich et al. 2015) and warmer temperatures slightly increased temperate species growth in our study. These relative increases in temperate species may mean that boreal species were reduced due to increased competitive pressures from temperate species (not just physiological range limits). Either way, our work supports the idea that the boreal regeneration bottleneck may start as early as seed germination and seedling emergence.

Importantly, we also show that warming has negative effects on exotic species emergence and survival at these sites. While past work has theorized that increased disturbance or rapidly changing environmental conditions may favor the establishment and growth of exotic species (Dukes and Mooney 1999; Bellard et al. 2013), experimental evidence for this has been varied (Hulme 2016). In areas where climate change factors create less favorable habitats, exotic species may be affected in similarly negative ways to native species (Sorte et al. 2012). Here, warming had
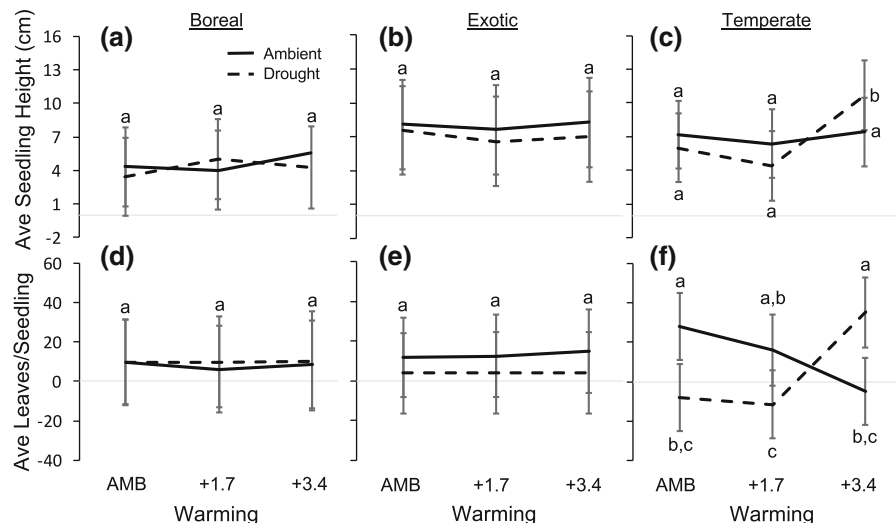
Fig. 3 Species group seedling height (a–c) and number of leaves per seedling (d–f). Measurements are from a random subset of seedlings growing in each plot in 2012, 2013, and 2014. Temperature had a positive effect on temperate seedling height and leaf number, but only when paired with drought (Table 3). Error bars represent standard error of the mean (averaged across time and plot according to our statistical

model). Post hoc Student's t tests were also performed. Significant differences between groups (within a single panel) are shown with lower case letters (e.g., for panel c, temperate seedlings were taller in the warmed drought plots than in any of the other treatment groups but they were not compared with any of the other panels for this display)
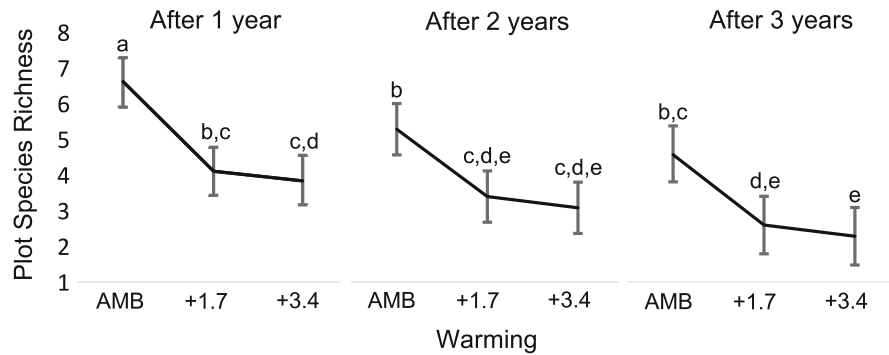
Fig. 4 Plot-level species richness, based on species identity of surviving seedlings in each plot at each census. Species richness was lower in warmed plots and lowest at the final census. Error bars represent standard error of the mean. Post-hoc Student's *t* tests were also performed. Significant differences between groups are shown with lower case letters. Biodiversity trends for individual cohorts match these trends and are shown in Fig. S3

an overall negative effect on exotic species emergence and survival, and for seedling emergence this was strongly exacerbated by drought. Importantly, both species that drive these trends (*R. cathartica* and *Berberis* spp.) are invasive within, and south of, the study region (Knight et al. 2007), and thus were expected to respond positively to warming. However, ability to invade may be more closely related to phenological responses (Fridley 2016) that were not measured here. It is currently unknown what limits *R. cathartica* at the northern edge of its range (Kurylo et al. 2007).

Post hoc comparisons demonstrate that temperate seedlings were largely unaffected by warming, but this was mostly due to variable results for temperate broadleaf species (Fig. S2) and significantly negative results for temperate conifers. Notably, some species-specific emergence responses to warmer temperatures do not follow the same pattern as in a similar experiment using growth chambers (Fisichelli et al. 2014b). In the growth chamber experiment, several species showed positive emergence responses to warming (e.g., *A. balsamea*, *R. cathartica*, *P. resinosa*, and *P. strobus*). These differences may be due to the differences in microhabitat conditions in the field versus the lab, and/or the much lower rates of emergence that occurred under field conditions overall (average of $< 5\%$ in the field vs. up to $\sim 90\%$ in the growth chamber experiment). For those species (mostly broadleaf) that emerged at higher rates in the growth chamber, some of the lower emergence (or early seedling survival) trends under field conditions

may have been related to seed predation (Bell and Clark 2016). Importantly, our census regime allowed us to capture new germination in the field, but if a species germinated and then died before our monthly census, this would be captured as merely failure to germinate. Thus, we suspect that lower emergence rates were also a reflection of low seedling survival rates due to herbivory from small mammals and pathogen infection. This may help explain the strong conifer (smaller seed) results, in comparison with highly variable broadleaf (larger seed) trends.

For species like *R. pseudoacacia*, responses to warming were somewhat neutral and nearly identical in field versus growth chamber comparisons. But for those species that emerged at similar rates in the field and in the growth chamber (e.g., *A. balsamea* and *R. cathartica*), but reversed their response to warming, this result is unexpected. In reality, and similar to our field experiment, the interactions between warming and moisture are correlated and will be dynamic in both space and time. For a germinating seed, the combined effects of temperature, and consequences for soil moisture and vapor pressure deficit will strongly affect survival (Wright et al. 2015; Cowles et al. 2016). While future projections of seasonal precipitation in the region show anywhere from a 17% decrease to an 18% increase in long-term average precipitation and uncertainty in spring precipitation predictions (Kunkel et al. 2013), recent regional ensemble climate models predict that overall soil moisture will be decreased even if seasonal rainfall increases (Seager et al. 2014). These models

demonstrate that increased temperatures will increase water losses due to evapotranspiration faster than spring precipitation will increase, even in the more severe model predictions. In our experiment, we intentionally manipulate both the direct (temperature) and indirect (soil moisture) effects of climate warming. Past work at this site has shown that soil moisture is indeed slightly lower (0.2–0.5 cm^3 H$_2$0/cm^3 soil) in warmed versus ambient plots (Rich et al. 2015) and thus simulates a more realistic warming scenario. Furthermore, climate extremes may be more important than climate averages for determining young seedling responses to climate change (Wright et al. 2014, 2015; Fisichelli et al. 2014a; Jentsch et al. 2016). Thus, manipulating the climate average (day/night temperatures in a growth chamber), and associated changes in moisture and humidity, may not have been as influential as the changes that occur during a particularly hot or dry period during the growing season: a 1.7 °C increase over ambient conditions during a period of unseasonably high temperatures or low soil moisture in the field (Rich et al. 2015) could be enough to result in immediate seedling mortality.

Growth

Temperate seedlings in our study grew more (taller and more leaves) when growing in warmer conditions. Reich et al. (2015) showed similar results for 3- to 5-year-old saplings growing in the same experiment: saplings near the southern edge of their range (boreal species) were negatively affected by warming, while saplings near the northern edge of their range (temperate species) were positively affected by warming. This was driven by increased photosynthetic rates in warmer plots for those species growing near the northern edge of their range. This may also be related to increased plasticity in hydraulic functioning, which may be more common in temperate species (McCulloh et al. 2016). However, our results demonstrate that at this earlier seedling stage, the positive effects of warming on growth are highly variable and lost under ambient rainfall conditions: the positive effects of + 3.4 °C warming on growth were only significant when seedlings were grown in drought plots (∼ 42% of ambient water removed). This is somewhat counterintuitive as warmer conditions are often considered favorable, as long as they are not paired with increased evapotranspiration and drought. Fisichelli et al. (2013)

demonstrated similar results in terms of emergence and leaf out: seedlings growing in the warmer, wetter plots emerged less and leafed out later. We predict that the negative effects of warming + moisture on early seedling growth and emergence may be due to increased pathogen abundance or infection in warmer, wetter conditions (Ibañez et al. 2007).

Biodiversity loss and concluding remarks

Warming temperatures and drought can have strong influences on seedling emergence, survival, and growth. At the boreal–temperate ecotone, climate change and more extreme conditions may cause a seedling-driven biodiversity bottleneck. Specifically, the inability of temperate species to fill the voided niches of boreal species would have long-term implications for forest composition, structure, and function. This research only focuses on a small portion of the plant life cycle and climate change impacts on other life history stages may enhance or counteract the trends found here. For example, changes in overstory longevity, seed production, seed dispersal, and disturbance regimes will also play roles in forest biodiversity. Further field-based studies are needed to examine these dynamics at ecotonal boundaries. However, if the observed patterns reflect broader trends, loss of forest diversity at ecotonal boundaries will likely cascade to further negative effects for these ecosystems (Lavorel 1999). Comprehensively assessing these changes in response to warming and drought, at every life history stage, is a priority for future research.

Author contributions AJW designed the seedlings study, collected the data, analyzed the data, and wrote the first draft of the manuscript; NAF helped design and conceptualize the seedling study and provided extensive guidance on the manuscript; CB, RR, RM, and PBR conceptualized and designed the B4warmed experiment; and KR and AS collected the data. All authors provided feedback on multiple versions of the manuscript.

References

Beckage B, Osborne B, Gavin DG, Pucko C, Siccama T, Perkins T (2008) A rapid upward shift of a forest ecotone during 40 years of warming in the Green Mountains of Vermont. Proc Natl Acad Sci 105:4197–4202. https://doi.org/10.1073/pnas.0708921105

Bell DM, Clark JS (2016) Seed predation and climate impacts on reproductive variation in temperate forests of the southeastern USA. Oecologia 180:1223–1234. https://doi.org/10.1007/s00442-015-3537-6

Bellard C, Thuiller W, Leroy B, Genovesi P, Bakkenes M, Courchamp F (2013) Will climate change promote future invasions? Glob Change Biol 19:3740–3748. https://doi.org/10.1111/gcb.12344

Bonner FT, Karrfalt RP (2008) The woody plant seed manual. USDA Forest Service Agriculture Handbook 727

Buckley YM, Catford J (2015) Does the biogeographic origin of species matter? Ecological effects of native and non-native species and the use of origin to guide management. J Ecol 104:4–17. https://doi.org/10.1111/1365-2745.12501

Cowles JM, Wragg PD, Wright AJ, Powers JS, Tilman D (2016) Shifting grassland plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity. Glob Change Biol 22:741–749. https://doi.org/10.1111/gcb.13111

Dukes J, Mooney H (1999) Does global change increase the success of biological invaders? Trends Ecol Evol 14:135–139

Fisichelli N, Frelich LE, Reich PB (2012) Sapling growth responses to warmer temperatures "cooled" by browse pressure. Glob Change Biol 18:3455–3463. https://doi.org/10.1111/j.1365-2486.2012.02785.x

Fisichelli NA, Frelich LE, Reich PB (2013) Climate and interrelated tree regeneration drivers in mixed temperate–boreal forests. Landsc Ecol 28:149–159. https://doi.org/10.1007/s10980-012-9827-z

Fisichelli N, Vor T, Ammer C (2014a) Broadleaf seedling responses to warmer temperatures "chilled" by late frost that favors conifers. Eur J Forest Res 133:587–596. https://doi.org/10.1007/s10342-014-0786-6

Fisichelli N, Wright A, Rice K, Mau A, Buschena C, Reich P (2014b) First-year seedlings and climate change: species-specific responses of 15 North American tree species. Oikos 123:1331–1340. https://doi.org/10.1111/oik.01349

Fridley JD (2016) Extended leaf phenology and the autumn niche in deciduous forest invasions. Nature 485:359–362. https://doi.org/10.1038/nature11056

Hulme PE (2016) Climate change and biological invasions: evidence, expectations, and response options. Biol Rev. https://doi.org/10.1111/brv.12282

Ibañez I, Clark J, LaDeau S, Lambers J (2007) Exploiting temporal variability to understand tree recruitment response to climate change. Ecol Monogr 77:163–177

Isbell F, Craven D, Connolly J, Loreau M, Schmid B, Beierkuhnlein C, Bezemer TM, Bonin C, Bruelheide H, De Luca E, Ebeling A, Griffin JN, Guo Q, Hautier Y, Hector A, Jentsch A, Kreyling J, Lanta V, Manning P, Meyer ST, Mori AS, Naeem S, Niklaus PA, Polley HW, Reich PB, Roscher C, Seabloom EW, Smith MD, Thakur MP, Tilman D, Tracy BF, Van Der Putten WH, Van Ruijven J, Weigelt A, Weisser WW, Wilsey B, Eisenhauer N (2015) Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature 526:574–577. https://doi.org/10.1038/nature15374

Jentsch A, Kreyling J, Beierkuhnlein C (2016) A new generation of climate change experiments: events, not trends. Front Ecol Environ 5(6):315–324

Knight KS, Kurylo JS, Endress AG, Stewart JR, Reich PB (2007) Ecology and ecosystem impacts of common buckthorn (Rhamnus cathartica): a review. Biol Invasions 9:925–937. https://doi.org/10.1007/s10530-007-9091-3

Kunkel KE, Stevens LE, Stevens SE, Sun L, Part EJ (2013) Regional climate trends and scenarios for the US national climate assessment. Part 3. Climate of the Midwest U.S. NOAA Tech. Rep. NESDIS 142–3

Kurylo JS, Knight KS, Stewart JR, Endress AG (2007) Rhamnus cathartica: native and naturalized distribution and habitat preferences. J Torrey Bot Soc 134:420–430

Lavorel S (1999) Ecological diversity and resilience of Mediterranean vegetation to disturbance. Divers Distrib 5:3–13

McCulloh KA, Petitmermet J, Stefanski A, Rice KE, Rich RL, Montgomery RA, Reich PB (2016) Is it getting hot in here? Adjustment of hydraulic parameters in six boreal and temperate tree species after three years of warming. Glob Change Biol. https://doi.org/10.1111/gcb.13323

Miller JR, Bestelmeyer BT (2016) What's wrong with novel ecosystems, really? Restor Ecol. https://doi.org/10.1111/rec.12378

Niinemets Ã (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For Ecol Manag 260:1623–1639. https://doi.org/10.1016/j.foreco.2010.07.054

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42

Parmesan C, Gaines S, Gonzalez L, Kaufman DM, Kingsolver J, Peterson AT, Sagarin R (2005) Empirical perspectives on species borders: from traditional biogeography to global change. Oikos 108:58–75

Pereira HM, Leadley PW, Proenca V, Alkemade R, Scharlemann JPW, Fernandez-Manjarres JF, Araujo MB, Balvanera P, Biggs R, Cheung WWL, Chini L, Cooper HD, Gilman EL, Guenette S, Hurtt GC, Huntington HP, Mace GM, Oberdorff T, Revenga C, Rodrigues P, Scholes RJ, Sumaila UR, Walpole M (2010) Scenarios for global biodiversity in the 21st century. Science. https://doi.org/10.1126/science.1196624

Pucko C, Beckage B, Perkins T, Keeton WS (2011) Species shifts in response to climate change: individual or shared responses? J Torrey Bot Soc 138:156–176. https://doi.org/10.3159/TORREY-D-10-00011.1

Reich PB, Sendall KM, Rice K, Rich RL, Stefanski A, Hobbie SE, Montgomery RA (2015) Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. Nat Publ Group 5:148–152. https://doi.org/10.1038/nclimate2497

Reich PB, Sendall KM, Stefanski A, Wei X, Rich RL (2016) Boreal and temperate trees show strong acclimation of

respiration to warming. Nature. https://doi.org/10.1038/nature17142

Rich RL, Stefanski A, Montgomery RA, Hobbie SE, Kimball BA, Reich PB (2015) 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. https://doi.org/10.1111/gcb.12855

Seager R, Neelin D, Simpson I, Liu H, Henderson N, Shaw T, Kushnir Y, Ting M, Cook B (2014) Dynamical and thermodynamical causes of large-scale changes in the hydrological cycle over north america in response to global warming. J Clim 27:7921–7948. https://doi.org/10.1175/JCLI-D-14-00153.1

Sorte CJB, Ibáñez I, Blumenthal DM, Molinari NA, Miller LP, Grosholz ED, Diez JM, D'antonio CM, Olden JD, Jones SJ, Dukes JS (2012) Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. Ecol Lett 16:261–270. https://doi.org/10.1111/ele.12017

Stocker TF, Qin D, Plattner GK, Alexander LV, Allen SK, Bindoff NL, Breon FM, Church JA, Cubasch U, Emori S, Forster P, Friedlingstein P, Gillett N, Gregory JM, Hartmann DL, Jansen E, Kirtman B, Knutti R, Krishna Kumar K, Lemke P, Marotzke J, Masson-Delmotte V, Meehl GA, Mokhov II, Piao SL, Ramaswamy V, Randall D, Rhein M, Rojas M, Sabine C, Shindell D, Talley LD, Vaughan DG, Xie SP (2013) Climate change 2013: The physical science basis. Cambridge University Press, Cambridge

Thomas CD, Cameron A, Green RE, Bakkenes M (2004) Extinction risk from climate change. Nature 427:145–148. https://doi.org/10.1038/nature02121

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P (2011) Climate change and plant regeneration from seed. Glob Change Biol 17:2145–2161. https://doi.org/10.1111/j.1365-2486.2010.02368.x

Walther GR (2010) Community and ecosystem responses to recent climate change. Philos Trans R Soc Lond B Biol Sci 365:2019–2024. https://doi.org/10.1098/rstb.2010.0021

Williams J, Post D, Cwynar L, Lotter A, Levesque A (2002) Rapid and widespread vegetation responses to past climate change in the North Atlantic region. Geology 30:971–974

Wright AJ, Schnitzer SA, Reich PB (2014) Living close to your neighbors—the importance of both competition and facilitation in plant communities. Ecology. https://doi.org/10.1890/13-1855.1

Wright A, Schnitzer SA, Reich PB (2015) Daily environmental conditions determine the competition–facilitation balance for plant water status. J Ecol 103:648–656. https://doi.org/10.1111/1365-2745.12397