RESEARCH PAPER

Distribution ranges and spring phenology explain late frost sensitivity in 170 woody plants from the Northern Hemisphere

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ABSTRACT

Aim Cold events determine the distributional range limits of woody species. Despite global warming, the magnitude of late frost events in boreal and temperate regions is not expected to change. Hence, the risk for late spring frost damage of woody species may increase with an earlier onset of the growing season. Here, we investigated biogeographical, phenological and phylogenetic effects on late frost sensitivity.

Location Ecological-Botanical Gardens Bayreuth, Germany (49°55′45″ N, 11°35′10″ E).

Methods We inspected 170 woody species in the Ecological-Botanical Gardens from across the entire Northern Hemisphere for frost damage after an extreme late frost event in May 2011 (air temperature −4.3 °C after leaf unfolding of all species). Distribution range characteristics, climatic parameters of place of origin and phenological strategy were linked to sensitivity to the late frost event.

Results The northern distribution limit and the range in continentality across the distributional ranges correlated negatively with a taxon's late frost sensitivity (pseudo-$R^2 = 0.42$, pseudo-$R^2 = 0.33$, respectively). Sensitivity to the late frost event was well explained by the climatic conditions within species' native ranges (boosted regression trees; receiver operating characteristic 0.737). Average (1950–2000) May minimum temperature in species' native ranges was the main explanatory variable of late frost sensitivity (51.7% of explained variance). Phylogenetic relatedness explained additional variance in sensitivity to the late frost event. Sensitivity to the late frost event further correlates well with species phenological strategy. Frost-tolerant species flushed on average 2 weeks earlier than frost-sensitive species.

Main conclusions Range characteristics and the prevalent climatic parameters across species native ranges are strongly related to their susceptibility to late spring frost damage. Further, more late frost-sensitive species unfolded their leaves later than more tolerant species and late frost tolerance is phylogenetically conserved. Thus, late frost sensitivity may challenge natural and human-assisted migration of woody species under global warming.

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INTRODUCTION

Due to climate warming, extreme cold events are generally expected to occur less frequently (IPCC, 2012), but their magnitude is likely to persist (Kodra et al., 2011). Further decrease in wintertime sea ice in the Barents–Kara seas could even increase the likelihood of extreme cold events in Europe (Petoukhov & Semenov, 2010). Such extreme cold events can cause considerable damage to plants with significant ecological and also economic consequences (Gu et al., 2008; Jalili et al., 2010).

In Central Europe, the start of the growing season has advanced over the last decades (Menzel & Fabian, 1999; Badeck et al., 2004). Over three decades, leaf-out has started 6 days earlier (Menzel & Fabian, 1999). However, extreme cold events (spring frosts) after an earlier onset of the growing season are increasing the risk of frost damage in the temperate zone (Inouye, 2008; Martin et al., 2010; Hufkens et al., 2012; Augspurger, 2013).

Trees are less able to cope with rapid climate changes compared with other plant functional types due to their conservative dispersal strategies and their longevity (Petit & Hampe, 2006). An important factor limiting adaptation to global change in temperate tree species might be late frost sensitivity (Kollas et al., 2014). Knowledge on the role of late spring frost sensitivity in controlling range limits is therefore essential for understanding current and future natural and human-assisted range shifts.

In general, the probability of frost damage differs between tree species and is modified by their phenological phase (Augspurger, 2009). Directly after bud burst, temperate woody plants respond sensitively to frost events starting around −3 to −5°C (Sakai & Larcher, 1987; Inouye, 2008; Martin et al., 2010; Kreyling et al., 2012b; Lenz et al., 2013). Recent studies suggest that the susceptibility of species to late frosts is influenced by their phenological strategy, i.e. the leaves of early flushing species tend to withstand lower temperatures than species with a late spring phenology (Lenz et al., 2013; Vitasse et al., 2014a). Species with a longer dormancy period avoid late frost damage at the price of a shorter growing season (Lockhart, 1983; Leinonen & Hänninen, 2002; Basler & Körner, 2012). In contrast, species with a short dormancy period should profit from a prolonged vegetation period, but have to invest more in frost resistance mechanisms.

In addition, drought tolerance of plant species can modify the impact of frost events due to the physiologically comparable mechanisms aimed at preventing dehydration of cells (Blödner et al., 2005; Beck et al., 2007). Similar to drought stress, frost leads to dehydration of plant tissues and cells by crystallization of water (Sakai & Larcher, 1987). Hence, the water balance across a species' native range can have an impact on its late frost sensitivity due to cross-stress tolerance between drought and frost (Walter et al., 2012). In consequence, differing drought tolerance between woody species is likely to also be reflected in varying late frost sensitivity.

Sudden late frost events can affect large areas and can cause widespread damage (Gu et al., 2008; Hufkens et al., 2012; Kreyling et al., 2012a; Lenz et al., 2013). A strong late frost event in spring 2007 caused severe damage to woody species and crops across the eastern United States, and led to the loss of young foliage, shoots and fruits as well as to widespread necrosis and desiccation of leaves (Gu et al., 2008). Another large-scale cold event during the early vegetation period occurred in May 2011, where large parts of Germany experienced an extreme late frost event. This frost event led to frost damage such as severe leaf damage and a shortened vegetation period, and meant that additional investment of resources in second leaf-out across species was necessary for recovery (Kreyling et al., 2012a).

Minimum temperatures in winter are assumed to limit the native ranges of woody species (Sakai & Weiser, 1973). Likewise, cold tolerance of tree species is closely related to the climate of their native ranges, with a study focusing solely on cold tolerance over winter and before bud burst (Kreyling et al., 2015) finding the strongest correlations in late winter and early spring. In general, it has long been acknowledged that late frost events pose a risk for woody species in temperate regions (Gayer, 1882; Ellenberg, 1963).

However, for a long time there were no studies quantifying the effect of late frost events on the distribution ranges of woody species. Just recently, Kollas et al. (2014) pointed out that it is not the absolute minimum temperature in winter that controls the native range limits but rather the low-temperature extremes during bud burst in springtime, which is the phenological stage where woody plants respond most sensitively to sudden freezing events. This is in line with Lenz et al. (2013), who found that freezing temperatures in spring might be one of the main driving factors for range limits due to the selective pressure controlling the beginning of the growing season. Given this potentially strong effect of late frost sensitivity on distribution ranges, the increased risk of late frost damage (Inouye, 2008; Martin et al., 2010; Hufkens et al., 2012; Augspurger, 2013) opposes the poleward and upward range shifts expected with global warming (Parmesan et al., 1999; Lenoir et al., 2008). However, studies quantifying the effect of distributional and underlying climatic characteristics of species native ranges on late frost sensitivity across a large spatial scale and multiple species are missing.

Here, we tested if late frost sensitivity of woody species can be explained by the climatic conditions in their native distributional ranges, in particular spring minimum temperature. In particular we hypothesized that woody species whose native ranges are characterized by low temperatures (spring, winter, annual) and low amounts of precipitation (summer, growing season, annual), are well adapted to late frost events. In addition, we tested if frost sensitivity is related to the order in which species leaf out each year. We expected early leafing species to develop high frost resistance, while phenologically late species should afford lower frost resistance to their leaves. Finally, we checked if phylogeny (members of certain genera) contributed additional power for explaining
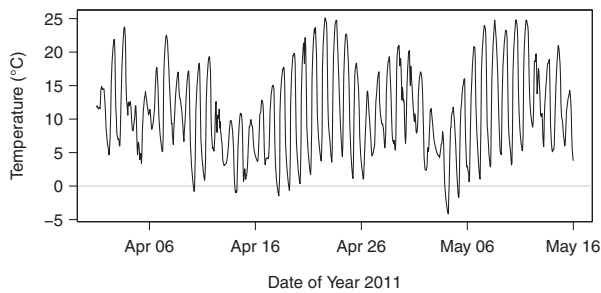
Figure 1 Air temperature (hourly means at +2m) from 1 April to 16 May 2011 showing the warm April preceding the late frost event on 4 and 5 May at the Ecological-Botanical Gardens of the University of Bayreuth, Germany. Data courtesy of Th. Foken, Department of Micrometeorology, University of Bayreuth.

the sensitivity to a late spring frost event. For this, we inspected 170 adult and established woody species growing in the Ecological-Botanical Gardens (EBG) of the University of Bayreuth for damage after one extreme frost event (air temperature below −4.3°C). This late frost event occurred naturally in May 2011 after the start of the growing season (Kreyling *et al.*, 2012a). We then tested if the observed frost damage could be explained by distributional and underlying climatic characteristics of these species native ranges.

METHODS

Ecological-Botanical Gardens Bayreuth and the late frost event in May 2011

The EBG of the University of Bayreuth, Germany (49°55′45″ N, 11°35′10″ E, 16 ha) is located at an elevation of 355 to 370 m a.s.l. The local climate represents a transition between oceanic and continental influences, with a long-term mean annual temperature of 8.2 °C and mean annual precipitation of 724 mm (Foken, 2007). As the EBG was founded in 1978, all tree specimens are of comparable age and have reached tree size with considerable growth in height. Thus, the EBG offers an implicit common garden setting to study late frost sensitivity of even-aged woody plant species.

Late frost events, i.e. frost events after the end of winter in spring or summer, occur occasionally. Such frost events can appear after bud burst of trees. The late frost event in May 2011 was the most extreme since the start of temperature recording on the site in 1997 and at the nearest station of the German Weather Service in 1961 (distance about 10 km; the second coldest event in 1976 reached −3.7 °C). Temperatures dropped to −10 °C close to the surface (+5 cm) and −4.3 °C at a height of 2 m on the early morning of 4 May (meteorological station at the EBG, coordinates as above; data courtesy of Th. Foken, Department of Micrometeorology, University of Bayreuth) (Fig. 1). This frost event happened after an extraordinarily warm April during which all studied species had started greening (Fig. 1). Bud burst was completed when the late frost event took place. Frost damage

became clearly visible over the following days. On 16 May we checked the new foliage and new needles of adult plant individuals of 170 woody species in the EBG (with heights between 1 and 15 m) – one to ten individuals per species – for visible frost damage (0 = no frost damage, 1 = at least one individual showing frost damage measured by leaf browning as an indicator).

Species distributional characteristics and underlying climatic conditions

For each species we obtained the native distribution range from various sources (data are given in Appendix S1 in Supporting Information). Based on the species distribution ranges, for each species we calculated the following distributional characteristics: southernmost occurrence, latitudinal and longitudinal distribution centroid as well as northernmost occurrence. For the climatic characterization of the distribution ranges, the current climatic conditions (averages over the time period 1950–2000) with a spatial resolution of 10 arcmin (obtained from WorldClim, http://worldclim.org; Hijmans *et al.*, 2005), were intersected with the native ranges. Continentality was chosen as a further parameter because a strong continental climate within a species' native range might lead to a higher frost tolerance due to required protection against cold winters, a higher risk of extreme late frost events and drought during summer (Czajkowski & Bolte, 2006). Continentality within a species' distribution range was quantified by using a simplified continentality index (high values equal high continentality; Iwanow, 1959 in Hogewind & Bissolli, 2011):

$$\text{continentality} = \frac{260 \times \text{annual temperature range}}{\text{latitude}}.$$

Spatial information about the annual temperature range was derived from Bioclim variable 7 (BIO7) from the WorldClim dataset, which is calculated as the difference between the maximum temperature of the warmest month (BIO5) and the minimum temperature of the coldest month (BIO6). Based on this gridded information about the annual temperature range and the latitude of the corresponding grid cells we calculated minimum, mean and maximum continentality as well as the range of continentality (maximum − minimum) for the distribution range of each species. All spatial analyses were conducted with the GIS software ArcGIS 10 (ESRI 2011, Redlands, CA, USA).

To test the influence of phylogenetic relatedness on the sensitivity to the late frost event, we pooled species-specific distributional characteristics for the 69 different genera under investigation. For all genera we calculated: northernmost and southernmost occurrence, maximum and minimum continentality, the average range of continentality (average of the species-specific ranges) as well as the average and variation (standard deviation) of species latitudinal and longitudinal distribution centroids. Of the 69 genera, only those genera (16 genera, 105 species) with more than three species were included in the genera-specific analyses (Appendix S1).

Table 1 Climatic parameters and their univariate (generalized linear model, GLM) and multivariate (boosted regression tree, BRT) relationship with the sensitivity of 170 woody species to the late frost event in May 2011 in the Ecological-Botanical Gardens, Bayreuth.

Climatic parameter	Aggregation across species range	P_{GLM}	Expl. var.$_{BRT}$
May minimum temperature	Mean	<0.001	51.7%
Temperature annual range	Standard deviation	<0.001	14.4%
Annual precipitation sum	Standard deviation	0.157	
Mean warmest month temperature	Standard deviation	0.025	18.7%
Sum of monthly precipitation (May–September)	Maximum	0.007	15.1%
De Martonne aridity index	Standard deviation	0.555	

The current climatic conditions (averages over the period 1950–2000) at 10-arcmin spatial resolution from WorldClim (http://worldclim.org; Hijmans *et al.*, 2005) were used for the analyses. Each single climatic parameter was assessed as the maximum (0.95 quantile), the mean, the minimum (0.05 quantile) and the standard deviation over all grid cells occupied by each respective species. After excluding collinearity (see Methods), six candidate climatic parameters were kept for further statistics in the stated aggregation across each species range. P_{GLM} provides their univariate *P*-value according to a binomial GLM. Expl. var.$_{BRT}$ provides the explained variance of those parameters, which showed significant univariate relations to late frost damage (P_{GLM} <0.001) and have thus been used in the binomial BRT model (ROC = 0.737).

To understand the underlying climatic processes that shape the general relationships between late frost sensitivity and distributional characteristics, we analysed climatic parameters of the species distribution ranges at the species level. Here, we initially considered nine climatic parameters. The six parameters which have been used for further analyses are shown in Table 1. Three climatic parameters (annual mean temperature, minimum temperature of the coldest quarter, and precipitation of the warmest quarter) were removed due to autocorrelation with the six remaining parameters (see below). For each of the climatic parameters we considered the maximum (0.95 quantile), mean, minimum (0.05 quantile) and standard deviation across each species' native range (resulting in 36 parameters). Minima and maxima were used to take extreme values into account. Extreme values might characterize the absolute limits of species occurrences more precisely than mean conditions (Zimmermann *et al.*, 2009). Standard deviations were chosen to characterize the spatial heterogeneity across species ranges and to investigate the potential impact of climatic variability. Such variability can be expected to lead to more conservative phenology, with strategies to avoid spring frost risk (e.g. later onset of leaf unfolding at the price of a shorter growing season) and higher investment in protection (Wang *et al.*, 2014). Dehydration tolerance of plants plays an important role not only during drought but also during frost events (Sakai & Larcher, 1987; Blödner *et al.*, 2005; Beck *et al.*, 2007). Therefore, precipitation of the warmest quarter, sum of monthly precipitation from May to September and the aridity index according to De Martonne (1926) were considered in addition to temperature and annual precipitation parameters [aridity index = mean annual precipitation sum (mm)/(mean annual temperature (°C) + 10)].

Species leaf-out strategies

Data on leaf-out dates for 110 of the 170 species were available from observational studies on woody species conducted in the Munich Botanical Garden from 2012 to 2015 (see Zohner & Renner, 2014 for methodological details). The sampling included a broad range of woody species from the Northern Hemisphere. Individuals grown in the garden are mostly wild collections that are acclimated, but not evolutionarily adapted. Hence, their leaf-out times reflect native phenological strategies. For analysis, the mean of a species' leaf-out date (from 2012 to 2015) was used. Leaf-out was defined as the day when three to four branches of a plant unfolded leaves and pushed out all the way to the petiole.

Statistical analysis

The effects of species distributional characteristics on late frost tolerance (at species as well as genus level) were tested by simple and mixed generalized linear models based on a quasi-binomial distribution. To estimate goodness of fit for the generalized linear models, we calculated a pseudo-R^2 according to Nagelkerke (1991) using the NagelkerkeR2()-function of the fmsb-R-package (version 0.5.1).

The influence of the climate within a species' native range on sensitivity to the late frost event was quantified by boosted regression trees (BRT) (Elith *et al.*, 2008). Before fitting BRTs, a reduction in dimensionality was applied by removing autocorrelated parameters. Candidate climate parameters were tested for collinearity with each other using Spearman's nonparametric correlation. Where pairs of variables were highly correlated ($q > 0.7$), a univariate binomial generalized additive model (GAM) was fitted to the data using each highly correlated variable. In order to obtain less correlated variables and a final minimal model, the variable within each pair that yielded the higher Akaike information criterion (AIC) value was omitted. For the six resulting climate parameters, we ran univariate binomial generalized linear models (GLMs) which resulted in four climate parameters that were significantly related to sensitivity to the late frost event: (1) mean over the species' range of the minimum temperature in May, (2) standard deviation over the species' range of the mean temperature of the warmest month, (3) maximum over the species' range of the sum of
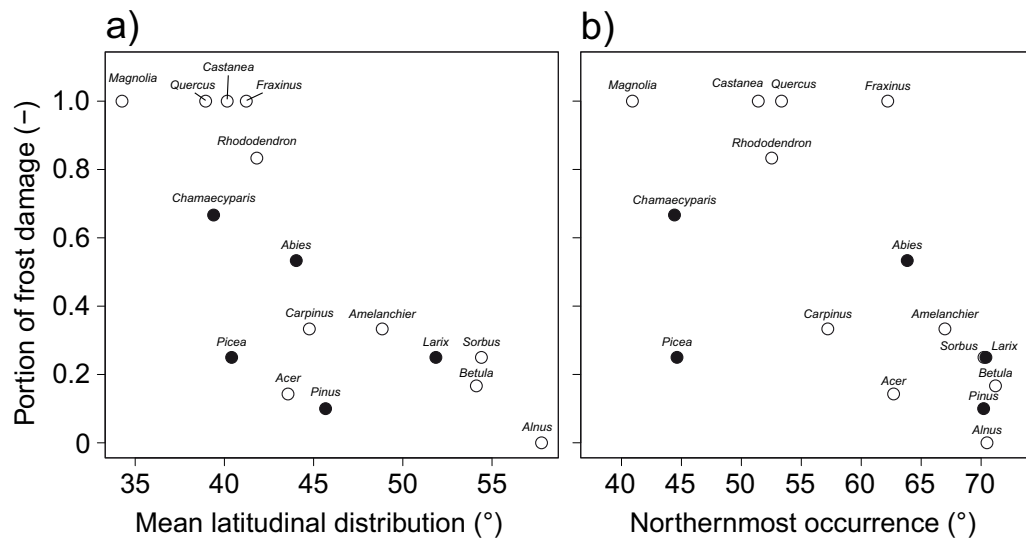
Figure 2 Relation between the probability of late frost damage for 16 Northern Hemisphere genera of 105 woody plant species and (a) the mean latitudinal distribution (species-specific latitudinal distribution centroid averaged for each genus, $P = 0.005$, pseudo-$R^2 = 0.59$) and (b) the northernmost occurrence (maximum latitudinal occurrence for each genus, $P = 0.022$, pseudo-$R^2 = 0.42$). The probability of late frost damage is depicted as the portion of species within each genus with visible late frost damage during May 2011. Filled symbols refer to coniferous species and open symbols to broad-leaved species.

precipitation from May to September, and (4) standard deviation over the species' range of the annual temperature range (Table 1). Only these four significantly explaining climatic parameters ($P < 0.05$) were considered in the subsequent BRT models.

Binomial BRTs were fitted according to Elith *et al.* (2008) with the selection of the final model being based on minimal estimated cross-validated deviance. This was obtained by setting the tree complexity to 5, the learning rate to 0.001 and the bag fraction to 0.9. The cross-validated receiver operating characteristic (ROC) score was used to express the correlation between climate within a species' native range and late frost damage. For each climatic parameter, its relative importance for explained variance was provided.

In addition to the climatic parameters of species native ranges the role of phylogenetic relatedness on late frost tolerance was tested with ANOVA analyses paired with post hoc multiple comparison tests. To omit statistical biases caused by small sample sizes we focused on a comparison of seven genera for which at least five species were investigated (*Abies, Acer, Betula, Fraxinus, Pinus, Quercus, Rhododendron*; see Appendices S1 & S2 for detailed information). Differences in the geographical distribution of these genera were tested by using Tukey honestly significant difference tests for multiple comparisons. Differences in late frost sensitivity were tested pairwise by using Wilcoxon rank sum tests for independent samples because of the binomial character of the tested variable. The level of significance was adjusted for these multiple tests by applying the Bonferroni–Holm correction.

All statistical analyses were executed with the software R 3.0.2 (R Development Core Team, 2013) and the additional packages mgcv v.1.7-26, gbm v.2.1, sciplot v.1.1-0, and popbio v.2.4.

RESULTS

The probability of leaf damage of the observed 170 woody plant species and 16 genera due to the studied late frost event significantly decreased with increasing latitudinal distribution centre (at species level, $P = 0.005$, pseudo-$R^2 = 0.15$, Appendix S2; at genus level $P = 0.005$, pseudo-$R^2 = 0.59$, Fig. 2a). This pattern was consistent for broad-leaved as well as coniferous genera as the effect of leaf morphology on late frost sensitivity was not significant in a generalized linear mixed effect model ($P = 0.09$). The same positive effect on sensitivity to the late frost event was found for the northernmost occurrence (species level, $P = 0.001$, pseudo-$R^2 = 0.12$; genus level, $P = 0.022$, pseudo-$R^2 = 0.42$; Fig. 2b), again with no significant difference between broad-leaved and coniferous genera ($P = 0.17$), but not for the southernmost occurrence (species level, $P = 0.13$, pseudo-$R^2 = 0.02$; genus level, $P = 0.48$, pseudo-$R^2 = 0.04$).

Besides the significant effects detected for the geographical ranges (distribution centre and northernmost occurrence), phylogenetic relatedness showed a strong effect on the frost tolerance of the investigated species. Species-specific frost tolerance was significantly better explained when including 'genus' as an additional explanatory variable besides the distributional variables (pseudo-$R^2 = 0.15$ vs. 0.86 for latitudinal distribution centre and pseudo-$R^2 = 0.12$ *vs.* 0.87). For instance, observed frost damage differed significantly between the genera *Quercus* (frost damage in all observed species) and *Pinus* (no frost damage in any observed species), despite their largely overlapping geographical distribution ranges (Appendices S2 & S3). Likewise, *Pinus* and *Acer* (frost damage in only 2 out of 14 species) differed significantly from *Fraxinus* (frost damage in all observed species) and *Rhododendron*

(frost damage in four out of five observed species) despite the distributional characteristics (latitudinal distribution centre, northernmost as well as southernmost occurrence) not differing significantly among these genera in our dataset (Appendix S3).

Longitude had no significant effect on sensitivity to the late frost event, neither the mean nor the variation of the longitudinal centroids ($P = 0.42$ and $P = 0.10$, respectively). The same was true for maximum and minimum continentality experienced by a species over its range within each genus ($P = 0.20$ and $P = 0.30$, respectively). Also species-specific mean continentality averaged for each genus showed no significant effect on sensitivity to the late frost event ($P = 0.23$). However, the probability of late frost damage significantly decreased with increasing species-specific range of continentality averaged for each genus ($P = 0.045$, pseudo-$R^2 = 0.33$). This means that the wider the range of continentality experienced by the species of a certain genus in their distribution ranges, the lower was the probability of late frost damage within this genus.

The species-specific probability of being damaged by the late frost event was well explained by the climatic conditions within the native distribution ranges (BRT cross-validated ROC score = 0.737). The probability of frost damage was best explained by the mean over the species' range of the May minimum temperature (51.7%) followed by the standard deviation over the species' range of the mean temperature of the warmest month (18.7%), the maximum over the species' range of the sum of precipitation from May to September (15.1%) and by the standard deviation over the species' range of the annual temperature range (14.4%) (Table 1). The probability of being damaged by the late frost event increased with increasing mean over the species' range of the May minimum temperature ($P < 0.001$), with decreasing standard deviation over the species' range of the mean temperature of the warmest month ($P = 0.025$), with increasing maximum over the species' range of the sum of precipitation from May to September ($P = 0.007$), and with decreasing standard deviation over the species' range of the annual temperature range ($P < 0.001$) (Fig. 3).

Species leaf-out dates (mean for 2012 to 2015) were highly correlated with sensitivity to the late frost event ($P < 0.001$) (Fig. 4). On average, the leaf-out dates of frost-resistant species preceded those of frost-sensitive species by 10 days.

DISCUSSION

The sensitivity of the 170 woody species studied to the late frost event in May 2011 was found to be significantly related to species distributions (latitude of species distributional centres, northern range limit). Furthermore, genera with wider ranges in their latitudinal distribution and in continentality turned out to be less vulnerable to the late frost event in May 2011. In addition to the biogeographical patterns, we found that the phenological strategy of species was highly adapted to sensitivity to the late frost event, with early leaf-ing species being less susceptible to late frost events. These patterns were consistent for broad-leaved and coniferous species.

Notably, many of the woody species studied here grew outside their native range. Thus, climate, community composition, photoperiod and soil conditions may not be at their preferred values. Still, frost sensitivity as well as phenological strategy of leaf-out can be assumed to be rather conservative traits so that our results bear implications beyond the single study site. We did observe clear geographical patterns by only considering the natural species distributions without further information on the precise origin of the studied ecotypes. To address this limitation, not just the mean of the climatic parameters within the native ranges but also the maxima, minima and the standard deviation have been used to characterize the distribution ranges. Hence, extreme values and spatial heterogeneity across species ranges are taken into account.

Species sets in botanical gardens represent a subjective sample of species able to tolerate the conditions at the specific garden. Despite this obvious bias, our results indicate that sensitivity to the studied late frost event could be significantly better explained by including genus as an additional explanatory factor besides the distributional variables. Further, the observed frost damage differed significantly between genera, even if the distributional characteristics did not due to the given subset of species within the genera. Hence, our study hints at phylogenetic relatedness having strong effects on the late frost tolerance, i.e. phylogenetic conservatism of late spring frost tolerance.

Up to now, more attention has been paid to the role of extreme cold events in winter and winter frost sensitivity as limiting factors for the ranges of tree species (Sakai & Weiser, 1973; Jalili *et al.*, 2010; Kreyling *et al.*, 2015). 'Winter hardiness zones' have been classified, reflecting distribution patterns related to the extreme minimum temperatures in tree species ranges (Roloff & Bärtels, 2006; Daly *et al.*, 2012). However, Lenz *et al.* (2013) and Kollas *et al.* (2014) found extreme frost events during bud burst in spring rather than minimum winter temperature to be the factor that was most limiting for species distribution. Focusing on the underlying climatic drivers, the probability of frost damage in our study was well explained by the climatic characteristics of species native ranges (BRT ROC = 0.737). Concerning specific climate parameters, late frost sensitivity was most strongly related to the May minimum temperature within the native range (> 50% of explained variance), which is at the beginning of the growing season of most species considered. Species with higher May minimum temperatures in their native range responded more sensitively to this particular late frost event. This tight link across 170 species from all over the Northern Hemisphere supports the conclusion of Lenz *et al.* (2013) and Kollas *et al.* (2014) that late frost sensitivity is an important consideration in projections of range shifts of woody species in the face of climate change.
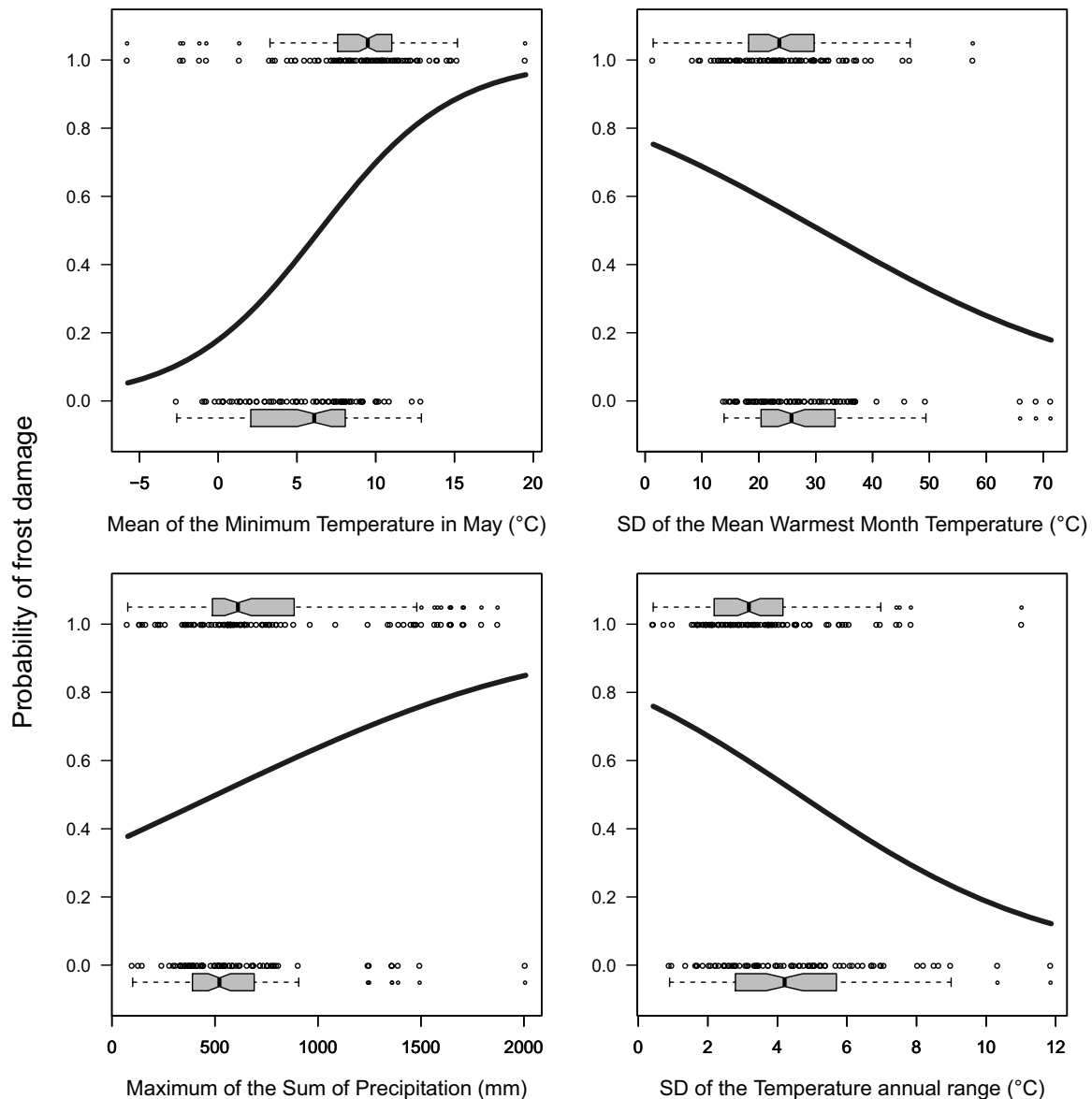
Figure 3 The univariate probability of species-specific late frost damage (no damage = 0, damage = 1) in relation to climatic characteristics of the species ranges of 170 woody species (only those parameters with $P < 0.05$ in univariate generalized linear models are shown; see Table 1).

In addition to species differences in sensitivity to the late frost event, phenological adaptation to climatic conditions in the native range of woody species could play an important role with regard to their response to late frost events. The timing of bud burst, which is a sensitive phase in the pheno-logical cycle, is crucial for the risk of frost damage in respect to cold events in the temperate latitudes during the spring (Sakai & Larcher, 1987; Inouye, 2008; Martin et al., 2010; Kreyling et al., 2012b; Augspurger, 2013; Vitasse et al., 2014b). By investigating the leaf-out strategies of a broad range of taxonomically distinct temperate woody species in relation to their sensitivity to the late frost event, we found that leaf unfolding dates were highly related to the frost sensitivity of the leaves: species resistant to the late frost event

leafed-out as much as 10 days earlier than susceptible species. This demonstrates that species finely adjust the time of leaf appearance – the most freezing-sensitive phenological phase – to their susceptibility to late frosts. By using a broad range of woody temperate species from various climates, our study thereby confirms similar patterns found for smaller and more regional subsets of species (Lenz et al., 2013; Vitasse et al., 2014a).

According to Lenz et al. (2013) freezing tolerance within species differs among phenological stages. Here, the strongest changes in frost sensitivity occurred before bud burst and there were none or only slight changes in both possible directions after leaf unfolding, depending on the individual species. Thus, a possible caveat of our approach is that not all
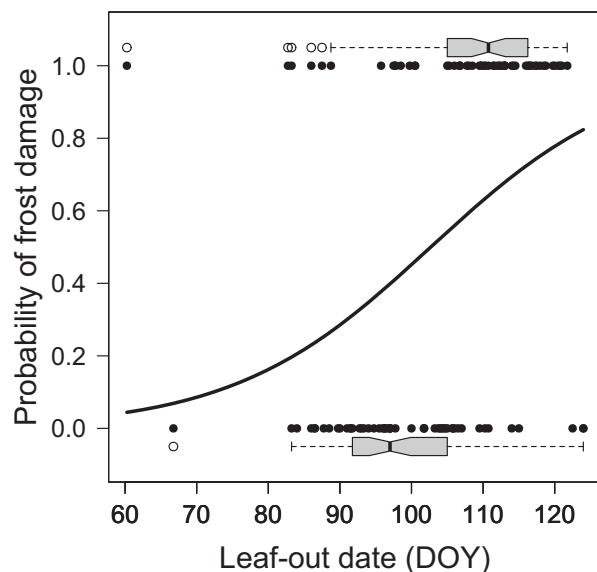
Figure 4 The univariate probability of species-specific late frost damage (no damage = 0, damage = 1) in relation to the leaf-out strategy of 110 woody species ($P < 0.001$; univariate generalized linear model). Species leaf-out dates (recorded as day of the year, DOY) are the average dates from 2012 to 2015 observed in the Munich Botanical Garden.

species were at exactly the same phenological stage when they were exposed to the freezing event. However, bud burst and leaf unfolding were completed in all studied species when they were hit by the late frost. Therefore, it is unlikely that contrasting phenological stages at the time of the frost event would be responsible for the observed pattern of early leafing species being more frost tolerant. Unfortunately, we lack phenological data for the 2011 study year, but infer species phenological strategies from leaf-out data collected from 2012 to 2015 (including the warmest recorded spring in Bavaria in 2014). The order of species-level leaf-out dates was found to be highly conserved over time (Panchen *et al.*, 2014; Zohner & Renner, 2014) and therefore our leaf-out data can be assumed to reflect also the sequence of leaf unfolding in the year 2011.

Which phenological safety mechanisms do frost-sensitive species use to avoid precocious bud development? Current studies suggest that chilling requirements are the main drivers to limit advanced budburst (Laube *et al.*, 2014). According to Fu *et al.* (2015), reduced chilling over winter potentially leads to an increased heat requirement in spring and consequently to a delayed tracking of climate warming in spring phenology. However, the impact of photoperiod as well as temperature cues on phenology have to be kept in mind, especially in times of global warming (Körner & Basler, 2010; Basler & Körner, 2012): late successional tree species have been observed to be photoperiod sensitive. Photoperiodic control of phenology can limit the phenological responses of late successional species to warming, particularly when a warm spring temperature would suggest

promoted development. Savage & Cavender-Bares (2013) found that northern species within the family Salicaceae were more strongly constrained by photoperiod as a cue for bud burst than southern species. The role of photoperiodic constraints on spring phenology indicated by their example clearly requires further attention, as it is of great importance for understanding the impacts of climate change on species migrations.

In our study, high spatial (and ecological) heterogeneity within a species' natural range was found to be linked to reduced observed damage as a response to the spring frost event. For a species as a whole, a high spatial heterogeneity of annual air temperature and climatic continentality within its range necessitates, among other things, protection against cold winters, extreme late frost events and summer drought (Czajkowski & Bolte, 2006). Moreover, species that tolerate high heterogeneity in terms of the warmest mean monthly temperature also need to be adapted to drought, as high temperatures during summer are likely be connected to a higher evapotranspirational demand and hence can cause drought stress (Dai *et al.*, 2004).

Likewise, low precipitation during the growing season (the maximum, i.e. 95% quantile, of the sum of monthly precipitation from May to September across the species' native range) in the native range reduced the probability of being damaged by the late frost event. Thus, water shortage experienced during evolution can play a role with regard to late frost sensitivity. This, again, can potentially be explained by cross-stress tolerance in the face of drought or frost (Walter *et al.*, 2012). Plant species that are adapted to drought are often also adapted to frost-induced water stress via physiological responses, such as accumulation of non-structural carbohydrates, to protect phenological, morphological or physiological traits (Inouye, 2000; Beck *et al.*, 2007).

In conclusion, the individual sensitivity of the 170 woody species observed to a late frost event after leaf unfolding can be explained by the species' natural latitudinal range, the spectrum of continentality and by specific climatic conditions, in particular the mean minimum temperature in May across the species' distribution.

Thus, late frost sensitivity appears to be a factor controlling species' distribution limits and is an important consideration in projections of range shifts of tree species or in concepts of assisted migration. Furthermore, we reveal in this study that late frost sensitivity appears to be synchronized with the species' phenological strategy.

Implications and outlook

Species are expected to respond to global warming with upward or poleward shifts of their distribution limits (Parmesan *et al.*, 1999; Lenoir *et al.*, 2008). In particular, tree species are found to lag behind the rapidity of warming, a fact commonly explained by their conservative dispersal strategies and long regeneration cycles (Petit & Hampe, 2006). Therefore, assisted migration is discussed as an option to

support the adaptation of forest stands to future climate conditions. However, the success of actively shifted populations might be jeopardized by extreme winter frost (Jalili *et al.* 2010) and late frost events (our results, but see also Kollas *et al.*, 2014), as these appear to be two of the most important factors controlling species native range limits.

Even though the late frost event in May 2011 was extreme (the most severe late frost event since records began on site in 1997 and locally in 1961), it was generally not lethal to any tree species in this study. However, late spring frost events can have strong ecological implications as they can reduce growth performance. For instance, tree ring widths dropped by up to 90% in *Fagus sylvatica* in the Alps in years with spring temperatures below $-3\ °C$ (Dittmar *et al.*, 2006) due to reduced growing season length and loss of resources like stored carbon and other nutrients (Lockhart, 1983; Gu *et al.*, 2008; Augspurger, 2009; Martin *et al.*, 2010). This can scale up to extreme late frost events altering biogeochemical cycles (Mulholland *et al.*, 2009). Resilience, however, appears remarkably high with tree rings in *F. sylvatica* in the Alps in the years after the frost events reaching equal increments as before (Dittmar & Elling, 2006). We therefore assume that the tight link between species distributions and late frost sensitivity observed in our study is not due to lethal effects but rather to loss of storage and a shortened growing season. In consequence, the link could be due to the reduced competitive power and potential carry-over effects on build-up of dormancy and winter hardening in autumn.

CONCLUSIONS

The sensitivity of 170 boreal and temperate tree species in the EBG of the University of Bayreuth to the late frost event in May 2011 was well explained by species geographical distributions and the underlying climatic conditions in species native ranges, in particular by spring minimum temperatures. Sensitivity to the late frost event was generally greater for species with lower northern range limits, lower variability in continentality and higher May minimum temperatures as well as higher precipitation during the growing season in their native ranges. Species phenological strategies appear to be well adjusted to late frost sensitivity. Early leafing species were more tolerant against the late frost event than species that started their development later in spring. Hence, our study emphasizes the ecological and evolutionary importance of late frost damage in tree species. Single extremes such as late frost events can potentially jeopardize natural and anthropogenic range shifts as a response to global warming and should therefore be acknowledged in further research, nature conservation or forestry.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Species list with information on distribution and frost damage.

Appendix S2 Distributional characteristics and late frost sensitivity of the investigated woody species.

Appendix S3 Differences in late frost sensitivity and geographical distributions between the investigated tree genera.

BIOSKETCH

Lena Muffler is interested in global change and the impact of extreme weather events, such as frost, late frost and drought, on ecosystems. Here, species responses to global warming are investigated by statistical modelling (species distribution modelling, multiple regression analysis), ecological experiments (reciprocal transplantation, climate manipulation) and field observations.

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