

The role of biotic interactions in altering tree seedling responses to an extreme climatic event

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Abstract

Questions: We addressed two poorly understood aspects of plant response to climate change: the impact of extreme climatic events and the mediating role of biotic interactions, through a study of heatwave effects on tree seedling survival rates and ability of the tree canopy to alter seedling responses.

Location: Mountain belt of the northern French Alps (Maurienne Valley).

Methods: The survival rates of two seedling cohorts from four tree species (*Abies alba*, *Acer pseudoplatanus*, *Fraxinus excelsior* and *Picea abies*) were measured during both the 2003 European heatwave and an average summer (2004) in deciduous broadleaf mountain forests. Seedlings were transplanted into two soil moisture conditions, and in experimental gaps or under the tree canopy.

Results: The heatwave strongly decreased tree seedling survival rates, while there was an important species-specific mediating role of biotic interactions. In the wettest conditions, the tree canopy strongly increased survival of *Abies*, buffering the negative impact of the heatwave. In contrast, in the driest conditions, the tree canopy decreased survival of *Picea* and *Acer*, amplifying the negative impact of the heatwave. We found evidence of increasing soil water stress in the understorey of the driest community, but further studies including vapour pressure deficit measurements are needed to elucidate the driving mechanism of facilitation.

Conclusions: The high species specificity of the mediating role of biotic interactions and its variation along stress

gradients leads to questions on our ability to predict large-scale responses of species to climate changes.

Keywords: Climatic changes; Competition; Facilitation; Heatwave; Survival

Introduction

Human activities have already had discernible effects on the Earth's climate, with global warming of 0.6°C during the past century (Jones et al. 2001; Brohan et al. 2006) and increased climate variability (Easterling et al. 2000; Beniston & Stephenson 2004). Climate models predict that these trends will continue in the future. The experts consensually expect a global increase of 3.0°C for a doubling in concentration of atmospheric CO₂ by the end of this century (Kerr 2004), and an increase in the frequency and intensity of extreme climatic events (ECE) such as heatwaves (Meehl & Tebaldi 2004; Meehl et al. 2007).

There is a considerable amount of research on the effects of human-induced environmental change, and on climate changes, in particular, on communities and ecosystems. Most of this research has focused on current and future global warming (see e.g. Parmesan & Yohe 2003; Thuiller et al. 2005; Walker et al. 2006). However, we are now aware that ECE may have the strongest impacts on terrestrial ecosystems (Ciais et al. 2005) because of their effects on the survival of species (Milbau et al. 2005). As a result, there is increasing interest in the biological and ecosystem effects of ECE (MacGillivray et al. 1995; Buckland et al. 1997; Rolland et al. 2000;

White et al. 2000; Mueller et al. 2005; Gitlin et al. 2006).

Climate changes are known to affect species through regional and local filters (Davis et al. 1998a; Guisan & Thuiller 2005). Biotic interactions are recognized as one of the most important local filters for community composition (Lortie et al. 2004). In a review, Brooker (2006) argued that plant–plant interactions may play a role in mediating the responses of species to anthropogenic environmental changes. However, because competition and facilitation vary along environmental gradients (Bertness & Callaway 1994; Callaway et al. 2002), the mediating role of biotic interactions may depend on regional and local environmental conditions. In arctic and alpine habitats, which are predicted to be among the most affected by global warming (Guisan & Theurillat 2000; Anon. 2005), climate change experiments including the role of biotic interactions have produced contrasting results. Those focusing on the beneficial effect of increasing temperature and nutrient availability found an increase in competition between species (Bret-Harte et al. 2004; Dormann et al. 2004; Klanderud 2005). In contrast, Wipf et al. (2006) showed that facilitation might increase under global warming as advances in snowmelt induce an increase in environmental stress. These results fit with the stress–gradient hypothesis (Bertness & Callaway 1994) that predicts a shift from competition to facilitation with increasing stress. However, there are a growing number of studies demonstrating that competition may, conversely, increase with increasing stress, in particular in water-limited environments (see meta-analysis of Maestre et al. 2005). It has also been argued that facilitation may wane in extremely stressful conditions (Michalet et al. 2006), as experimentally demonstrated in the dry Andes (Kitzberger et al. 2000). Given such uncertainty and the potential importance of these processes, further experiments are needed to understand how climate change may alter biotic interactions and to assess their mediating role for species responses to climate changes.

The 2003 European heatwave is considered to be one of the most important ECE of current climate change (Meehl & Tebaldi 2004). Therefore, this represented a good opportunity to improve our understanding of ECE effects on plant communities. We took advantage of an experiment, originally set up in November 2002 to study variation in biotic interactions along environmental gradients within forests, to explore the role of the tree canopy in altering species responses to ECE. Specifically, we explored: (i) how the 2003 heatwave affected tree

seedling survival; (ii) whether the tree canopy buffered or amplified the impact of ECE on different tree species; and (iii) how environmental conditions indirectly affected the impact of ECE through changes in the intensity and direction of the tree canopy effect.

These objectives were addressed through an analysis of the responses to the 2003 European heatwave of four dominant tree species in the French Alps mountain belt. The heatwave effect was assessed by comparing tree seedling survival rates during the heatwave with survival during an average summer (2004). To test the effect of environmental and biotic filters, we compared seedling responses to the heatwave under two contrasting soil moisture conditions, and both within forests and in experimental gaps. Two conifers (*Abies alba* and *Picea abies*) and two broadleaf trees (*Acer pseudoplatanus* and *Fraxinus excelsior*) were used to assess any species specificity in tree seedling responses to the heatwave, as already observed in herbaceous (MacGillivray et al. 1995; White et al. 2000; Marchand et al. 2006) and forest ecosystems (Mueller et al. 2005; Gitlin et al. 2006).

Materials and Methods

Study area and meteorological conditions

The experiment was performed in the northern French Alps, on the southern slopes of the Maurienne valley (St Michel de Maurienne, 45°7'N, 6°3'E). This part of the valley has a rain-shadowed temperate climate (Michalet et al. 2003); mean annual temperature (1950–2000) is 7.2°C and mean precipitation (1950–2000) is 946 mm at elevations of 1360 m (Meteo France, Le Thyl recording station, France). All experimental sites were located in secondary forests at elevations between 1000 and 1400 m. Two community types have been described along a water availability gradient, driven by topography and stream web density: mesoxeric and mesohygric types (Grossi et al. 1995). Both communities are dominated by *Acer pseudoplatanus* and *Fraxinus excelsior*, but the mesoxeric type is also characterized by the presence of *Quercus pubescens*, while the mesohygric type is characterized by the presence of *Alnus glutinosa*. *Abies alba* and *Picea abies*, two potential late-successional species of this forest, are rare within secondary forests, but form scattered mature patches in the vicinity of the experimental sites.

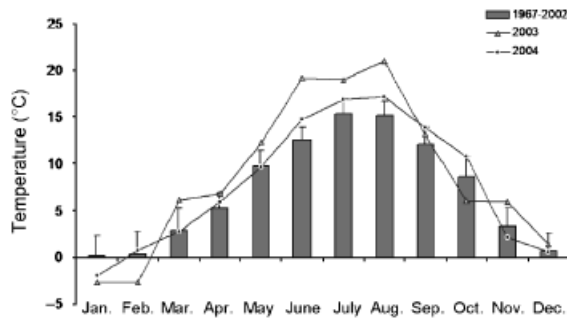


Fig. 1. Monthly temperature for 2003 and 2004 (curves) and means (± 1 SD; $n = 35$) for 1967–2002 (bars). Data from the Meteo-France recording station, Le Thyl-St Michel de Maurienne, 1360 m a.s.l.

As in other parts of Europe (Beniston & Stephenson 2004), the heatwave of summer 2003 was recorded at the meteorological station (Meteo France, Le Thyl) close to our study sites (same slope, same elevation, and < 1 km away), with monthly means 3 to 5°C above the average for 1967–2002 from June to August (Fig. 1). In contrast, summer 2004 had monthly mean temperatures that were not significantly different from the long-term average (Fig. 1), and thus could be taken as an average summer for our study purposes. In addition, precipitation in both 2003 and 2004 was not significantly different from the average in our study site (data not shown).

Experimental design

We used 2-year-old bare-rooted seedlings of two deciduous trees, *Acer pseudoplatanus* and *Fraxinus excelsior*, and two evergreen conifers, *Picea abies* and *Abies alba*. Seedlings were grown in a local nursery (Pépinières Puthod, Le petit Bornand, France), from seeds harvested in neighbouring forests. Two cohorts of seedlings of the four species were planted within the same design; in November 2002 for the heatwave cohort and in November 2003 for the cohort of the average summer. We used a completely randomized split-plot design (Underwood 1997), with community as main effect and tree canopy as sub-effect. For the community effect, we randomly selected eight sites, four sites as replicates of the mesoxeric community type and four sites as replicates of the mesohygric type. The size of each site was 1 ha. The tree canopy treatment (gap versus forest) was applied in each of the eight sites by fencing (1.5-m high to exclude large herbivores) a 20 m \times 20 m plot in both the experimental gap and the adjacent forest. Experimental gaps were estab-

lished in mid-autumn 2002, by removing all trees from areas of 40 m \times 40 m and were maintained during the experiment by removal of stool shoots. To limit the biotic interactions to tree canopy effects, and to avoid indirect interactions with understorey species (Pagès & Michalet 2003), we removed all herbaceous species and shrubs from each of the 16 plots by hand-pulling each month. Twelve seedlings of each target species were randomly transplanted into each plot, with at least 50 cm between individuals.

Data collection

For each cohort, seedling survival was measured in each of the 16 plots at the beginning (May) and at the end (October) of the growing season. Survival rates were calculated per plot as the proportion of seedlings alive at the end of the experiment relative to those alive at the beginning of the growing season.

Soil moisture measurements were carried out twice in each of the 16 plots, in late August 2003 and 2004, at least 3 days after the last precipitation event. Two soil samples per plot were collected at depths of 3 to 10 cm, and weighed before and after oven drying at 105°C for 2 days. Soil moisture was expressed as a percentage of fresh mass, and the two values per plot were averaged. Because the heatwave obviously induced premature senescence of leaves in the tree canopy (P. Saccone, personal observation), we quantified light transmission in the understorey. At noon in July 2003 and 2004, during a sunny day, we measured the fraction of photosynthetically active radiation (PAR; 400–700 nm) absorbed by the forest canopy, using a portable quantum sensor (LICOR, Li-188b, Lincoln, Nebraska). Twelve PAR measurements per plot were done 10 cm above the ground. The twelve values per plot were averaged and the understorey light availability of each forest plot was expressed as the percentage of incident PAR in the gap plot of each site.

Data compilation

We analysed the results using two forms of data. First, we used raw seedling survival rates to compare heatwave impacts on seedling survival rates to factors such as community and tree canopy. Second, because a preliminary analysis showed significant differences in survival rates among species during the average summer of 2004 (data not shown), we used a response index, the natural log-transformed response ratio (LnRR; Hedges et al. 1999), to

quantify the responses of the four species per plot to the heatwave (HW) and to tree canopy effects:

$$\text{LnRR}_{\text{HW for survival}} = \text{Ln} \left(\frac{(\text{cohort 2003 survival rate} + 1)}{(\text{cohort 2004 survival rate} + 1)} \right).$$

$$\text{LnRR}_{\text{canopy for survival}} = \text{Ln} \left(\frac{(\text{survival rate in the forest} + 1)}{(\text{survival rate in the gap} + 1)} \right).$$

A “one” was added to both the numerator and the denominator because the survival data contained zeros (Suding 2001).

We also used this response index to assess the heatwave impact on soil moisture and light availability per plot under the tree canopy:

$$\text{LnRR}_{\text{HW for soil moisture}} = \text{Ln} \left(\frac{(\text{soil moisture in 2003})}{(\text{soil moisture in 2004})} \right).$$

$$\text{LnRR}_{\text{HW for light availability}} = \text{Ln} \left(\frac{(\text{understorey light availability in 2003})}{(\text{understorey light availability in 2004})} \right).$$

Values of LnRR are symmetrical around zero, with positive values indicating positive effects of the heatwave or of the tree canopy on the studied variables and, conversely, negative values indicating negative effects.

Statistical analyses

First, we analysed differences in raw seedling survival rates using a split-split-plot ANOVA (Gotelli & Ellison 2004), with community as the main plot effect, tree canopy as sub-plot effect, and year and species as sub-sub-plot effects. We found strong significant interactions between species and tree canopy and year treatments (Table 1), indicating that the effect of year and tree canopy on seedling survival rates differed significantly among species. We then analysed differences in $\text{LnRR}_{\text{HW for survival}}$ using a split-split plot ANOVA with community as the main plot effect, tree canopy as sub-plot effect, and species as sub-sub-plot effect, and analysed differences in $\text{LnRR}_{\text{canopy for survival}}$ using a split-plot ANOVA with community as the main plot effect and year and species as sub-plot effects. We also found strong interactions between species and tree canopy and species and year treatments (Table 1), indicating that the effect of tree canopy on $\text{LnRR}_{\text{HW for survival}}$ and the effect of year on $\text{LnRR}_{\text{canopy for survival}}$ differed significantly among

Table 1. Results of two split-split-plot ANOVAs on seedling survival rates and $\text{LnRR}_{\text{HW for survival}}$ and of the split-plot ANOVA on $\text{LnRR}_{\text{canopy for survival}}$. Only results of the effects of interactions between species treatment and community, tree canopy and year are shown.

	Source of deviation	MS	df	F	P
Survival rates	Year×Species	0.41	3	10.99	<0.0001
	Trees×Species	0.24	3	6.41	0.0006
	Community×Species	0.02	3	0.65	0.5849
	Error	0.02	84		
$\text{LnRR}_{\text{HW for survival}}$	Trees×Species	0.18	3	13.68	<0.0001
	Community×Species	0.02	3	1.41	0.2569
	Error	0.01	36		
$\text{LnRR}_{\text{canopy for survival}}$	Year×Species	0.18	3	8.32	0.0002
	Community×Species	0.02	3	0.72	0.5449
	Error	0.02	42		

species. Thus, to facilitate interpretation of the results, they are presented using within-species analyses.

To test the overall effects of community, tree canopy and year, we analysed survival rate differences per species using a split-split-plot ANOVA, with community as the main plot effect, tree canopy as sub-plot effect and year as sub-sub-plot effect. To assess changes in heatwave impacts on seedling survival rates, differences in $\text{LnRR}_{\text{HW for survival}}$ were analysed per species using a split-plot ANOVA, with community as the main plot effect and tree canopy as sub-plot effect. In addition, we used one-way ANOVAs to analyse, for each species and community, the significance of the tree canopy effect on $\text{LnRR}_{\text{HW for survival}}$ and the significance of the year effect on $\text{LnRR}_{\text{canopy for survival}}$.

Within-community differences in $\text{LnRR}_{\text{HW for soil moisture}}$ between gaps and forests were analysed using one-way ANOVAs. Between-community differences below the tree canopy in $\text{LnRR}_{\text{HW for light availability}}$ were also analysed using a one-way ANOVA.

Finally, we tested the direct relationship between species responses to the heatwave and its effect on soil moisture using a linear regression model. A similar relationship between species responses to the heatwave and its effect on understorey light availability could not be conducted because of the low number of replicates.

In all ANOVA models, community, tree canopy and year were considered as fixed factors. In the split-split-plot and the split-plot ANOVA models, site nested within community and tree canopy×site nested within community were considered as random factors in order to use them as main plot and sub-plot error terms, respectively. One-sample *t*-tests with *P*-values adjusted using sequential Bonferroni corrections were conducted to test significant deviations from zero of the index values. An arc-sin transformation (Underwood 1997) was applied to raw seedling survival rates

to meet the assumptions of ANOVA, and other data sets were checked for normality and homoscedasticity. All analyses were carried out using JMP 5.0.1 (SAS Institute, Cary, NC, USA).

Results

Overall effects of year, community and tree canopy on seedling survival rates

For all species, year had the strongest effect on seedling survival rates (Table 2), with a dramatic decrease from 2004 to 2003. Survival rates ranged from 83.3% for *Picea* to 98.4% for *Fraxinus* in 2004, versus from 31.3% for *Abies* to 72.9% for *Fraxinus* in 2003. There was also an overall community effect (Table 2), with higher mean survival rates in the mesohygric community than in the mesoxeric community. However, this effect was more significant for *Abies* and *Fraxinus* than for the other two species (Table 2). In contrast, the overall tree canopy effect was only significant for *Abies*, which had a higher mean survival rate in forests than in gaps (Table 2).

Heatwave impacts on seedling survival (LnRR_{HW for survival})

Abies was significantly negatively affected by the heatwave (negative LnRR_{HW for survival} values, one sample *t*-tests, Fig. 2d). In addition, the heatwave impact was strong for this species in gaps and in the mesoxeric community (significant tree canopy and community effects, Table 3), but low and not significant in the understorey of mesohygric forests (Fig. 2d). In contrast, for *Acer* and *Picea*, the nega-

tive effects of the heatwave were stronger in forests than in gaps (significant and marginally significant tree canopy effect, respectively, Table 3), and especially in the mesoxeric community (see results of one-way ANOVAs, Fig. 2a and c). However, the community×tree canopy interaction was only marginally significant for *Acer* and not significant for *Picea* (Table 3). Finally, for *Fraxinus* there was no tree canopy effect and a lower negative impact of the heatwave in the mesohygric community than in the mesoxeric community (significant community effect, Table 3 and Fig. 2b).

Heatwave impact on adult-tree seedling interactions

During the average year (2004), the tree canopy had no effect on seedling survival rates for the four species in both the mesoxeric and mesohygric communities (Fig. 3). In contrast, during the heatwave (2003), the tree canopy of the mesohygric community induced a strong and significant facilitative effect (positive LnRR_{canopy for survival} values) on the survival of *Abies* seedlings (one sample *t*-test; Fig. 3d), and there was a highly significant year effect in this community (one-way ANOVA, Fig. 3d). In contrast, although no significant tree canopy effect was observed for *Acer* and *Picea*, there were significant (2004) and marginally significant (2003) decreases in LnRR_{canopy for survival} in the mesoxeric community (one-way ANOVAs, Fig. 3a and c). This indicates an increase in the negative effect of the tree canopy during the heatwave. Finally, tree canopy effects for *Fraxinus* were null in all treatments (Fig. 3b).

Table 2. Results of four within-species split-split-plot ANOVAs for the effects of community, tree canopy and year on seedling survival rates. Only single factor effects are shown.

Source of deviation	<i>Acer</i>				<i>Fraxinus</i>			
	MS	df	F	P	MS	df	F	P
Community	0.53	1	18.38	0.0052	0.16	1	7.38	0.0348
Error (main plot)	0.03	6			0.02	6		
Trees	0.10	1	0.89	0.383	0.07	1	0.54	0.4916
Error (sub-plot)	0.11	6			0.12	6		
Year	0.95	1	22.07	0.0005	1.95	1	40.59	<0.0001
Error (residual)	0.04	12			0.05	12		
Source of deviation	<i>Picea</i>				<i>Abies</i>			
	MS	df	F	P	MS	df	F	P
Community	0.23	1	4.29	0.0838	0.45	1	25.37	0.0024
Error (main plot)	0.05	6			0.02	6		
Trees	0.01	1	0.02	0.8924	0.68	1	5.08	0.065
Error (sub-plot)	0.16	6			0.13	6		
Year	2.57	1	50.75	<0.0001	6.20	1	101.58	<0.0001
Error (residual)	0.05	12			0.06	12		

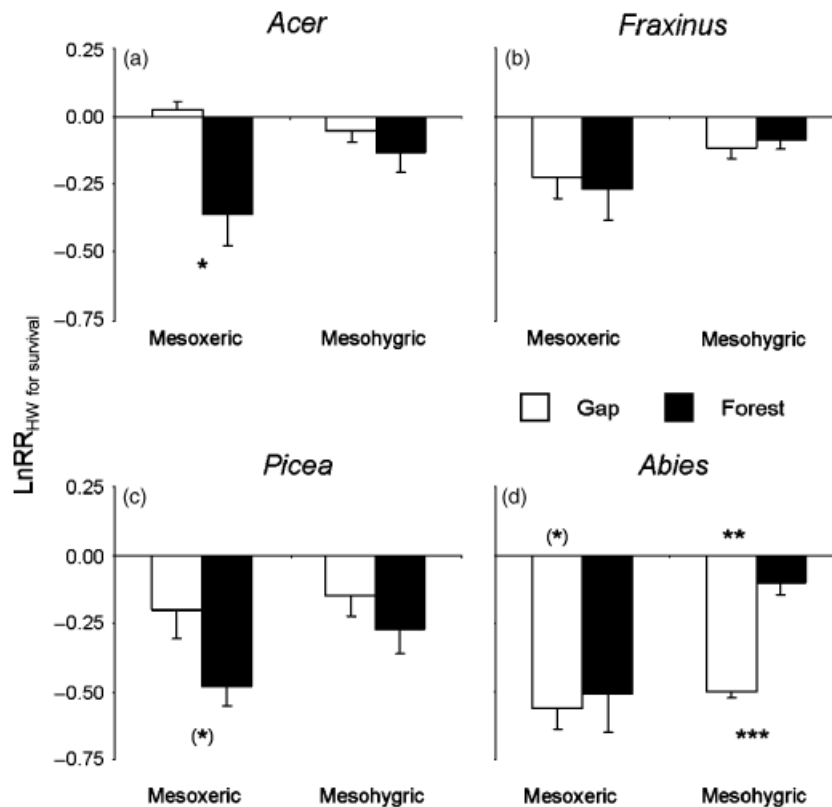


Fig. 2. Heatwave impact on survival rates (mean ± 1 SE of LnRR_{HW} for survival; $n = 4$) of seedlings of (a) *Acer*, (b) *Fraxinus*, (c) *Picea* and (d) *Abies* transplanted into gaps and forests within mesoxeric or mesohygric communities. Asterisks between bars indicate significant tree canopy effects per community in one-way ANOVAs. Asterisks above bars indicate significant differences from zero of the index values (one-sample t -tests with adjusted P -values). (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3. Results of four within-species split-plot ANOVAs for effects of community and tree canopy on the impact of the heatwave on seedling survival rates (LnRR_{HW} for survival).

Source of deviation	<i>Acer</i>				<i>Fraxinus</i>			
	MS	<i>df</i>	<i>F</i>	<i>P</i>	MS	<i>df</i>	<i>F</i>	<i>P</i>
Community	0.02	1	0.80	0.4058	0.08	1	16.87	0.0063
Error (main plot)	0.03	6			0.01	6		
Trees	0.21	1	11.67	0.0142	0.01	1	0.01	0.9341
Community \times Trees	0.10	1	5.31	0.0608	0.01	1	0.11	0.7472
Error (residual)	0.02	6			0.04	6		
Source of deviation	<i>Picea</i>				<i>Abies</i>			
	MS	<i>df</i>	<i>F</i>	<i>P</i>	MS	<i>df</i>	<i>F</i>	<i>P</i>
Community	0.07	1	3.07	0.1303	0.22	1	7.67	0.0324
Error (main plot)	0.02	6			0.03	6		
Trees	0.16	1	4.00	0.0924	0.21	1	7.63	0.0327
Community \times Trees	0.03	1	0.63	0.4575	0.12	1	4.42	0.0802
Error (residual)	0.04	6			0.03	6		

Heatwave impact on soil moisture and light availability and relation to heatwave impact on seedling survival

In all treatments, the heatwave induced significant or marginally significant decreases in soil

moisture (one sample t -tests; Fig. 4). In the mesoxeric community, the decrease in soil moisture due to the heatwave was significantly stronger below the tree canopy than in the gaps (one-way ANOVA; Fig. 4), whereas no difference in values of LnRR_{HW} for soil moisture were observed between

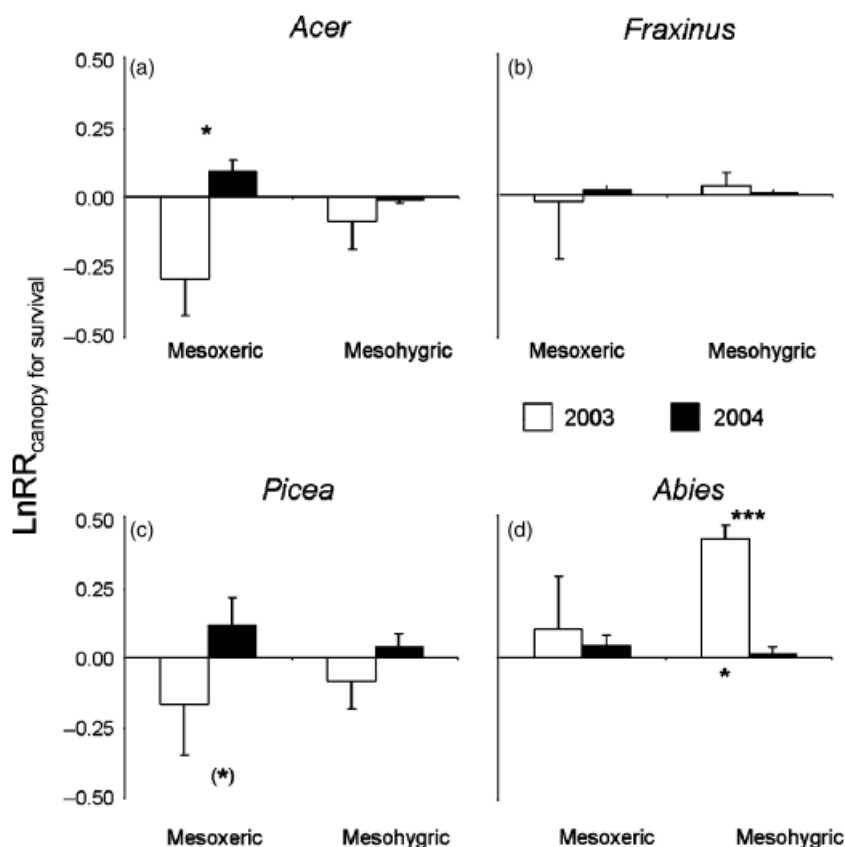


Fig. 3. Tree canopy effects on survival rates (mean \pm 1 SE of LnRR_{canopy for survival}; $n = 4$) of the 2003 and 2004 seedling cohorts of (a) *Acer*, (b) *Fraxinus*, (c) *Picea* and (d) *Abies* within mesoxeric or mesohygric communities. Asterisk between bars indicates significant year effects per community in one-way ANOVAs. Asterisk below bars indicates significant differences from zero of the index values (one-sample t -tests with adjusted P -values). * $P < 0.05$, *** $P < 0.001$.

gaps and forests in the mesohygric community. This result showed that, in the mesoxeric community, trees amplified the heatwave impact on soil moisture.

Light availability in the understorey was significantly positively affected by the heatwave only in the mesoxeric community (one-way ANOVA and one-sample t -test; Fig. 5).

The effect of the heatwave on soil moisture was significantly positively correlated to its effects on the survival of *Acer* and *Picea* seedlings (Table 4). In contrast, we found no relation between effect of the heatwave on soil moisture and its effects on the survival of *Abies* and *Fraxinus* seedlings (Table 4).

Discussion

The overall low seedling survival rate during the 2003 heatwave, compared to survival rates during the 2004 average summer, indicates that the ECE had a strong impact on our target species, as also

observed by Buckland et al. (1997) for herbaceous species during the 1995 extreme drought in UK. However, we observed species-specific responses to the heatwave. Overall, *Abies* was more negatively affected by the heatwave than the other species, highlighting the inequality of species responses when faced with a heatwave event, as previously shown for both herbaceous (MacGillivray et al. 1995; White et al. 2000) and forest communities (Mueller et al. 2005; Gitlin et al. 2006).

Abies also showed the most contrasting responses under the four experimental conditions; the negative effect of the heatwave was high in gaps of both communities and below the canopy of the mesoxeric forests, but its survival rate was not affected within mesohygric forests. *Acer* and *Picea* showed similar but less contrasting responses among experimental conditions, and their responses were opposite to those of *Abies*. *Acer* and *Picea* were negatively affected by the heatwave below the canopy of the mesoxeric forests, but no effect was observed either in gaps of this community or in the two

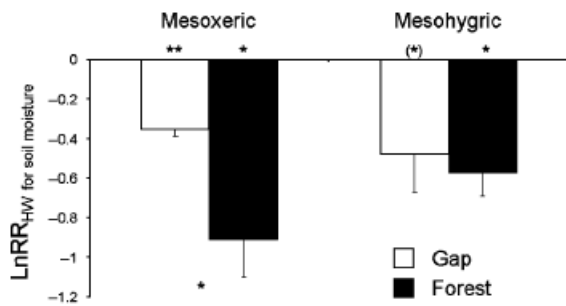


Fig. 4. Heatwave impact on soil moisture (mean \pm 1 SE of LnRR_{HW} for soil moisture; $n = 4$) in gaps and forests within mesoxeric and mesohygric communities. Asterisk between bars indicates significant tree canopy effects per community in one-way ANOVAs. Asterisks above bars indicate significant differences from zero of the index values (one-sample t -tests with adjusted P -values). (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$.

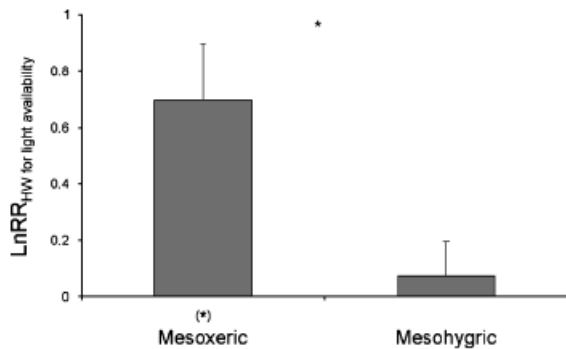


Fig. 5. Heatwave impact on understorey light availability (mean \pm 1 SE of LnRR_{HW} for light availability; $n = 4$) in forest plots of the mesoxeric and mesohygric communities. Asterisk between bars indicates a significant difference LnRR_{HW} for light availability between communities in one-way ANOVA. Asterisk below the mesoxeric bar indicates significant difference from zero of the index value (one-sample t -test with adjusted P -value). (*) $P < 0.1$.

canopy conditions of the mesohygric community. *Fraxinus* did not respond to the effect of the tree canopy in the two communities.

The mediating role of biotic interactions

One important result of our experiment was the high species specificity of the mediating role of biotic interactions. For *Abies*, the heatwave effect was significantly buffered by the tree canopy of the mesohygric community; whereas, for *Picea* and *Acer*, it was amplified by the tree canopy of the mesoxeric community. These contrasting tree canopy effects suggest that different mechanisms may operate in each community and for the different species.

Table 4. Results of linear regressions between specific LnRR_{HW} for survival and LnRR_{HW} for soil moisture following the equation: LnRR_{HW} for survival = $a + b \text{LnRR}_{\text{HW}}$ for soil moisture.

	Estimated parameters			
	a	b	r^2	P
<i>Abies</i>	-0.37	0.09	0.02	0.6503
<i>Acer</i>	0.09	0.37	0.43	0.0056
<i>Fraxinus</i>	-0.10	0.12	0.07	0.3192
<i>Picea</i>	-0.12	0.27	0.20	0.0796

Variation in soil moisture and light availability in our experimental plots may reveal some of the mechanisms and resources involved in these interactions. The mesoxeric tree canopy significantly increased the negative effect of the heatwave on water availability, as compared to the gaps, whereas this negative effect did not occur in the mesohygric community. In addition, light availability significantly increased during the heatwave in the former community but not in the latter. The increase in light availability in the mesoxeric community might have been driven by a reduction in canopy leaf area (P. Saccone personal observation) in response to soil water stress, in order to reduce transpiration, as described in other forest communities during drought periods (e.g. Delzon & Loustau 2005). In contrast, in the mesohygric community, where water coming from the stream web likely outweighed uptake by adult trees, the tree canopy did not amplify the negative effect of the heatwave on environmental conditions. This evidence of increased soil water stress in the mesoxeric forest may explain the slightly amplified heatwave effect of the tree canopy for *Acer* and *Picea* seedlings, as well as the occurrence of positive relationships only for these species between LnRR_{HW} for survival and LnRR_{HW} for soil moisture. These results are consistent with those of Valladares & Pearcy (2002), who showed that drought was more critical in the shade than in the sun for *Heteromeles arbutifolia* seedlings in central California (see also Abrams & Mostoller 1995).

However, the facilitation of *Abies* seedling survival by the mesohygric tree canopy, which buffered the heatwave impact for this species, could not be explained by our environmental measurements. Two mechanisms were likely to be responsible for this positive effect of the mesohygric tree canopy, a decrease in light stress (photoinhibition) or/and in VPD (Holmgren et al. 1997; Michalet 2007). During the average summer, the tree canopy did not facilitate survival of *Abies* seedlings while light interception was similar, which does not support the

photoinhibition hypothesis. Furthermore, *Abies alba* has a well-known sensitivity to atmospheric water stress (Rolland et al. 1999), its stomata closing at a very low VPD (0.3 kPa and minimum needle water potential of -1.8 MPa, Guicherd 1994). This ecophysiological behaviour may explain the very low survival rate of *Abies* in gaps (high VPD) and its ability to decrease its mortality rate below the forest canopy under some conditions. This occurred in the mesohygric forests but not in the mesoxeric forests, likely because competition for soil water outweighed this positive effect in the latter community but not in the former. Maestre et al. (2003) also observed this effect for *Pistacia lentiscus* seedlings transplanted below the grass *Stipa tenacissima*.

This buffering effect of the tree canopy did not occur for the three other species. In contrast, the survival rates of *Acer* and *Picea* seedlings were rather reduced by the tree canopy during the heatwave. Stomata of *Picea* have been shown to be less sensitive to VPD than to soil water stress (Wieser & Emberson 2004). Together with its well-documented low shade tolerance (Pagès et al. 2003), this may explain why *Picea* never took advantage of the cooler climate found below the forest canopy in our experiment. Furthermore, *Picea* might have been affected by lower soil water availability due to the water uptake of the adult trees under specific conditions (as in the mesoxeric community in 2003). *Acer* showed a very similar response as *Picea* to the tree canopy in our experiment, which is consistent with its ecophysiology. Tissier et al. (2004) showed that *Acer pseudoplatanus* had very low cavitation resistance as compared to other maples, and Lemoine et al. (2001) showed that the steepness of the slope of the vulnerability curve (speed to reach total embolism) for *Acer pseudoplatanus* was typical of a water-demanding species. As a result, *Acer*, similarly to *Picea*, was primarily affected by the additional decrease in soil moisture induced by the tree canopy, in particular under the driest conditions (mesoxeric community in 2003).

The absence of negative effects of the tree canopy for *Fraxinus* is also consistent with ecophysiological studies demonstrating its high cavitation resistance (Cochard et al. 1997), and low reduction in sap flow during drought periods (Holscher et al. 2005), compared to species such as *Acer* (Lemoine et al. 2001).

Heatwave impact on biotic interactions

Our results highlight the important mediating role of biotic interactions and are consistent with the

predictions of Brooker (2006). However, this mediating role was complex due to species-specific variation in biotic interactions between years and communities. Most of the few climate changes studies on the mediating role of biotic interactions support predictions of the model of Bertness & Callaway (1994). They found increasing competition as climate change decreased stress (e.g. Davis et al. 1998a; Klanderud 2005) and increasing facilitation as stress increased with climate changes (Wipf et al. 2006). In our experiment, facilitation increased for *Abies* with increasing stress from 2004 to 2003 in the mesohygric community, but did not occur in the most severe conditions – the mesoxeric community in 2003. In contrast, the strongest negative effects of the tree canopy were observed for *Picea* and *Acer* in the 2003 severe mesoxeric conditions.

These results are in opposition to the model of Bertness & Callaway (1994), which predicts a decrease in competition and an increase in facilitation with increasing environmental severity. However, a number of experimental studies have demonstrated that competition increases with soil water stress (Davis et al. 1998b; Tielbörger & Kadmon 2000), consistent with the model of Taylor et al. (1990). In addition, Michalet et al. (2006) proposed a model where facilitation decreases in extremely severe environments because nurse species are no longer able to improve the habitat for their protégés. Our results for *Abies* support the model of Michalet et al. (2006), because facilitation was not observed in either the least or the most severe environmental conditions, but occurred under intermediate stress conditions in the mesohygric community in 2003. However, the most important negative effect of the tree canopy for both *Picea* and *Acer* was observed in the most severe conditions, a result that is more in agreement with the model of Taylor et al. (1990). This latter result rather suggests that the decrease in facilitation for *Abies* in the most stressed conditions was related to increased competition for water, which outweighed the benefit of facilitation, as also found by Pugnaire & Luque (2001) and Brooker & Callaghan (1998).

Conclusion

Our results emphasize the mediating role of biotic interactions on the responses of species to climate changes, as previously suggested by Brooker (2006). However, we also highlighted the complexity of this mediation, which was mainly due to its strong species specificity and context dependency, therefore

limiting our current ability to predict how large-scale species responses to ECE will be altered by biotic interactions. Further experiments using different functional groups of target species, together with ecophysiological measurements and more detailed environmental measurements, are urgently needed in the context of an increasing risk of ECE (Easterling et al. 2000; Meehl et al. 2007). In addition, this is also a strong opportunity for plant community ecologists to greatly improve their knowledge of how both the importance and direction of biotic interactions vary along environmental gradients.

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