Climate change in experimental ecology

The impact of warming and drought on key European tree, heath, and grassland species

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Contents

1 Short summary of the thesis

During climate change rising mean air temperatures will be accompanied by climatic extremes such as drought. The frequency and magnitude of those extreme weather events are expected to increase in the future. Additionally, climatic extremes may have stronger effects on plants than a gradual temperature shift. Therefore, investigations of plant response to extreme weather events are needed to understand ecological and economic implications better. Apart from the consequences of climate change for plants, this thesis also asks the question: Can we simulate global warming reliably with our available warming techniques? In particular, the work in hand is intended to give answers to four research questions in this context (corresponding to four manuscripts).

1. How reliable are our experimental evidences and techniques (passive warming versus warming by transplantation) (**manuscript 1**)?
2. Is the leaf palatability of *Quercus pubescens* influenced by warming and drought (**manuscript 2**)?
3. Does drought response of *Fagus sylvatica* differ between central and marginal provenances (**manuscript 3**)?
4. Does stress response of plant communities and species (heath and grassland) differ if drought reoccurs (**manuscript 4**)?

Concerning the first of the above stated research questions, the comparison of the two warming techniques "passive warming" and "warming by transplantation" revealed that the reliability of the experimental results and therefore the resulting predictions for future plant response depend on the chosen technique. Experimental passive warming techniques are complicated in execution and should consider further parameters besides temperature increases such as: evapotranspiration, photosynthetically active radiation, and wind speed in order to improve the knowledge of warming technique effects (**manuscript 1**).

The climatic manipulations "warming" and "drought" were simulated in the EVENT 3 experiment to investigate their impact on the leaf palatability of *Quercus pubescens* of four European provenances from Germany, Bulgaria, Hungary, and Italy (second research question). This was done in a palatability experiment using the leaf consumption of the forest pest *Lymantria dispar* (gypsy moth) as response parameter (**manuscript 2**). Surprisingly, "warming" and "drought" did not effect the leaf palatability. However, leaf palatability was negatively correlated with an increasing density of trichomes (i.e. leaf hairs) and Bulgarian leaves were preferred compared to Hungarian and Italian oak leaves. This study suggests that the attractiveness of the four tested European *Q. pubescens* provenances might be stable in a changing climate at least as far as *L. dispar* is concerned.

Regarding the third research question, beech seedlings originating from three marginal (Bulgaria, Spain, and Poland) and three central (Germany) *Fagus sylvatica* provenances out of its geographic range were exposed to drought in two different soil types (sand, loam) (**manuscript 3**). Drought had a negative impact on all tree growth parameters, especially in sandy substrate. The provenances differed in their response to drought and soil type, but marginal *F. sylvatica* provenances generally did not withstand drought exposure better than central provenances. The performance of the Bulgarian *F. sylvatica* provenance might indicate a trade-off between growth and drought tolerance, as the overall total increment was low but stable under drought conditions. Furthermore, local adaptation to summer drought could be detected with respect to mortality. Regarding forest management, focusing only on drought-resistant marginal provenances might not be beneficial, as those provenances could be less adapted to other climatic parameters. Therefore, intermixing Central European forests with drought-resistant tree provenances might be an option to ensure forest resistance and yield in the future.

Beside the impact of warming and drought on tree species, the fourth research question assessed the response of temperate grassland and heath communities as well as single species to a very severe drought event (more than half of the growing season) (**manuscript 4**). The plants experienced previously within their life history different precipitation manipulations ("drought", "heavy rain", "regular watering", and "ambient control" conditions) in addition to natural drought occurrences in the EVENT 1 experiment over a six-year period. Differences in drought stress resistance and the influence of plant community composition on the ecological stress memory of single plant species were tested. Recurrent mild drought stress over several years seems to improve plant resistance and thus lead to less tissue die-back compared to plants with hardly any drought experience in the face of a very severe drought. Additionally, plant community composition might have an influence on the ecological stress memory of single plant species.

To sum up, the findings of this dissertation showed that the design of the chosen warming technique has to be thoroughly considered to make reliable predictions of plant response to climate change. Moreover, warming and drought did not impact the leaf palatability of different *Q. pubescens* provenances. On the other hand, drought influenced the growth of central and marginal *F. sylvatica* provenances negatively. Beyond that, drought occurrences do not have to be extreme themselves to promote drought resistance of heath and grassland communities and single species.

Kurze Zusammenfassung der Doktorarbeit

Im Zuge des Klimawandels werden steigende mittlere Lufttemperaturen von Klimaextremen wie Dürre begleitet sein. In der Zukunft wird eine Zunahme in der Häufigkeit und in der Größenordnung dieser extremen Wetterereignisse erwartet. Des Weiteren könnten Klimaextreme stärkere Effekte auf Pflanzen haben als eine graduelle Temperaturveränderung. Daher werden Untersuchungen der Reaktionen der Pflanzen auf extreme Wetterereignisse benötigt, um ökologische und ökonomische Implikationen besser verstehen zu können. Abgesehen von den Konsequenzen des Klimawandels für Pflanzen, wird in dieser Dissertation ebenso die Frage gestellt: Können wir die globale Erwärmung mit unseren zur Verfügung stehenden Erwärmungstechniken verlässlich simulieren? Insbesondere beabsichtigt die vorliegende Arbeit vier Forschungsfragen in diesem Zusammenhang zu beantworten (korrespondierend zu vier Manuskripten).

1. Wie verlässlich sind unsere experimentellen Evidenzen und Techniken (passive Erwärmung gegenüber Erwärmung durch Transplantation) (**Manuskript 1**)?
2. Wird die Schmackhaftigkeit von *Quercus pubescens* Blättern durch Erwärmung und Dürre beeinflusst (**Manuskript 2**)?
3. Ist die Dürrereaktion von zentralen und marginalen *Fagus sylvatica* Herkünften unterschiedlich (**Manuskript 3**)?
4. Unterscheidet sich die Stressreaktion von Pflanzengemeinschaften und -arten (Heide und Grasland), wenn Dürre sich wiederholt ereignet (**Manuskript 4**)?

Bezüglich der ersten Forschungsfrage erbrachte ein Vergleich der zwei Erwärmungstechniken "passive Erwärmung" und "Erwärmung durch Transplantation", dass die Verlässlichkeit der experimentellen Ergebnisse und folglich die daraus resultierenden Prognosen für zukünftige Pflanzenreaktionen von der gewählten Technik abhängen. Experimentelle passive Erwärmungstechniken sind kompliziert in ihrer Umsetzung und sollten weitere Parameter neben Temperaturerhöhungen berücksichtigen, wie z. B.: Evapotranspiration, photosynthetisch aktive Strahlung und Windgeschwindigkeit, um die Kenntnisse der Effekte von Erwärmungstechniken zu verbessern (**Manuskript 1**).

Die klimatischen Manipulationen "Erwärmung" und "Dürre" wurden im EVENT 3 Experiment simuliert, um deren Auswirkung auf die Schmackhaftigkeit von Blättern von vier europäischen *Quercus pubescens* Herkünften aus Deutschland, Bulgarien, Ungarn und Italien zu untersuchen (zweite Forschungsfrage). Dies wurde in einem Fraßversuch getan, bei dem der Blattkonsum des Waldschädlings *Lymantria dispar* (Schwammspinner) als Untersuchungsvariable diente (**Manuskript 2**). Überraschenderweise hatten "Erwärmung" und "Dürre" keine Auswirkung auf die Schmackhaftigkeit der Blätter. Jedoch war die Schmackhaftigkeit der Blätter negativ mit der Zunahme der Dichte von Trichomen (d. h. Blatthaaren) korreliert und die bulgarischen Blätter wurden im Vergleich zu den ungarischen und italienischen Eichenblättern bevorzugt. Diese Studie deutet darauf hin, dass die Attraktivität der vier getesteten europäischen *Q. pubescens* Herkünfte in einem sich wandelnden Klima stabil bleiben könnte, jedenfalls soweit es *L. dispar* betrifft.

Hinsichtlich der dritten Forschungsfrage wurden junge Buchen, die aus drei marginalen (Bulgarien, Spanien und Polen) und drei zentralen (Deutschland) *Fagus sylvatica* Herkünften aus deren Verbreitungsgebiet abstammen, einer Dürre in zwei unterschiedlichen Bodenarten (Sand, Lehm) ausgesetzt (**Manuskript 3**). Dürre hatte eine negative Auswirkung auf alle Baumwachstumsparameter, insbesondere im sandigen Substrat. Die Herkünfte unterschieden sich in ihrer Reaktion auf die Dürre und auf die Bodenart, allerdings widerstanden marginale *F. sylvatica* Herkünfte generell nicht besser der Dürreexposition als zentrale Herkünfte. Das Verhalten der bulgarischen *F. sylvatica* Herkunft könnte ein "Trade-off" zwischen Wachstum und Dürretoleranz indizieren, da das Wachstum der Herkunft insgesamt gering, jedoch stabil unter Dürre war. Weiterhin wurde eine lokale Anpassung an Sommerdürre in Bezug auf die Mortalität entdeckt. Im Hinblick auf die Waldbewirtschaftung wäre der alleinige Fokus auf dürreresistente, marginale Herkünfte nicht unbedingt von Vorteil, da diese Herkünfte an andere klimatische Parameter weniger angepasst sein könnten. Daher stellt das Mischen von zentraleuropäischen Wäldern mit dürreresistenten Baumherkünften eine Option dar, um die Waldresistenz und den Ertrag in der Zukunft zu sichern.

Neben der Auswirkung von Erwärmung und Dürre auf Baumarten wurden in der vierten Forschungsfrage die Reaktionen von Grasland- und Heidegemeinschaften ebenso wie von einzelnen Arten aus der gemäßigten Klimazone auf ein sehr strenges Dürreereignis (mehr als die Hälfte der Vegetationsperiode) untersucht (**Manuskript 4**). Die Pflanzen erfuhren innerhalb ihrer vorherigen Lebensgeschichte über sechs Jahre unterschiedliche Niederschlagsmanipulationen ("Dürre", "Starkregen", "regelmäßige Bewässerung" und "Umgebungsklima" = Kontrolle) sowie natürliche Dürreereignisse im EVENT 1 Experiment. Es wurden Unterschiede in der Dürrestressresistenz und der Einfluss der Zusammensetzung der Pflanzengemeinschaft auf das ökologische Stressgedächtnis von einzelnen Pflanzenarten untersucht. Über mehrere Jahre wiederkehrender milder Dürrestress schien die Pflanzenresistenz im Bezug auf Dürre zu verbessern, und führte dadurch zu einem geringeren Absterben von Blattgewebe während einer sehr strengen Dürre im Vergleich zu Pflanzen mit fast gar keiner Dürreerfahrung. Weiterhin könnte die Zusammensetzung der Pflanzengemeinschaft einen Einfluss auf das ökologische Stressgedächtnis der einzelnen Pflanzenarten gehabt haben.

Zusammenfassend konnte im Rahmen dieser Dissertation gezeigt werden, dass das Design der gewählten Erwärmungstechnik sorgfältig bedacht sein sollte, um verlässliche Prognosen der Pflanzenreaktion auf den Klimawandel machen zu können. Zudem hatten Erwärmung und Dürre keine Auswirkung auf die Schmackhaftigkeit der Blättern von unterschiedlichen *Q. pubescens* Herkünften. Jedoch beeinflusste dahingegen Dürre das Wachstum von zentralen und marginalen *F. sylvatica* Herkünften negativ. Darüber hinaus müssen Dürreereignisse ihrerseits nicht extrem sein, um die Dürreresistenz von Heide- und Graslandgemeinschaften und einzelnen Arten zu fördern.

2 Background of the thesis

Climate Change and extreme weather events

The impact of past and current anthropogenic activities have changed global climate beyond natural forcings (Trenberth 2011a; IPCC 2013). An increase of carbon dioxide by 40% in comparison to pre-industrial values, impacts on the ozone layer by industrial chemicals, pollution by aerosols, and changes in other greenhouse gases lead to heating of the climate system (IPCC 2013). Therefore, globally averaged surface temperatures (land and ocean) have increased by 0.85 [0.65 to 1.06] °C from 1880-2012 (IPCC 2013). The accumulation of the greenhouse gases, with CO2 leading the way, is still uncertain. These uncertainties result in four different climate predictions for the mid and late 21st century (RCP2.6, RCP4.5, RCP6.0, and RCP8.5 scenarios) (Fig.1).

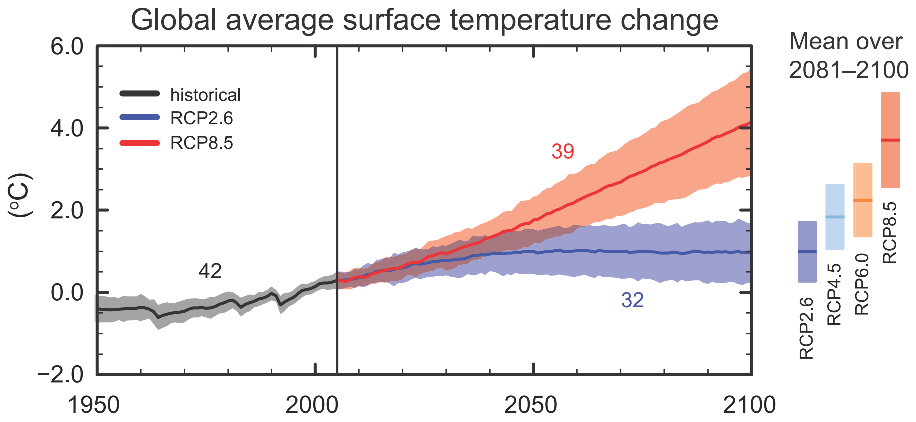

Fig. 1: "CMIP5 multi-model simulated time series from 1950 to 2100 for change in global annual mean surface temperature relative to 1986–2005. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) and RCP8.5 (red). Black (grey shading) is the modelled historical evolution using historical reconstructed forcings. The mean and associated uncertainties averaged over 2081–2100 are given for all RCP scenarios as colored vertical bars. The numbers of CMIP5 models used to calculate the multi-model mean is indicated." (figure and caption reproduced from IPCC 2013)

A global mean surface temperature change is likely in the range of 0.4-1.6°C (RCP2.6), 0.9-2.0°C (RCP4.5), 0.8-1.8°C (RCP6.0), and 1.4-2.6°C (RCP8.5) for the mid 21st century (2046-2065 relative to 1986-2005). Focusing on the late 21st century (2081-2100 relative to 1986-2005) global mean surface temperature increases between 0.3-1.7°C (RCP2.6), 1.1-2.6°C (RCP4.5), 1.4-3.1°C (RCP6.0), and 2.6-4.8°C (RCP8.5) are likely (IPCC 2013). Regarding Germany, a mean temperature increase between 0.8 and 1.3°C is currently predicted for 2021–2050 relative to 1971-2000 (Wagner et al. 2013) and an increase between 2.5-3.5°C is predicted by 2100 (relative to 1961-1990) (Jacob et al. 2008).

Apart from the predicted higher global mean temperatures, extreme climatic events such as drought, heat waves, frost, and heavy rain, are expected to increase in magnitude and frequency in the near future (Meehl et al. 2000; Beniston & Stephenson 2004; Sillmann & Roeckner 2008; O'Gorman & Schneider 2009; IPCC 2012, 2013). The relevance of climatic extremes has been recognized and is a very important issue in ecological research topics and in the global change agenda (Easterling 2000a; Jentsch & Beierkuhnlein 2008; Smith 2011; IPCC 2012, 2013).

Definitions of climatic extremes A change in the mean as well as an increase of the standard deviation of a climatic parameter will produce varyingly strong extreme climatic events (Meehl et al. 2000). An extreme climatic event can be defined, for instance, as "an episode or occurrence in which a statistically rare or unusual climatic period alters ecosystem structure and/or function well outside the bounds of what is considered typical or normal variability" (Smith 2011). Beyond the ecological point of view, climatologists consider an extreme climatic event as "the occurrence of a value of a weather or climate variable above (or below) a threshold value near the upper (or lower) ends of the range of observed values of the variable [...]". Additionally, extreme climatic events can be defined in relation "to their probability of occurrence" or "to a specific (possibly impact-related) threshold" (IPCC 2012). However, no accurate definition of an "extreme" exists (e.g. Stephenson et al. 2008). The definition by Smith (2011) focuses on ecosystem alterations toward climatic extremes and covers therefore only a certain scale. Most organisms can acclimate to an extreme weather event wherein a temperature of 40°C can be extreme in one year and normally tolerated by the organisms in another year (Gutschick & BassiriRad 2003). Thus, definitions of extreme weather events based on the reaction of organisms and ecosystems, respectively, have to be considered with caution. On the contrary, definitions of extreme climatic events based on climate variables could be more reliable because they are easier to characterize and the degree of extremeness is more straightforward to determine.

Heat waves The impact of an extreme event depends on its duration, frequency, magnitude, and abruptness (Jentsch et al. 2007) and its influence on plant species, communities, populations, or ecosystems can be very variable (Smith 2011). How powerful extreme weather events can be became apparent in 2003 (Schär et al. 2004) and 2010 (Barriopedro et al. 2011), when Europe and Russia experienced extraordinary heat waves combined with drought. Both heat waves have revealed the potential of extreme events and showed the ecological and societal impacts (Ciais et al. 2005; García-Herrera et al. 2010). The exceptional summer of 2003, for instance, caused a 30 per cent reduction in gross primary production in Europe (Ciais et al. 2005), forest fires, losses of forest cover and wildlife, an increase in air pollution, a loss of livestock, and a very high elderly mortality across Europe (UNEP 2004). Economic losses due to the 2003 heat wave exceeded US$ 10 billion (Munich Re 2004). In addition, the extreme hot summer of 2010 in Russia caused approximately 25% annual crop failure, forest fires in a land area of over 1 million ha, economic losses of about US$ 15 billion, and a death toll of more than 55,000 (see Barriopedro et al. 2011 supplemental material).

Cold extremes Despite heat waves and drought, evidence suggests that cold extremes may persist into the 21st century in many regions, although the frequency of those extremes are probable to decline (Kodra et al. 2011). Moreover, in Europe, shorter frost seasons are expected and fewer frost days are predicted to occur more scattered in time (Jylhä et al. 2008). Focusing on late spring frost risks, an earlier beginning of the growing season, which is already initiated by global warming, can lead to an increase in late frost damage on flower buds of common perennial herbaceous wildflower species (Inouye 2008). However, an advanced leaf onset due to simulated global warming reduced the late frost leaf injury of *Fagus sylvatica* L. seedlings and showed the importance of timing of frost events with respect to the phenological development (Kreyling et al. 2012a).

Heavy rain Furthermore, more often occurring heavy rain events are found in most land areas worldwide (Easterling et al. 2000b; Peterson et al. 2002; Klein Tank & Können 2003; Klein Tank et al. 2006) and model projections strengthen a heavy rain increase in the context of climate change (Groisman et al. 2005; Alexander et al. 2006; IPCC 2012, 2013). However, the impact of heavy rain events on the water balance of ecosystems may differ. Mesic ecosystems are expected to experience more drought stress because of increases of intervals between heavy rain events. On the contrary, xeric ecosystems may benefit due to a decline in losses of evaporation and thus more available soil water in view of larger but less frequent heavy rain events (Knapp et al. 2008).

In respect of the impact of extreme events, the Intergovernmental Panel on Climate Change (IPCC) issued a risk management report to deal with more frequently occurring extreme weather events and disasters to advance climate change adaptation. The report provides information about the changes in extreme events, the impact on the natural physical environment, as well as on ecosystems, and human systems. A determination of the risks of climatic extremes is contained and a risk management system on the local, national, and international level is presented (IPCC 2012).

Impact of extreme events Focusing on ecosystems, extreme events such as severe droughts or heat waves can lead to rapid mortality of plant species and populations (Breshears et al. 2005; Bigler et al. 2006; Gitlin et al. 2006; Bigler et al. 2007; Miriti et al. 2007), can change ecosystem function and large-scale and/or long-term community structures (MacGillivray et al. 1995; White et al. 2000; Haddad et al. 2002; Ciais et al. 2005; Mueller et al. 2005), and can result in ecotone boundary shifts (Allen & Breshears 1998). On the other hand, extreme events might affect only plant individuals or a few populations with minor effects on the ecosystem (van Peer et al. 2001; van Peer et al. 2004; Marchand et al. 2005; Marchand et al. 2006a; Marchand et al. 2006b; Milbau et al. 2005; Bokhorst et al. 2008; Kreyling et al. 2008a; Arnone et al. 2011; Jentsch et al. 2011). The impact of extreme weather events varies strongly between different plant species, populations, communities, or ecosystems and is furthermore plant idiosyncratic (Lloret et al. 2012). Therefore, impacts of climatic extremes might be very difficult to predict, as plant adaptations to recurrent climatic extremes are also possible (Gutschick & BassiriRad 2003; Walter et al. 2011; Walter et al. 2013; **manuscript 4**). Current research should focus on plant strategies to deal with recurrent extreme events. Focusing on plant mechanisms such as an "ecological stress memory", which enable plants to withstand recurrent climatic extremes (Walter et al. 2013), might be very helpful to understand plant response to forecasted climate scenarios. In addition, Sutherland et al. (2013) state the important question: "What are the magnitudes and durations of carry-over effects of previous environmental experiences on an individual's subsequent life history and consequent population dynamics?", which is one out of the "100 important questions of fundamental importance in pure ecology" and strengthen the importance of ecological stress memory research. Moreover, research on stabilizing processes, which can reinforce plant community resilience, in response to extreme events could render new insights with regards to future climate. In detail, phenotypic variability and plasticity, tolerance, site quality variability or facilitative interactions might reduce and counteract the effects of extreme events (Lloret et al. 2012). Plant species, which exhibit high phenotypic plasticity, for example, might be more prepared to cope with rapid environmental changes (Meier & Leuschner 2008; Berg & Ellers 2010; Nicotra et al. 2010; Richter et al. 2012).

Insect infestations Climate change could be accompanied by insect herbivore infestations as pest species may benefit from warmer conditions (Vanhanen et al. 2007). Temperature rises promote the development rates of insect herbivores positively (Bale et al. 2002; Netherer & Schopf 2010). Additionally, insect calamities might follow extreme events such as drought and may cause greater damages on already stressed plants (White 1984; Huberty & Denno 2004; Rouault et al. 2006; Netherer & Schopf 2010).

In the following, the impact of alterations in precipitation regimes with a special focus on extreme drought events will be presented.

Drought

Global warming influences precipitation and precipitation patterns in a complex way. Heating promotes evaporation, which leads to a drying of the soil surface and thus increases the duration and intensity of drought events. Moreover, the water holding capacity of air rises by about 7% per 1°C according to the Clausius-Clapeyron equation, whereby water vapor in the atmosphere increases (Trenberth 2011b). Thus, the hydrological cycle is changing (Allen & Ingram 2002; Trenberth et al. 2003; Groisman et al. 2004), and drought events could occur more often in the future (Christensen & Christensen 2004; Trenberth 2011b; IPCC 2013). Precipitation seems to increase over Northern mid to high latitudes, equatorial East Africa, and parts of equatorial South America. A decrease in precipitation is predicted over Southern Europe, the Mediterranean, the Middle East, Southern Africa, and parts of the Southern US (Orlowsky & Seneviratne 2012).

In the last decades, Europe experienced several droughts, remarkably in 1976, 1988-1992, 1997 (Bradford 2000), 2003 (Schär & Jendritzky 2004; Rebetez et al. 2006), and 2010 (Barriopedro et al. 2011). An increasing number of drought events throughout the 20th century (Dai et al. 2004; Trenberth et al. 2007) could be measured by the Palmer drought severity index (PDSI). In particular, Dai (2011) detected an increase of the global percentage of dry areas by about 1.74% per decade from 1950 to 2008. Furthermore, very dry land areas (with PDSI less than -3.0) have more than doubled since 1970s (Dai et al. 2004). However, evaluations of predicted changes in dryness depend on the choice of the drought index. In addition, model predictions of soil moisture alterations are less solid than predictions of "consecutive dry days" (Orlowsky & Seneviratne 2012).

For Europe, especially in the Mediterranean droughts are expected to occur earlier in the year and last longer (Beniston et al. 2007). Moreover, the North-Eastern, South-Western, and Southern parts of Germany will most likely experience more drought periods in the future (Schönwiese et al. 2005; Jacob 2009).

Vegetation response to climate change

Climate change impacts plant species, communities, and ecosystems (Parmesan et al. 2000; Walther et al. 2002). As a consequence thereof, plant species extinction (Smith et al. 2009), a loss of biodiversity (Sala et al. 2000; Alkemade et al. 2011), higher rates of biological invasion (Walther 2000; Walther et al. 2002; Jiménez et al. 2011), plant species shifts towards higher altitudes or towards the poles (Parmesan & Yohe 2003; Walther et al. 2005; Murphy et al. 2010), as well as alterations in phenology (Peñuelas & Filella 2001; Fitter 2002; Jentsch et al. 2009; Nagy et al. 2012) and primary productivity (Ciais et al. 2005; Barriopedro et al. 2011) may become more pronounced. Beyond that, the net primary production has increased globally, especially in Amazonia (Nemani et al. 2003). Evidence suggests that a combination of different factors, such as atmospheric CO_2 fertilization, increases in solar radiation associated with a decline of cloud cover (Nemani et al. 2003), and a reduced water consumption via CO_2 induced decrease in leaf conductance (Morgan et al. 2004) led to an increase of terrestrial net primary production.

Loss of plant species Regarding forecasted plant species losses, extinction rates range between 3% and 6% for plant species with the ability to dispersal and between 9% and 21% without dispersal in relation to the respective projection and strength (minimum, mid-range or maximum) of climate change in Europe (Thomas et al. 2004).

Losses and shifts in biodiversity Moreover, high losses and shifts in biodiversity are predicted due to global warming in Northern Europe with a more than 35% share of new species within the entire species composition in 2100. On the contrary, Southern Europe will experience a species loss of approximately 25% due to the climatic conditions as forecasted for 2100. Major alterations are predicted for the arctic and tundra regions as well as for the Mediterranean scrubland and natural grassland/steppe (Alkemade et al. 2011). However, the predicted impact of climate change made by Alkemade et al. (2011) is based only on an average climate data set, whereby extreme events with their varying amplitude and timing are neglected. Including those aspects the effect of climate change on biodiversity could be worse. Therefore, the predictions of Alkemade et al. (2011) are considered with caution. However, the forecasted shifts in biodiversity of Alkemade et al. (2011) are in accordance with observations (e.g. Walther et al. 2002; Parmesan & Yohe 2003; EEA 2012).

Biological invasion Beyond plant species shift towards higher altitudes and extinctions, climate change is also accompanied by biological invasion. Global warming leads to a decrease in frost days per year, for example, and results in the occupation of suitable sites for exotic vegetation due to the displacement of indigenous vegetation (Walther 2000). Range expansions of exotic plant species are observed (Taylor et al. 2012) and predicted (Kriticos et al. 2003; Wang et al. 2011; Taylor et al. 2012) due to warmer climatic conditions. Moreover, extreme drought events can disturb plant communities and thus promote invasion of exotic plant species (Jiménez et al. 2011).

Shifts in phenology Global warming leads to shifts in plant phenology (Peñuelas & Filella 2001). An earlier onset of spring induced by temperature increases leads to earlier shooting and flowering of plants (Walther et al. 2002). Across the Northern Hemisphere spring occurs earlier by 2.8 days decade^{-1} (Parmesan 2007). Across Europe an average advancement of spring/summer by 2.5 days decade^{-1} could be detected (Menzel et al. 2006). Moreover, an increase of the average annual growing season by 10.8 days has occurred since the early 1960s in Europe (Menzel & Fabian 1999). Alterations in plant phenology might disrupt plant-pollinator interactions and could entail serious consequences such as plant and pollinator extinctions (Memmott et al. 2007). On the other hand, changes in phenology can also lead to disruptions between defoliating insects and host plants (Visser & Holleman 2001), which might reduce the defoliating risk for host plants.

Plant response to extreme events In the past the main focus of climate change research has been on shifts in average conditions. More difficult is the assessment of the response of vegetation to extreme weather events (Jentsch & Beierkuhnlein 2008). Figure 2 visualizes the classical point of view that extreme events lead to abrupt changes in vegetation (a), whereas gradual changes in climate cause a gradual response (d). The crossed relations (b and c) might seem counterintuitive but there is theoretical and empirical evidence in their favor (Lloret et al.

2012).

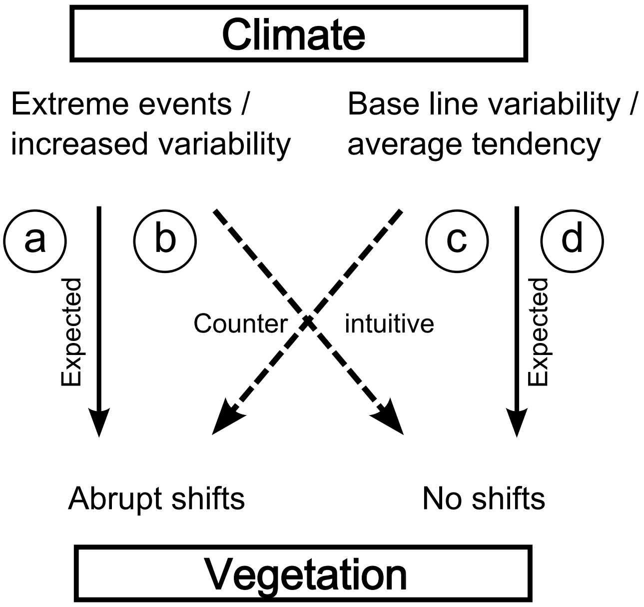

Fig. 2: “Conceptual model of vegetation shift response to climate. Abrupt shifts could occur under extreme events or increased climatic variability (case a) or under gradual climatic change involving trends in mean climatic parameters and leading to a turning point (case c). Alternatively, no shifts (but gradual vegetation changes may happen on the long term) would be observed if climate change involves gradual changes in the average climatic tendency (case d), or in spite of extreme events or increased climatic variability (case b). While cases a and d are expected from classical studies of the impact of climatic changes on vegetation, cases b and c, are counterintuitive but there is both theoretical and empirical evidence supporting their importance.” (figure and caption reproduced from Lloret et al. 2012)

Species diversity (Kahmen et al. 2005), functional diversity (Kreyling et al. 2008a; Kreyling et al. 2008b), and environmental factors (Peñuelas et al. 2004) seem to be important key factors in plant response to extreme weather events. Furthermore, severity and recurrence of extreme weather events, such as drought, are crucial to plant response (Jentsch & Beierkuhnlein 2008). Indeed, plants are able to withstand extreme weather events due to stabilizing processes such as phenotypic variability and plasticity, tolerance, biological interactions (e.g. facilitation), or site quality. A reduced plant mortality risk or higher rates of survival and recruitment, respectively, might be a result of those stabilizing processes. In terms of vegetation shifts, vegetation can also exhibit persistence accompanied with only slight alterations after the extreme event (Lloret et al. 2012).

Plant responses to extreme weather events are highly variable and depend on the respective growth form. Herbs and grasses might respond different to changing climatic conditions compared to woody species and trees. The long lifespan of perennial species such as trees makes a rapid response to climate change more difficult (Lindner et al. 2010). However, some tree species or populations might cope with those climatic alterations on the basis of phenotypic plasticity (Richter et al. 2012). The expected extinction risk due to habitat losses might be higher in temperate mixed/deciduous forests and shrublands than in pastures and croplands (Thomas et al. 2004). Therefore, in the following, the response of grassland, heath, and tree species to warming and drought, representing the main research of this thesis, will be considered separately. Additionally, a special emphasis will be on plant biomass as this reflects the main response parameter of the dissertation.

The impact of warming and drought on grassland

Aboveground biomass European grassland ecosystems, mostly managed as pastures and hay meadows, are important economic resources in European agriculture. The expected climatic alterations may impair agricultural crops, whereby Northern Europe might experience a crop productivity increase while Southern Europe will experience crop productivity decreases (Falloon & Betts 2010). The impact of climate change, such as higher mean temperatures, has already shown variable results in aboveground plant productivity in grasslands. Rustad et al. (2001) found increases and decreases in grassland productivity subjected to experimental warming, whereas De Boeck et al. (2007) detected only a decline, and Beierkuhnlein et al. (2011) found no alterations. However, Rustad et al. (2001) examined mostly natural grasslands in their meta-analysis (covering 2-9 years), De Boeck et al. (2007) used artificially assembled grassland model ecosystems (over one growing season), and Beierkuhnlein et al. (2011) carried out their study in a common garden experiment (potted plant individuals, over one growing season). Regarding the different findings of these studies, the scale of observation might have led to the observed differences in plant responses. Focusing on long-term observations, Craine et al. (2012) detected across 27 years only in a certain period of the growing season (critical period during 25 days; July 9 – August 2) a decline in grassland productivity due to high temperatures. Thus, grassland response to higher temperatures might be very variable depending on the timescale.

Beside temperature increases, drought events can also cause a reduction in biomass production (Grime 2000; Ciais et al. 2005; Kahmen et al. 2005; Beierkuhnlein et al. 2011). Nevertheless, variable results in aboveground net primary productivity due to drought are observed. Cherwin & Knapp (2012), for instance, found no drought impact on semi-arid grasslands at the southern site (warmest and wettest) along a north-south gradient compared to the central and northern site (coolest and driest). However, the plant performance at the southern site could not be explained by edaphic gradients or limitation of other resources, but may be a response to higher precipitation during the growing season. Gilgen & Buchmann (2009) also detected different responses of grasslands to drought at different altitudes across Switzerland. The results of Gilgen & Buchmann (2009) reflect that annual precipitation might play an important role with respect to the observed plant response to drought. Thus, alterations in annual precipitation will also be relevant in the context of more frequently occurring drought events. Moreover, drought can reduce productivity of grasslands, but the effects can decrease within the progressing growing season (Craine et al. 2012). On the other hand, aboveground biomass remains surprisingly stable under single or recurrent extreme drought events (Kreyling et al. 2008a; Jentsch et al. 2011). Additionally, grass species seem to remember drought stress when subjected to recurrent drought. Walter et al. (2011) found hints for a "drought-memory" in *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl plants. An increase in percentage of living biomass in plants was found, which experienced a previous drought compared to plants without drought experience during a second late drought event within one growing season. Furthermore, such legacy effects in aboveground net primary production were also detected in desert grasslands over a period of three years. Here, the species experienced different two-year pre-exposures (five levels of annual precipitation) and a reversed exposure in the third year. The two-year pre-exposure explained 20% of the inter-annual variability of aboveground net primary production in the third year (Reichmann et al. 2013). However, drought events often had no long-term effects on plant productivity in mesic grasslands (Naudts et al. 2011).

Belowground biomass Regarding belowground biomass, a similar uncertainty of plant response subjected to drought exists. In general, increased root growth into deeper soil layers can be observed under drought stress (Kahmen et al. 2005; Ehdaie et al. 2012) and thus increases belowground biomass. However, shifts in root biomass in both directions, to higher or lower rates, are known in response to drought (Wedderburn et al. 2010; Weißhuhn et al. 2011; Fiala et al. 2012). Additionally, belowground biomass could also show no response to drought stress (Gilgen & Buchmann 2009; Jentsch et al. 2011; **manuscript 4**). Grassland species can respond differently to drought stress (Bessler et al. 2009) and therefore compensation within grassland communities might be an option to stabilize community root biomass (Gilgen & Buchmann 2009).

Warming can also impact root growth of grassland species. Pilon et al. (2013) (Clermont Climate Change Experiment) detected an increase in root growth of grassland monoliths due to warming by transplantation along an altitudinal gradient over a three-year examination. In contrast, De Boeck et al. (2007) found a decrease in root productivity in experimental grassland under warming. On the other hand, Edwards et al. (2004) found fluctuations in root number and mass within one growing season, whereby experimental soil warming induced a growth increase in roots during spring and revealed a root mortality increase during autumn and winter.

To sum up, the impact of warming and extreme drought events on the biomass production of grasslands seems variable. The respective scale of research observations, namely long-term observations versus different more short-term experimental approaches might play a major role with regards to plant response. Therefore, predicting alterations in grassland biomass production due to warming and drought seem quite difficult and also depend on the respective European region. Expanding experimental research approaches with a focus on recurrent drought events might be important as plants might 'remember' drought and thus could tolerate or adapt to the changing environmental conditions (**manuscript 4**).

The impact of warming and drought on heath

Aboveground biomass Heath ecosystems are important nature reserves in Europe and function as a habitat for partially endangered faunal species. The expected higher mean air temperatures induced by global warming will also impact on heath. Peñuelas et al. (2004) (CLIMOOR and VULCAN research projects) investigated alterations in aboveground biomass production in shrublands along a north-south European gradient (UK, Denmark, the Netherlands, and Spain) with regard to experimental warming over a period of two years. The aboveground plant biomass tended to increase at the northern sites, especially in the UK, compared to the southern sites (Peñuelas et al. 2004). Additionally, the same north-south gradient picture was found in a longer time series (seven years of experimentation) (Peñuelas et al. 2007). In contrast, including data of the extreme hot year 2003 into the analysis, the north-south gradient effect vanished and the warming effect was not significant (Peñuelas et al. 2007). Apart from the influence of the extreme conditions in 2003, the northern sites seem to benefit from warmer conditions in the near future, as temperature is the most limiting factor in the North (Körner & Larcher 1988). In addition, Wahren et al. (2013) (part of the International Tundra Experiment — ITEX Network) detected an increase of mean canopy height and of forb and shrub cover in an alpine heath with regard to experimental warming over a seven-year study period, whereas graminoid cover decreased. By contrast, Kongstad et al. (2012) (CLIMAITE experiment) found no experimental warming impact on aboveground biomass of heath dominated by *Calluna vulgaris* (L.) Hull and *Deschampsia flexuosa* (L.) Trin. over a period of three years in Denmark.

Drought leads to a decline in aboveground biomass productivity in heath (Gordon et al. 1999; Peñuelas et al. 2004; Peñuelas et al. 2007). However, plant resistance against climatic alterations, such as drought, might depend on succession status, whereby plant communities near the equilibrium state are less susceptible to disturbance (Kröel-Dulay et al. submitted). Furthermore, drought can decrease plant aboveground biomass, but the drought effect compared to control can also disappear 1-2 months after the drought stress period (Kongstad et al. 2012). However, Kreyling et al. (2008a) revealed no decrease in aboveground net primary production in experimental heath communities when subjected to extreme drought. Albert et al. (2012) found contrasting results in an experimental heath community; drought had no impact on the aboveground biomass of *C. vulgaris* (evergreen dwarf shrub), but reduced aboveground biomass of *D. flexuosa* (grass species) within one growing season. Both heath species revealed a different strategy to cope with drought, whereby *C. vulgaris* preserved shoot biomass and thus showed a higher resilience than *D. flexuosa*. Ransijn et al. (submitted) found no significant effect of an experimental drought on the competitive balance between *C. vulgaris* and *D. flexuosa*. In particular, the aboveground biomass depended on the dominance of either of the two plant species much more than on the drought exposure. Moreover, experimental heath communities exhibited a high resistance to a very severe drought event (57% of the growing season without precipitation) with respect to tissue die-back, whereby the heath communities and species with previous drought experience revealed a lower tissue die-back than plants with hardly any drought experience (**manuscript 4** – EVENT 1 experiment).

Belowground biomass Furthermore, drought can also impact belowground biomass of heath species. Andresen et al. (2010) detected an increase in fine root biomass of *C. vulgaris* with regard to drought. Focusing on experimental warming, *C. vulgaris* revealed an increase in fine root biomass, while fine root biomass of *D. flexuosa* tended to decline. However, Arndal et al. (2013) found no alterations in root biomass of *C. vulgaris* due to drought, but a significant root increase when exposed to experimental warming. It is known that root length growth correlates positively with soil temperature (Pregitzer et al. 2000) and thus may explain the root response of *C. vulgaris* due to experimental warming.

This short overview of the response of heath to warming and drought demonstrates the importance of the scale of observation as already mentioned for research results in grasslands. In addition, the European region is also important in the context of plant biomass responses, as Northern Europe, for example, might benefit from higher mean air temperatures compared to Southern Europe. Different growth forms in heath communities, e.g. shrubs versus graminoids, could also experience different impacts due to warming and drought. Expanding research efforts with regards to experimental warming and recurrent pulsed drought events could result in a more detailed knowledge about how heath communities and species might respond to predicted future conditions.

The impact of warming and drought on tree species

Tree species might be especially vulnerable to a changing climate. Perennial species exhibit a long life-span, which will exacerbate adaptation to rapidly changing environmental conditions in an appropriate time period (Lindner et al. 2010). However, phenotypic plasticity might be a possibility for tree species to deal with climate change (Richter et al. 2012). Additionally, an adaptation to a warmer climate via rapid alterations in gene frequency is known for *F. sylvatica* (Jump et al. 2006). Negative and positive effects on trees are associated with climate change (Allen et al. 2010). For example, tree growth stimulation by CO_2 fertilization, an increase of water use efficiency or an extension of growing seasons are positive forecasts (Bachelet et al. 2003; Scholze et al. 2006). On the other hand, a decline in growth, an increase in mortality and stress induced by climatic alterations as well as forest insect infestations (Ayres & Lombardero 2000; Lucht et al. 2006; Scholze et al. 2006; Lloyd & Bunn 2007) are also predicted. However, the impact of climate change on tree species depends on the regional context (Lindner et al. 2010). In the following, the focus will be on the temperate zone (subdivided in temperate oceanic and continental zone) of Europe, which represents the geographic position of research interest of the dissertation.

Temperate oceanic zone In the temperate oceanic zone of Europe, rising temperatures are projected to enhance tree growth in the northern and western parts (in less water-limited areas), and will lead to a decline in southern and eastern parts (in water-limited areas) (Lindner et al. 2010). For Germany, positive scenarios associated with precipitation increases predict an increase up to 7% in forest productivity (for three out of four main species), and under drier scenarios a decline of 4% to 16% in forest productivity (Lasch et al. 2002). Moreover, extreme events such as drought are expected to become more hazardous for tree species in the future (Lindner et al. 2010). Higher temperatures will also promote the development of insect herbivores. Therefore, more often occurring forest insect infestations are expected in the near future, especially for bark beetles (Lindner et al. 2010). Range shifts and expansions of pest species such as *Lymantria dispar* L. (gypsy moth) (preferring leaves of *Quercus* species (Lazarevic et al. 2002)) and *Lymantria monacha* L. (nun moth) (preferring leaves of *Picea abies* (L.) H. Karst. (Heiermann & Schütz 2008)) (Karolewski et al. 2007), *Thaumetopoea pityocampa* Denis & Schiffermüller (pine processionary moth) (feeding on pine foliage) (Battisti et al. 2005; Robinet et al. 2007) or of thermophilic pathogens, such as *Biscogniauxia mediterranea* (De Not.) O. Kuntze (preferring pines and oaks) (Desprez-Loustau et al. 2007) are expected in the temperate oceanic zone. A decline of *F. sylvatica* due to *Phytophthora* spp. (plant-damaging Oomycetes (water molds)) infection in combination with the wet and extreme dry conditions in 2002/2003 could already be detected in Germany (Jung 2009).

Temperate continental zone In the temperate continental zone, forest growth depends mainly on water availability (Maracchi et al. 2005). Thus, the predicted stronger water-limitations will influence tree productivity in the future. Especially, conifer productivity is projected to decrease in continental and Central Europe (Lexer et al. 2002). However, forest response to a warmer climate depends on site conditions and varies between tree species. *F. sylvatica*, for example, which is susceptible to drought, may lose dominance on certain sites (Geßler et al. 2007). On the other hand, *Quercus pubescens* Willd., which shows physiological adaptation to warm and dry climatic conditions (Damesin & Rambal 1995; Haldimann et al. 2008), may reveal a higher resilience to climate change, but is more preferred by insect herbivores, such as *L. dispar* (Wellenstein & Schwenke 1978). Mass outbreaks of pest species are also very likely in this climatic zone (Lindner et al. 2010). For instance, infestations of spruce forests with *Ips typographus* L. (European spruce bark beetle) or *Pityogenes chalcographus* L. (six-dentated bark beetle) will be promoted by abiotic disturbances such as drought (Wermelinger 2004). In addition, an assortment of fungal diseases and pest insects (e.g. *Armillaria* spp., *L. dispar*, *Tortrix viridana* L., *Agrilus* spp., *Scolytus* spp.) are suspected to promote oak die-back in combination with unfavorable climatic conditions such as prolonged drought (Balci & Halmschlager 2003).

The impact of climate change on tree species depends on factors such as climatic alterations on geographical location, site conditions, and on the tree species itself. On the other hand, trees can show local adaptation to drought stress or phenotypic plasticity to withstand drought events. In the following, local adaption and within-species diversity of tree populations in the context of climate change will be discussed.

Within-species diversity and local adaptation[1] of trees to environmental conditions in the context of climate change

Tree species can show adaptation to climatic conditions at specific sites within their entire geographic range (Czajkowski & Bolte 2006; Rose et al. 2009; Mimura & Aitken 2010; Kreyling et al. 2012a). Those local adaptation to climatic conditions is strongly linked to genetic and phenotypic differences in plant species as well as in tree species (Joshi et al. 2001; Hufford & Mazer 2003; McKay et al. 2005; Savolainen et al. 2007; Bennie et al. 2010). Especially tree species with a wide geographic range, such as *F. sylvatica*, which covers a large range of climatic conditions, reveals local adaptation to drought at the eastern distribution boundary (Czajkowski & Bolte 2006; Rose et al. 2009). Environmental conditions as

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[1] Local adaptation means that local individuals have a higher fitness at their home site in comparison with that of nonlocal individuals of the same species (Biere & Verhoeven 2008)

well as diminished genetic exchange due to geographic isolation at the margin of the geographic range induce a stronger selective pressure on marginal populations compared to those located in the center (Choler et al. 2004; Kawecki 2008; Paul et al. 2011). Thus, local adaptation seems more likely to occur at marginal sites of the geographic range. However, investigations of local adaptation at marginal sites are rare. Coulleri (2010), for example, found evidence for local adaptation in *Ilex dumosa* Reissek in a marginal population due to superior plant performance in its native area compared to worse performance of this species in other climatic environments as well as in comparison to populations of the center. By contrast, Savolainen et al. (2007) assume local adaptation in *Pinus sylvestris* L. populations due to relative fitness measurements in central parts of its geographic range and a breakdown in local adaptation close to the Northern range margins in Europe.

Tree provenances Conducting provenance trials has a long tradition and started more than 200 years ago, where foresters collected seeds and seedlings from different provenances for common garden experiments (Langlet 1971). Provenance trials were performed in order to test for differences such as: primary production, effects of drought, drought resistance or local adaptation (e.g. García-Plazaola & Becerril 2000; Oleksyn et al. 2000; Peuke et al. 2002; Nielsen & Jørgensen 2003; Czajkowski & Bolte 2006; Peuke et al. 2006; Kreyling et al. 2012b; Wellstein & Cianfaglione 2014; **manuscript 3**). In the context of climate change, the concept to intermix forest stands with drought-resistant provenances from other locations, which already experience predicted climate change conditions in their home site, is taken into consideration (Bolte et al. 2009; Bolte & Degen 2010). Especially for the deciduous forest tree *F. sylvatica*, which naturally dominates Central European forests (Rose et al. 2009). For example, the extreme dry year of 2003 revealed a drought adaptation in Greek beech populations (Fotelli et al. 2009). Regarding beech forests, the usage of those drought-resistant populations and provenances, respectively, are in the focus to promote forest resistance in the face of climate change (Bolte et al. 2009; Bolte & Degen 2010).

Furthermore, trees are able to tolerate a variety of climatic conditions due to genetic constitution, genetic adaptation, as well as phenotypic plasticity (Morgenstern 1996; Savolainen et al. 2007; Richter et al. 2012). Phenotypic plasticity appears to be an important mechanism for trees to deal with rapid climatic change (Nicotra et al. 2010). Therefore, focusing only on drought-resistant tree provenances might miss the target to ensure tree stand persistence in future. Intermixing tree stands with drought-resistant tree provenances and provenances with high phenotypic plasticity could promote forest resistance through local within-species diversity.

Introduction of non-native tree species Besides promoting within-species diversity of native tree stands, an introduction of non-native tree species might also be an option to maintain silvicultural yield in the face of climate change. Non-native tree species such as *Pseudotsuga menziesii* (Mirb.) Franco, *Larix kaempferi* (Lam.) Carrière, *Quercus rubra* L., *Robinia pseudoacacia* L., *Pinus strobus* L., *Pinus nigra* J. F. Arnold, *Abies grandis* (D. Don) Lindl., or *Castanea sativa* Mill. are already cultivated in Germany, whereby *P. menziesii* covers the greatest part with 1.7% of the German forest area (Kölling 2013). In addition, several other non-native tree species are favored for tentative plantings in Bavaria (Germany), namely *Abies borisii-regis* Mattf., *Abies bornmuelleriana* Mattf., *Carya glabra* (Mill.) Sweet, *Fagus orientalis* Lipsky, *Pinus echinata* Mill., *Pinus peuce* Griseb., *Pinus ponderosa* Dougl., *Pinus tabulaeformis* Carr., *Pinus virginiana* Mill., *Quercus mongolica* Fisch. ex Turcz., and *Tilia tomentosa* Moench (Bolte et al. 2009; Schmiedinger et al. 2009). However, cultivation of non-native tree species could be associated with risks for native tree stands such as changes in host-pathogen systems (Bolte et al. 2009). Additionally, the potential of an invasion by the non-native tree species in the new habitat as well as the competitive character of the new tree species have to be considered carefully before cultivation (Bolte et al. 2009; Kölling 2013).

To sum up, intermixing native tree stands with drought-resistant non-local tree provenances as well as promoting and maintaining within-species diversity of native tree stands should be a primary goal in present and future forestry to ensure native tree stand resistance and yield in the context of ongoing climate change. Therefore, searching for suitable and drought-resistant non-local tree provenances within the geographic range (e.g. center versus margin) of the respective tree species is an important research objective, which is also in the focus of the work in hand besides the impact of warming and drought on tree species (**manuscript 3**).

Limitations of experimental climatic simulations

Experimental climatic simulations are a useful tool to investigate plant response to predicted global warming and extreme weather events. Techniques such as transplantation to a warmer site (e.g. Joshi et al. 2001; Link et al. 2003; Turetsky et al. 2008; Gonzalo-Turpin & Hazard 2009; Saarinen & Lundell 2010; Haggerty & Galloway 2011; **manuscript 1**) or passive warming (e.g. Henry & Molau 1997; Marion et al. 1997; Beier et al. 2004; Dabros et al. 2010; De Boeck et al. 2012; Kreyling et al. 2012b; Thiel et al. 2012; **manuscript 1**) are applied to simulate global warming. Each technique exhibits advantages and drawbacks, and may reach its limits, especially in making reliable forecasts (Leuzinger et al. 2011). Regarding warming by transplantation a transplantation shock (Tetsumura et al. 1998) or herbivory (Kile et al. 2013), for instance, could influence plant response at the transplanted site in addition to new environmental conditions. These side effects could make it difficult to evaluate plant responses at the transplanted site, as their impact might obscure the main effect of interest. Moreover, warming by transplantation requires more efforts and costs compared to experimental approaches without transplantation design.

On the contrary, plants subjected to passive warming techniques do not experience a step change of the entire environment. Yet, passive warming with curtains of aluminum for nighttime warming, for example, also has drawbacks, such as edge effects (increased heat loss near the edges compared with plot center) (Beier et al. 2004).

However, Rustad et al. (2001) found no differences in their meta-analysis in effect sizes of their response variables soil respiration, net N mineralization, and aboveground plant productivity between different experimental warming techniques (field chamber, overhead infrared lamps, greenhouse, night-time warming, and electrical heat-resistance ground cables). In this case, plant response seems to be more susceptible to differences between biomes, vegetation types, and experimental sites than to the magnitude of experimental warming over a period of 2-9 years. Focusing only on one factor, i.e. experimental warming, in a comparison of different experimental warming techniques leads to the impression that they do not differ in their magnitude and impact on plant performance. Backhaus et al. (2014) (**manuscript 1**) conducted for the very first time a direct comparison of warming by transplantation and passive warming and included drought as a further climatic factor. Contrary to Rustad et al. (2001), Backhaus et al. (2014) (**manuscript 1**) revealed significant differences when comparing plant growth under warming by transplantation and under passive warming in one growing season. However, the differences between the warming techniques are mainly attributed to the combination of warming and drought. Plant growth differed between the two warming techniques, whereby plants under passive warming achieved a significant higher height increment than plants subjected to warming by transplantation or ambient conditions (i.e. without warming manipulation) all exposed to extreme drought. Thus, the single-factor effects were not additive in this study and the interaction of both climatic manipulations exhibited interesting aspects in plant performance. Similar non-additive effects of different climatic parameters in experimental approaches could also be observed by other authors (Shaw et al. 2002; De Boeck et al. 2011; Larsen et al. 2011; Kreyling et al. 2012a). Indeed, the findings of Backhaus et al. (2014) (**manuscript 1**) reflect only short-term plant responses and might be expanded with results over several years. Further experimental warming approaches could also include temperature extremes besides elevated mean air temperatures and investigations of thresholds with regard to plant response (Kreyling & Beier 2013). The implementation of gradient/regression-type experiments (Beier et al. 2012; Kreyling et al. 2013) might be useful to investigate thresholds of plant sensitivity (Kreyling & Beier 2013).

Reliability of future predictions Making reliable predictions with experimental results is difficult, as experiments mostly cover a small temporal and spatial scale. Consequently, up-scaling of experimental results to a realistic climate change scenario is complicated and might be error-prone (Leuzinger et al. 2011; Wolkovich et al. 2012). As the effect size of plant response dampens in the context of longer time periods and larger spatial scales, short-term experiments may overestimate the impact of climate change (Leuzinger et al. 2011). Extending our knowledge of plant response to climate change needs research combinations of long-term experiments on field-scale with a regression/gradient approach (Beier et al. 2012), for instance, combined with ecosystem models and short-term laboratory experiments to improve the up-scaling and thus the reliability of predictions (Kreyling et al. 2013). Additionally, comparing those combinations of short- and long-term experimental approaches with observational studies could improve the confidence of our predictions on plant responses (De Boeck et al. in revision). Furthermore, the detection of artifacts and flaws of experimental techniques are urgently needed in the context of reliable future predictions of plant response. Beyond that, the consideration of ecosystem characteristics (i.e. spatial scale and heterogeneity, biological complexity) as well as environmental factors and drivers (i.e. island effects, interdependence of manipulation factors, stepwise versus gradual changes, time scales or treatment choices) in the experimental design should be made carefully to avoid or restrict experimental artifacts and flaws (De Boeck et al. in revision).

Coordinated distributed experiments Coordinated distributed experiments (CDEs) at an international scale, which are a useful tool to improve databases for climate change meta-analyses (Fraser et al. 2013), might also help to reveal experimental artifacts and flaws. Moreover, long-term CDEs at different geographic locations could help to examine if plant response is based on climatic manipulation or on site-specific factors such as soil characteristics or plant species composition, especially in respect of precipitation manipulation experiments (Beier et al. 2012). Regarding the manipulation of precipitation in experiments, Beier et al. (2012) pointed out in their review that only one meta-analysis (Wu et al. 2011) exists in this context, as the manipulation of precipitation is quite complex and therefore difficult to compare. It is important to improve future precipitation manipulation experiments with CDEs or gradient/regression-type experiments and to include extremity, variability as well as interactions with other climatic drivers into the experimental design (Beier et al. 2012; Thompson et al. 2013).

Finally, a single experimental approach can only cover a certain task or hypotheses and those results should not be generalized or over-interpreted in the context of climate change, especially if the experiment was conducted over a short research period. An experimental framework such as CDEs is needed to make extrapolations and future projections more reliable with respect to plant response.

3 Synopsis of the thesis

Objectives of the thesis

The impact of warming and drought on key European tree, dwarf shrub, and grass species of special importance in plant ecology including aspects of biotic interactions were in the focus of the dissertation in hand. The variation in plant response to warming and drought depends on factors such as different geographic locations (e.g. North versus South Europe), scales of observation (long-term versus short-term experiments), site conditions, different experimental techniques, and on the properties of the respective plant species itself as already mentioned in the introduction section. In this context, four different research questions were chosen to investigate the impact of warming and drought in this dissertation:

1. How reliable are our experimental evidences and techniques (passive warming versus warming by transplantation) (**manuscript 1**)?

2. Is the leaf palatability of *Quercus pubescens* influenced by warming and drought (**manuscript 2**)?

3. Does drought response of *Fagus sylvatica* differ between central and marginal provenances (**manuscript 3**)?

4. Does stress response of plant communities and species (heath and grassland) differ if drought reoccurs (**manuscript 4**)?

Main findings and research outlook

Manuscript 1

Experimental approaches to examine the impact of forecasted warmer conditions and the limitations of those warming techniques are presented in **manuscript 1** (passive warming versus warming by transplantation). Basic knowledge about limitations of experimental techniques and predictions of short-term experiments exists (Marion et al. 1997; Leuzinger et al. 2011; Beier et al. 2012; De Boeck et al. 2012; Wolkovich et al. 2012). However, a direct comparison of passive warming and warming by transplantation along climatic gradients has not been done so far. Therefore, a comparison of both techniques within the EVENT 3 experiment, which is located in the Ecological-Botanical Garden of the University of Bayreuth (experimental warming site) as well as at the campus Siebeldingen of the University Koblenz-Landau (transplantation site), was carried out. The experimental sites differed in their long-term mean annual temperature by 2°C, whereby the experimental warming site in Bayreuth exhibit 8.2°C and the transplantation site in Siebeldingen 10.2°C. The plant growth of *F. sylvatica* seedlings (potted individuals) was examined under both warming techniques and in combination with extreme drought conditions during one growing season.

Contrary to the expectations that both warming techniques will lead to the same increase in height increment, we found differences in height increment of *F. sylvatica* seedlings between the two warming techniques, which were mainly attributed to warming in combination with an extreme drought. Thus, the results of the research question *"How reliable are our experimental evidences and techniques (passive warming versus warming by transplantation)?"* showed that the choice of the technique is crucial in the context of making reliable and comparable future predictions. Regarding future research, new solutions in experimental techniques are obviously needed (e.g. Beier et al. 2012; De Boeck et al. 2012) to improve the reliability of the results. The findings of **manuscript 1** indicate that further parameters such as evapotranspiration, photosynthetically active radiation, and wind speed in addition to temperature should be taken into consideration in further research studies with experimental warming. Thereby, the knowledge of how the warming technique and the mentioned parameters influence plant performance could be advanced. Moreover, experimental approaches such as the comparison of passive warming with warming by transplantation should be enlarged over a longer time span and by further response parameters. The comparison of long-term experiments to short-term experiments can yield deeper insights and thus might be an option to improve our future predictions of plant response to climate change.

Manuscript 2

In consideration of more frequently expected pest insect outbreaks in forests in the future, the impact of warming and drought on the leaf palatability of four different *Q. pubescens* provenances from Bulgaria, Germany, Hungary, and Italy were investigated in Bayreuth (EVENT 3 experiment). The provenances exhibit differences in the mean annual temperature and precipitation as well as in altitude, whereby the Bulgarian (620 mm mean annual precipitation – MAP) and Hungarian (587 mm MAP) *Q. pubescens* provenances originate from drier sites compared to the German (950 mm MAP) and Italian (876 mm MAP) ones. Palatability experiments are common, but the usage of different provenances is very rare in that context. Regarding the topic of tree stand mixtures to promote forest resistance (see subchapter *Within-species diversity and local adaptation of trees to environmental conditions in the context of climate change* in the introduction), the investigation of different tree provenances in a palatability experiment can support the assessment of suitable provenances with regard to climate change. Introduced drought-resistant tree provenances might withstand prolonged drought events, but the impact of insect herbivores at the new location is not well understood. In particular, it is still unclear if local or non-local provenances are more prone to insect infestations.

For the palatability experiment we used caterpillars of the generalist forest pest *L. dispar* to examine the influence of "warming", "drought", and "warming combined with drought" on the leaf palatability of *Q. pubescens*. Consumed leaf dry material, density of trichomes (i.e. leaf hairs), and the specific leaf area were recorded. Surprisingly, the research question *"Is the leaf palatability of Quercus pubescens influenced by warming and drought?"* revealed that the climatic manipulations did not affect the palatability of *Q. pubescens* leaves within the time of observation. Apart from this, the leaf palatability was related to the density of trichomes. Regarding the provenance aspect, we detected a higher value of consumed leaf dry material of Bulgarian tree seedlings, which had the lowest density of trichomes. The study suggests that "warming", "drought", and "warming combined with drought" might not alter the leaf palatability of *Q. pubescens* with regard to expected climatic alterations. In addition, it might be possible that the four European *Q. pubescens* provenances could be spared from more frequently occurring *L. dispar* infestations in the future. Finally, the results of the study should be considered as hints for future events and might be expanded with a palatability experiment under field conditions, where the insect herbivore would also experience the climatic alterations. Additionally, the usage of more than four European *Q. pubescens* provenances could also be helpful in order to expand the knowledge of how warming and drought will influence the leaf palatability of pubescent oak in the future.

Manuscript 3

The aspects local drought-adaptation of tree provenances and the possibility to enhance tree stand resistance with drought-resistant tree provenances in the context of climate change were in the focus of **manuscript 3**. The response of three central (all from Germany) and three marginal (Bulgaria, Spain, and Poland) *F. sylvatica* provenances to drought was investigated in two different soil types (loam, sand) at the EVENT 3 experimental site in Siebeldingen. The aim was to detect differences between *F. sylvatica* provenances from the center and the margin of the species' geographic range with respect to drought adaptation, whereas a lower drought sensitivity was expected in the marginal provenances. The response parameters height/diameter increment and leaf damage by drought-stress were chosen and the summer heat moisture index (Tuhkanen 1980) was calculated. Drought influenced height/diameter increment of *F. sylvatica* seedlings negatively and lead to leaf damage, whereas the negative effects were exacerbated on sandy substrate. Therefore, the interaction of climatic conditions and abiotic parameters such as soil type are important with regard to a changing climate. The provenances revealed differences in their reaction to drought and soil type. A minor decline in diameter increment could be found for the Bulgarian *F. sylvatica* provenance, whereas two German provenances revealed strong decreases due to drought. Focusing on soil type, *F. sylvatica* provenances from Bulgaria and Spain showed a relatively stable diameter increment on sand in comparison to the loamy substrate, whereby the three German and the Polish provenances performed better on loam. In the case of height increment, the Bulgarian *F. sylvatica* provenance showed a positive reaction to sand, while all other provenances revealed a negative or no response to sand compared to loam. Moreover, local adaptation to summer drought could be found with regards to mortality. In sum, the Bulgarian *F. sylvatica* provenance with an overall low total increment may exhibit a trade-off between growth and drought-tolerance. Regarding the research question *"Does drought response of Fagus sylvatica differ between central and marginal provenances?"*, the findings of the study exhibited differences between those provenances in the overall context, but did not imply that marginal *F. sylvatica* provenances are less drought sensitive than central provenances.

Evidence suggests that drought-resistant provenances may perform better under water-limitations, but could lose their advantage under high precipitation compared to provenances, which are able to phenotypic plasticity (Richter et al. 2012). Additionally, the introduction of single highly drought-resistant provenances to promote forest resistance with regards to climate change appears to be accompanied by risks, as tree provenances from drought-prone areas do not automatically imply adaptation to other abiotic or biotic parameters such as frost (Kreyling et al. 2012a) or insect infestations. Short- and long-term multi-factorial experiments are needed to improve the current knowledge of adaptation to abiotic and biotic factors of non-local tree provenances. However, long-term experiments require time, which is lacking in forest management during ongoing climate change. Therefore, expanding the search for suitable non-local drought-resistant provenances to intermix local tree stands with might be an option to ensure forest resistance in the face of climate change.

Manuscript 4

The impact of recurrent extreme weather events on plant communities and species is a gap in knowledge. Moreover, the role of stress history with regards to subsequent stress events is not well understood. The response of temperate grassland and heath communities and species to a very severe drought event were tested in the EVENT 1 experiment in 2011 (**manuscript 4**). Three different grassland species (*Arrhenatherum elatius*, *Holcus lanatus* L., *Plantago lanceolata* L.) and two heath species (*Calluna vulgaris*, *Vaccinium myrtillus* L.) contained in plant community compositions with different plant functional groups were chosen for examination. The plant species experienced four different precipitation manipulations ("ambient control", "drought", "heavy rain", and "regular watering") and natural drought occurrences over a period of six years (2005-2010) previous to the very severe drought. Aboveground biomass, tissue die-back, reproductive biomass, ratio of reproductive to total biomass, belowground biomass, and root to shoot ratio were investigated. In the seventh manipulation year we tested if a six-year long pre-exposure to different drought occurrences influenced the stress resistance of plant communities and species during a very severe drought event (exceeding the duration of projected drought scenarios). A better performance of plants formerly subjected to drought was expected. Furthermore, an alteration of this ecological stress memory effect of single plant species by plant community composition was assumed.

Plant communities and single species, which were exposed to "regular watering" with hardly any drought experience (only two drought days in 2010), revealed the highest amounts of tissue die-back during the very severe drought compared to plants with a preceding mild or extreme drought stress history. However, root to shoot ratio of plant communities did not show an ecological stress memory effect. Findings in tissue die-back of *P. lanceolata* and *V. myrtillus* and reproductive biomass of *P. lanceolata* in the different pre-exposures exhibited hints for an impact of plant community composition on the ecological stress memory.

In summary, recurrent mild drought stress over a period of six years seems to improve drought resistance of different heath and grassland communities and species in the face of a very severe drought event. In particular, the study revealed differences within the different pre-exposures of the heath and grassland communities and species, whereas the preceding drought experiences do not have to be extreme themselves in order to promote plant drought resistance. This adds evidence in favor of a positive answer to the research question *"Does stress response of plant communities and species (heath and grassland) differ if drought reoccurs?"*. Moreover, the influence of the plant community composition on the respective plant species represents a new aspect in ecological stress memory research, as further studies mostly focused on single plant species in that context (Goh et al. 2003; Molinier et al. 2006; Whittle et al. 2009; Cuk et al. 2010; Walter et al. 2011). Extending the current knowledge of ecological stress memory by investigations of thresholds, longevity of effects, and the role of biodiversity with respect to the influence of plant community composition should be considered in future research projects.

In conclusion, the work in hand examined the impact of warming and drought on key European tree, dwarf shrub, and grass species and answered four important research questions in respect of climate change. The thesis revealed two novel aspects in view of plant response to simulated climate change. First of all, warming by transplantation and experimental passive warming differed in their impact on plant response and thus with regard to reliability and validity of future predictions (**manuscript 1**). Moreover, the ecological stress memory effect of single plant species seems to be modified by community composition (**manuscript 4**).

Regarding experimental approaches, the optimization of warming techniques as well as the detection of flaws and limitations in that context is a challenging task for future projects in order to make convincing forecasts of plant response. However, time may be a limiting factor as climate change is in progress. Therefore, the topic of intermixing tree stands with drought-resistant tree provenances seems a useful tool to secure forest resistance and yield in future. Furthermore, the investigation of tree response to climatic alterations coupled with biotic interactions such as the impact of forest pest insects is urgently needed to secure forest stability and yield.

Nevertheless, experimental approaches on field-scale over a longer period (several growing seasons) and short-term experiments as well as the knowledge of observational studies are very valuable for forest management strategies. In addition, experimental approaches such as coordinated distributed experiments (Fraser et al. 2013) should be supported to ensure the reliability of meta-analyses and thus of future predictions of plant response. Especially climatic variability and recurrent weather extremes might be integrated in future climatic manipulations to examine plant response with regards to mechanisms such as an ecological stress memory. Investigating the impact of warming, drought, and their interaction on plant species is still an important topic in climate change research as plant communities or species might respond differently to ongoing climatic alterations.

4 References of introduction and synopsis

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5 List of manuscripts and declaration of own contribution

Concept: Idea for the study, development of experimental design, and development of concepts for research articles
Data acquisition: Responsibility for organization and execution of data acquisition, performing the measurements together with the help of technicians, students, and interns
Data analysis: Database production, statistical analysis of data, illustration of tables and figures
Writing: Writing the manuscripts, including literature research
Editing: Improvement and revision of the manuscript, proof-reading, and grammar editing

Manuscript 1:
Title: A transplantation experiment along climatic gradients suggests limitations of experimental warming manipulations
Authors: **Sabrina Backhaus**, Juergen Kreyling, Carl Beierkuhnlein, Constanze Buhk, Laura Nagy, Daniel Thiel, Anke Jentsch
Status: published
Journal: Climate Research, 2014, 60: 63–71 (doi: 10.3354/cr01219)
Own contribution: concept 10%, data acquisition 50%; data analysis 70%, writing of the manuscript 90%; editing 40%

Manuscript 2:
Title: Warming and drought do not influence the palatability of *Quercus pubescens* Willd. leaves of four European provenances
Authors: **Sabrina Backhaus**, Dominik Wiehl, Carl Beierkuhnlein, Anke Jentsch, Camilla Wellstein
Status: published
Journal: Arthropod-Plant Interactions, 2014, 8: 329–337 (doi: 10.1007/s11829-014-9313-4)
Own contribution: involvement in the preparation of the concept 60%; data acquisition 50%; data analysis 60%; writing of the manuscript 80%; editing 70%

Manuscript 3:
Title: Different reactions of central and marginal provenances of *Fagus sylvatica* to experimental drought
Authors: Daniel Thiel, Juergen Kreyling, **Sabrina Backhaus**, Carl Beierkuhnlein, Constanze Buhk, Kolja Egen, Gerhard Huber, Monika Konnert, Laura Nagy, Anke Jentsch
Status: published
Journal: European Journal of Forest Research, 2014, 133 (2): 247-260
(doi: 10.1007/s10342-013-0750-x)
Own contribution: involvement in the preparation of the concept 25%; support of the student during the diploma thesis and of the data acquisition 25%; data analysis 0%; writing of the manuscript 0%, editing 10%

Manuscript 4:
Title: Recurrent mild drought events increase resistance toward extreme drought stress
Authors: **Sabrina Backhaus**, Juergen Kreyling, Kerstin Grant, Carl Beierkuhnlein, Julia Walter, Anke Jentsch
Status: published
Journal: Ecosystems, 2014, 17: 1068–1081 (doi: 10.1007/s10021-014-9781-5)
Own contribution: concept 10%; organization of data acquisition 90%; manual data acquisition 30%; data analysis 90%; writing of the manuscript 90%; editing 60%

6 Manuscripts

Manuscript 1: A transplantation experiment along climatic gradients suggests limitations of experimental warming manipulations

Climate Research, 2014, 60: 63–71 (doi: 10.3354/cr01219)

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Abstract

Transplantation to a warmer site and experimental passive warming are powerful tools for predicting plant responses to climate change. Both techniques are widely applied for the study of plant species and community response to temperature increase. We investigated differences in height increment of *Fagus sylvatica* seedlings between 2 different techniques: experimental warming (passive warming) and transplantation to a warmer site. Additionally, the plants were exposed to an extreme drought to further examine the influence of the different warming techniques in combination with an additional climatic driver. We found significant differences between the 2 warming techniques for height increment, which were mainly attributed to the case with additional drought exposure (significant interaction between warming and drought). Surprisingly, when subjected to drought, experimental warming had no negative effect on height increment of seedlings, while transplantation decreased height increment by 32 % when subjected to drought. Growth did not show a linear dependence on the magnitude of warming. Differences between the warming techniques can therefore not be explained by differences in realized temperature increases. The results of this study emphasize the complexity of simulating global warming, as required for accurate prediction of shifts in plant performance. The role of co-varying parameters, such as evapotranspiration, photosynthetically active radiation, and wind speed, in addition to experimental temperaturee increases should be acknowledged when analyzing ecological responses to climate warming.

Keywords: Beech, EVENT experiment, Experimental manipulation, Global warming

Introduction

Simulating global warming is currently a fundamental topic in ecological climate change research (Leuzinger et al. 2011). Different techniques are used in this context, such as transplantation to a warmer site (e.g. Joshi et al. 2001, Link et al. 2003, Turetsky et al. 2008, Gonzalo-Turpin & Hazard 2009, Saarinen & Lundell 2010, Haggerty & Galloway 2011) or passive warming techniques (Henry & Molau 1997, Marion et al. 1997, Beier et al. 2004, Dabros et al. 2010, De Boeck et al. 2012, Kreyling et al. 2012a, Thiel et al. 2012). However, the question of which technique is more realistic and useful when predicting the effects of warmer temperatures in the future is difficult to answer. Obvious limitations in experimental warming techniques exist: passive warming can induce chamber overheating, alter moisture, light, gas concentrations and wind, or underestimate simulated temperature at tissue level (Marion et al. 1997, De Boeck et al. 2012). Furthermore, edge effects, such as an increased heat loss near the edges compared with the plot center, occur for passive nighttime warming by aluminum curtains (Beier et al. 2004). Regarding warming by transplantation, the respective plant species experience a step change of the entire environmental conditions in comparison to passive warming techniques. In addition, a transplantation shock (Tetsumura et al. 1998), the size of monoliths or new biotic interactions — especially herbivory (Kile et al. 2013) — might impact on the plant performance. These side-effects can blur the true warming response, yet they might also reveal ecologically important processes by their holistic nature of change.

In this study, we focus on the different effects of experimental warming (passive warming by wind shelters and black floor covers) versus warming by transplantation (across several hundred kilometers) in comparison to ambient conditions. To our knowledge no study has so far directly compared passive warming and warming by transplantation.

Climate change includes more than gradual warming; more frequent and stronger extremes are also an important aspect to consider (IPCC 2012). These extreme events are thought to have high ecological importance (Easterling et al. 2000, Jentsch et al. 2007). Recent multi-factor climate change experiments (which include different manipulations such as warming and drought as single factors and in combination) imply that single-factor effects are often not additive, i.e. the interaction of climate parameters results in unexpected effects (Shaw et al. 2002, De Boeck et al. 2011, Larsen et al. 2011, Kreyling et al. 2012b). Therefore, in the present study, we tested potential limitations of warming manipulations not only as a single factor, but also in combination with drought extremes.

The impact of global warming on ecosystems is indisputable (IPCC 2007); however, developing an accurate prediction of the consequences of climate change is very challenging. It is difficult, for example, to scale up experimental results, which describe a certain temporal and spatial scale, to realistic scenarios (Leuzinger et al. 2011, Wolkovich et al. 2012). A dampening of effect size is known to occur with increased scale and treatment complexity, which leads to an overestimation of the influence of a changing climate based on small-scale and short-scale experiments (Leuzinger et al. 2011). Inconsistencies in climate predictions might also be caused by experimental artifacts. A thorough investigation of potential artifacts, for example from the warming technique used, is needed to improve these predictions. Therefore, we compared the performance of *Fagus sylvatica* L. (European beech) seedlings in response to 2 different warming techniques: experimental warming (passive warming by wind-shelters and black floor covers) and transplantation to a warmer site. Both warming techniques were compared to ambient conditions. We chose *F. sylvatica* because this deciduous forest tree is naturally dominant in Central European forests.

We hypothesized that (1) experimental warming and warming of similar magnitude by transplantation would lead to the same increase in height increment. As warming by transplantation depends on actual weather conditions, it cannot be controlled in its magnitude. To circumvent this potentially confounding effect, we expected that the effect sizes of our response variable to correlate linearly with the magnitude of warming, irrespective of warming technique. Furthermore, we assumed that (2) different warming techniques would not influence the temperature sensitivity of plant growth under additional pulsed drought stress.

Materials and methods

Experimental sites

The main experimental site was established in Bayreuth, Germany, in the Ecological-Botanical Garden of the University of Bayreuth (49°55'19' N, 11°34'55' E) in March 2010 (Beierkuhnlein et al. 2011, EVENT 3). The long-term mean annual temperature at this site is 8.2°C and the long-term mean annual precipitation is 724 mm with a precipitation peak in December/January and July/August (data: German Weather Service, www.dwd.de/klimadaten).

The second experimental site was established in April 2010 next to the campus Siebeldingen (University Koblenz-Landau) at the experimental sites of the Julius Kühn-Institut (JKI) (Federal Research Center for Cultivated Plants) (49°13'03' N, 8°02'47' E), Germany. The long-term mean annual temperature at the site is 10.2°C, i.e. 2.0°C warmer than the main experimental site, and the long-term mean annual precipitation is 643 mm, which is distributed bimodally with a major peak in May/June and in November/December (data: German Weather Service, www.dwd.de /klimadaten).

Plant material

We used 7 *Fagus sylvatica* provenances — 3 from Germany, 3 from Bulgaria, and 1 from Hungary — in order to improve the generality of the observations. Specific geographic origin of the provenances is provided (Table A1 in the Appendix). Despite differing in their general performance, the provenances showed no difference in their sensitivity to experimental warming at the main experimental site (Kreyling et al. 2012b) and at the transplantation site (tested in a pre-analysis). The seedlings were cultivated at the Bavarian Institute for Forest Seeding and Planting (ASP) in Teisendorf, Germany from February 2009 to March 2010. In March 2010 the seedlings were transported bare-root to Bayreuth and individually planted in 12 l plastic pots filled with sandy silt (pH 7.73, total C 1.58 %, total N 0.13 %). The plants were planted and watered until they were saturated on March 23. Further watering was applied on March 25 and 29. On April 12, 2010, 126 planted seedlings were transported to the second experimental site in Siebeldingen. In total, there were 252 planted seedlings in Bayreuth. Nine seedlings per provenance and treatment (ambient, ambient with drought, experimental warming, experimental warming with drought, warming by transplantation, warming by transplantation with drought) were selected randomly from all plants alive at planting date. The irrigation simulated the local daily 30 yr average precipitation, which was applied twice a week using collected rainwater at the main experimental site and groundwater at the transplantation site.

Experimental design

At the main experimental site in Bayreuth, the 4 climate treatments resulted from fully crossed twofold factorial combination of 2 temperature regimes, i.e. ambient and experimental warming, and 2 moisture regimes, i.e. ambient and drought, conducted from April to October 2010. Each of the 4 climate treatments (ambient, ambient with drought, experimental warming, experimental warming with drought) were replicated 3 times, resulting in 12 experimental units in total. Each provenance was represented by 3 plants in each experimental unit (7 provenances × 4 climate treatments × 3 plants per experimental unit × 3 replications = 252 plants in total). The available plants were assigned randomly to the experimental units. Each experimental unit (10.5 m × 7 m) was covered by a single rain-out shelter with a steel frame (GlasMetall Riemer GmbH) and covered by a transparent polyethylene sheet (0.2 mm, SPR5, Hermann Meyer KG). The edges of the rain-out shelters were at a height of 80 cm and the polyethylene sheet permitted nearly 90% penetration of photosynthetically active radiation. Due to the sensitivity to solar radiation of *Fagus sylvatica* seedlings, shading nets (Quadra 105 ME, 105 g, DM-Folien GmbH) were attached inside of the polyethylene sheet, resulting in a penetration of about 55% of the photosynthetically active radiation, which resembles natural forest floor conditions in beech stands (Ellenberg 1996). The warming treatment was mainly achieved by passive warming via additional wind breaking around the shelters and black floor covers (in comparison to white floor covers in the ambient and ambient with drought treatments). The windbreaker nets (type Z, 330 g m^{-2}, 70 % wind speed reduction, DM-Folien GmbH) were installed around the experimental units reaching up to the roof height of 80 cm aboveground. Note that all climate treatments including ambient were installed under rain-out shelters in order to control precipitation. In addition, each experimental unit had 8 evenly distributed infrared (IR) heating lamps (IOT/90, 250 W, 230 V, Elstein) placed at 2 m height, theoretically resulting in roughly 30 W m^{-2}. However, these affected warming only marginally (by 0.1°C in comparison to passive warming alone; Thiel et al.

2012). The applied experimental warming (passive warming, IR-heating) increased the average air temperature at plant height by 1.6°C.

At the transplantation site in Siebeldingen only 1 rain-out shelter (see above) was set up. Below this shelter, all 126 plants were kept completely randomized in 2 climate treatments: warming by transplantation and warming by transplantation with additional drought (× 7 provenances, × 9 replications).

At both sites the air temperature at plant height was measured hourly. At the main experimental site in Bayreuth, 2 thermistores (B57863-S302-F40, EPCOS) connected to a dl2 datalogger (Delta) were used per experimental unit. At the transplantation site in Siebeldingen, 2 Tinytag Plus 2 Data Loggers (Gemini Data Loggers, Chichester) were used (Fig. 1). At both sites, soil moisture was measured hourly at a depth of 5 cm with 3 Ech2o EC-5 moisture sensors (Decagon Devices) per climate treatment (Fig. 2). Regarding the entire experimental time period (April to October 2010), 'experimental warming' resulted in a temperature increase by 1.9°C in comparison to 'ambient', while 'warming by transplantation' resulted in a temperature increase by 3.2°C (Fig. 1; Table 1). The air temperature sums (see Section 2.5) of both warming techniques and ambient conditions are also provided in Table 1.

The drought manipulation took place from 14 May until 16 or 17 July 2010. The plants at the main experimental site in Bayreuth reached the stopping criterion (20% of the plants showed strong drought damage; 76 to 100% of the leaves damaged) one day earlier than at the transplantation site in Siebeldingen. After the drought the plants received additional water (600 ml per pot) on 16, 19, and 23 July 2010.

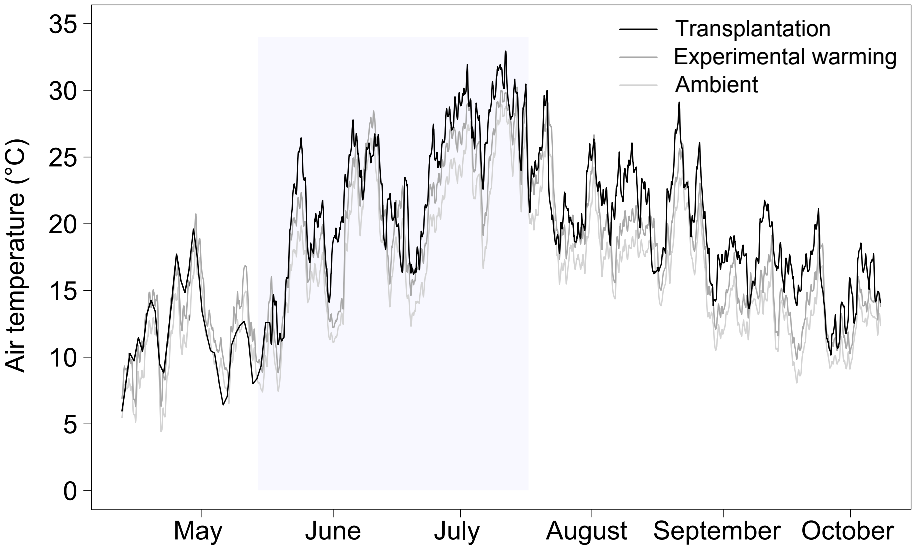

Fig. 1 Air temperature at plant height in the different warming treatments over the course of the experiment in 2010. The grey area represents the drought period

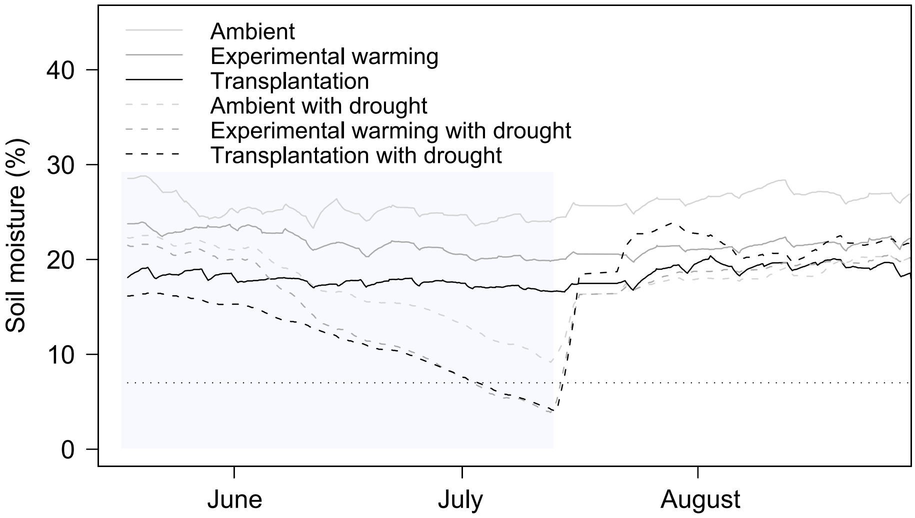

Fig. 2 Soil moisture at a depth of 5 cm for the different warming treatments over the course of the experiment in 2010. The grey area represents the drought period. The permanent wilting point (pF = 4.2) is indicated by the dotted black line

Table 1 Mean air temperature (C°) and air temperature sum of both warming techniques and ambient conditions during the period from the start of the experiment to the final height measurements for height increment (April to October 2010)

Mean air temperature (C°)			Air temperature sum				
Transplantation	Experimental warming	Ambient	Transplantation	Experimental warming	Ambient	Ambient in combination with drought	Exp. warming in combination with drought
19.3	18.0	16.1	3387	3158	2830	2874	3123

Response variable

We used height increment (height measured in October minus height measured in April/May) as our response variable.

Statistical analysis

Analysis of variance (ANOVA) combined with linear mixed effect models was applied to test for main effects and interactions of the treatments ‘warming technique’ (levels: ‘ambient’, ‘experimental warming’, and ‘warming by transplantation’) and drought (levels: yes or no) on height increment. The provenance identities were included as a random factor in the mixed models, as no significant interactions of this factor with ‘warming technique’ were present in a pre-analysis. Data on height increment were square root transformed to improve the normality of residuals and the homogeneity of variances prior to analysis (Faraway 2006). In case of significant main effects of the linear mixed effect model, pair-wise post-hoc comparisons (Tukey’s test) were performed according to Hothorn et al. (2008).
The air temperature sum was calculated as the sum of daily mean temperatures from 12 April to 8 October 2010. All daily mean temperatures were above a 5°C threshold value for all treatments (ambient, ambient with drought, experimental warming, experimental warming with drought, and transplantation) at both experimental sites. Using growing degree days with the commonly applied threshold would therefore have no impact on the results. The respective air temperature sums are provided in Table 1. Warming by transplanting to a different site does not allow for a pre-defined amount of warming as weather conditions often vary from the long-term mean conditions at the given site. In order to enable a direct comparison between the 2 warming techniques, we therefore needed to acknowledge the slight differences between realized warming in both techniques. This was done by

assuming the most simple, i.e. a linear, response to warming and checking if this assumption is met by the data. For a parameter A the linear construction yields

$$A(T) = \frac{A_{exp} - A_{amb}}{T_{exp} - T_{amb}} \times (T - T_{amb}) + A_{amb},$$

where A_{amb} and A_{exp} are the parameter means for the cases "ambient" and "experimental warming". T_{amb} and T_{exp} are the corresponding temperature sums. The assumption of linearity is tested by comparing the predicted parameter mean for the "transplantation" temperature sum $A\left(T_{trans}\right)$ with the experimentally determined mean for this site.
All statistical analyses were conducted with the statistical software R v. 2.11.1 (R Development Core Team 2010) including the packages ‘nlme’ (Pinheiro et al. 2012) and ‘multcomp’ (Hothorn et al. 2008).

Results

Warming technique

Regarding height increments of all *Fagus sylvatica* plants, there was a significant difference between the 2 warming techniques (Table 2; post-hoc test: p = 0.0016). Furthermore, a difference between the observed temperature effect and the one based on expected linear extrapolation was observed for ‘warming by transplantation’ (see arrow in Fig. 3A).

Table 2 *Fagus sylvatica*. ANOVA results for the effects of warming technique and drought (experimental warming, warming by transplantation, ambient, experimental warming with drought, warming by transplantation with drought, and ambient with drought) on height increment. All values are significant (p < 0.05)

Factor	F	P
Warming technique	6.4	**0.0019**
Drought	31.0	**< 0.0001**
Interaction warming technique:drought	11.2	**< 0.0001**

Warming technique in combination with drought

When additionally subjected to drought, ‘experimental warming’ was significantly different from ‘warming by transplantation’ and from ‘ambient’ (Table 2; Fig. 3B), resulting in 56 % relative growth difference between warming techniques. Plants warmed by transplantation only showed a height increment average of 36 %, whereby the plants of the ‘experimental warming’ had a height increment average of 92 %. When subjected to drought there was no significant difference between the plants of the ‘warming by transplantation’ and the plants under ‘ambient’ conditions (Fig. 3B). The ‘experimental warming’ in combination with drought had no negative effect on height increment, contrary to the transplanted and ambient plants subjected to drought, which showed a decrease in height increment by 32 % (‘warming by transplantation’) and 50 % (‘ambient’), respectively. Again, the observed temperature effect in the ‘warming by transplantation’ differed more strongly in height increment than expected (see arrow in Fig. 3B).

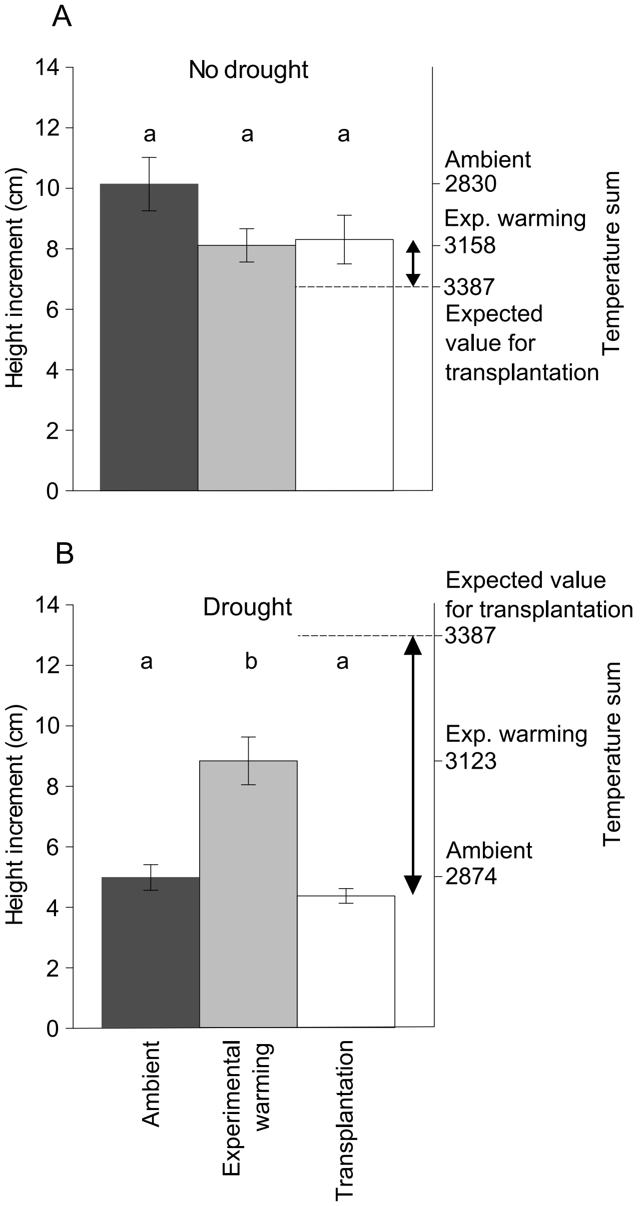

Fig. 3 Height increment (cm) of the *Fagus sylvatica* seedlings in response to the different warming techniques without drought (A) and under drought (B) (mean ± SE). The respective temperature sum is indicated on the right hand side. The temperature sums for ’ambient’ and ’experimental warming’ are positioned at the respective height of the corresponding bar. Relative to these 2 points, the expected value for ’warming by transplantation’ is shown, together with the dashed horizontal line, at the position where the response effect size would be expected based on the linear extrapolation. The vertical arrow indicates the difference between the expected and actually realized response for the case of ’warming by transplantation’. Lower case letters represent significant differences as revealed by the post-hoc test of the interaction warming technique:drought.

Discussion

Influence of warming technique

Plant performance (i.e. height growth) of juvenile beech trees differed among warming techniques, i.e. ‘experimental warming’ (mainly passive warming with marginal effectiveness of IR radiation) and ‘warming by transplantation’ (across several hundred kilometers). This finding contradicts our first hypothesis: experimental warming and warming of similar magnitude by transplantation would lead to the same increase in height increment. In particular, we compared the plant response of the transplantation technique to an expected response for the achieved warming based on a linear extrapolation in the temperature sum between the ‘experimental warming’ and ‘ambient’ conditions. This was done because the 2 warming techniques yielded different mean air temperatures due to unpredictable weather conditions at both sites. The linear extrapolation was done to take this into account and to test whether the temperature difference had explanatory power in this experiment. The transplanted plants experienced a temperature that was, on average, 1.3°C higher than under the ‘experimental warming’ conditions over the period the growth took place (Fig. 1; Table 1). Nevertheless, no linear dependence on the magnitude of warming could be found (Fig. 3). In summary, the respective effect sizes of both warming techniques for height increment could not be explained by the difference in temperature sum between the 2 warming treatments. Warming reduced height growth in our experiment, which can be explained by the reduced soil moisture in the warmed pots — leading to a generally mild yet chronic drought stress in comparison to ambient conditions (McLaughlin & Downing 1995).

The lack of linear dependence of plant growth on temperature sum suggests further complexity. Factors such as the vapor pressure gradient (VPG) from inside the leaves to the air outside (Kimball 2005), the duration, intensity, and spectral distribution of the radiation, the water potential in the soil, the content of atmospheric CO_2 as well as gravity and pressure effects (wind, flow of water, pressure of snow) may have impacts on plant performance (Larcher 2003). A different VPG in the case of infrared heating leads to an increased water loss (Kimball 2005). As testing the VPG was not at our disposal, we compared the soil moisture content of the different sites and treatments. Only under the control treatment was soil moisture higher in the ‘experimental warming’ pots compared to the pots of the transplanted plants without the drought treatment. Under drought conditions, both techniques showed a similar decline in soil moisture (Fig. 2). This suggests that the evapotranspiration was similar in the pots of the 'experimental warming' plants. Thus, evapotranspiration might have played a secondary role associated with the difference between warming techniques in our study.

Another potentially important climatic parameter is solar radiation. The fraction of photosynthetically active radiation (PAR) within the solar radiation depends on time and location (Amthor 2010) and might be one reason for the differences between warming techniques. In this study, the curves of the solar radiation (short-wavelength radiation of the global radiation; daily sums) were close together at both sites (see Fig. A1 in the Appendix). The average of the daily sums of the solar radiation was 4324 W m^{-2} at the 'experimental warming' site and 4869 W m^{-2} at the 'warming by transplantation' site during the time span of the experiment (April to October 2010). The resulting difference of about 12 % in solar radiation could contribute to the difference between both warming techniques.

Another possible explanation for the difference between the warming techniques could be the 70 % reduction of wind speed due to passive warming. This is, however, an unavoidable side effect of passive warming and can thus be taken as a further argument against these techniques. Future studies will show to what extent the above-mentioned parameters (evapotranspiration, photosynthetically active radiation, and wind speed) may be responsible for the observed differences in warming techniques.

In addition, other factors of importance, such as species-specific effects, are improbable explanations, as we used *Fagus sylvatica* from 7 different provenances, and thus covered a broad spectrum of intraspecific variability. Furthermore, the interaction of warming technique and provenance was not significant (F = 1.4; p = 0.162). Overall, our results strengthen the argument that an interpretation of global warming experiments should be made very carefully in relation to climate change predictions.

Influence of warming technique interacting with drought

'Experimental warming' and 'warming by transplantation' had different effects on plant performance when superimposed by drought (Fig. 3B). In our study, the plants in the 'experimental warming' revealed high resilience, showing no decrease in height increment, whereas the plants grown under ambient conditions and those transplanted had a growth reduction under drought. An explanation for the growth stimulation after drought in the 'experimental warming' could be a compensatory growth after the re-watering phase at the end of the drought. Spieß et al. (2012) carried out a long-term drought during 2 growing seasons and detected a strong compensation growth in *Quercus robur* L. (pedunculate oak) after re-watering. However, in our experiment only the plants subjected to 'experimental warming' under drought showed an increase in growth. The plants of 'ambient' (drought exposed plants without the warming treatment) and the transplanted plants did not respond in the same way. Hence, further research is needed to understand the complex response of plants under drought and heat stress. Nevertheless, our results strengthen the conclusions from several multi-factor experiments (Shaw et al. 2002, De Boeck et al. 2011, Larsen et al. 2011, Kreyling et al. 2012b), i.e. that different climatic drivers are not additive. The significant interaction of warming and drought in our study, which implies non-additivity of both factors, further depended on the warming technique, a factor which complicates the comparability of different warming techniques.

Conclusions

In climate change experiments, the selected warming technique, i.e. experimental warming (mainly passive warming with marginal effectiveness of IR-radiation) versus warming by transplantation influences plant performance differently. Thus, prediction of global warming effects on plant performance is highly influenced by the choice of technique. These differences among warming techniques were further exacerbated when warming was combined with manipulation of another climate parameter, here an extreme drought event. The findings of this study suggest that experimental warming should include the control or consideration of further parameters such as evapotranspiration, photosynthetically active radiation, and wind speed, aside from temperature, in order to provide a deeper insight.

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Appendix

Table A1 *Fagus sylvatica*. Geographic origin of the 7 provenances used in the experiment

Provenance	Country	Latitude	Longitude
Gotze Delchev	Bulgaria	N 41°38’08"	E 23°35’08"
Petrochan	Bulgaria	N 43°15’18”	E 23°14’20"
Strumjani	Bulgaria	N 41°40’55"	E 23°00’42"
Kisbárapáti	Hungary	N 46°35’19"	E 17°51’12"
Hengstberg	Germany	N 50°08’00"	E 12°11’00"
Elchingen	Germany	N 48°27’21"	E 10°03’48"
Weildorf	Germany	N 47°50’45"	E 12°52’56"

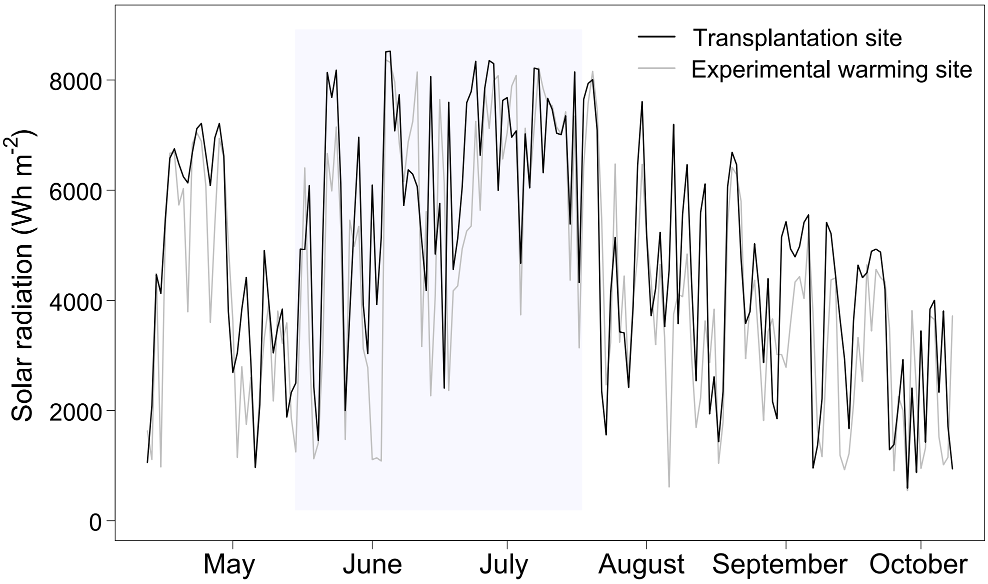

Fig. A1 Solar radiation (short-wavelength radiation of the global radiation; daily sums) at the different sites over the course of the experiment in 2010. The grey area represents the drought period

Manuscript 2: Warming and drought do not influence the palatability of *Quercus pubescens* Willd. leaves of four European provenances

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Abstract

In the context of global warming, the impact of extreme drought events on trees and biotic interactions with herbivore insects is widely unknown. A faster range expansion of insects in a changing climate could lead to mass propagations of pests in forests. Therefore, the aim was to investigate the influence of climatic alterations on leaf palatability. We exposed juvenile *Quercus pubescens* Willd. individuals of four European provenances (Bulgaria, Germany, Hungary, and Italy) to warming and drought. In addition, we conducted a palatability experiment with the pre-exposed *Q. pubescens* leaves and the caterpillars of the generalist forest pest *Lymantria dispar* L. (gypsy moth). Consumed leaf dry material, density of trichomes, and specific leaf area were examined. Surprisingly, neither warming nor drought affected the leaf palatability, but palatability was related to the density of trichomes. The Bulgarian provenance of *Q. pubescens*, which had the lowest density of trichomes, was most palatable. These findings suggest that global warming and drought might not lead to more frequent infestations of the four tested European *Q. pubescens* provenances by *L. dispar* caterpillars in the future.

Keywords: Climate change, Feeding experiment, Insect herbivory, Leaf traits, Provenance trial, Extreme weather events

Introduction

Under global warming, extreme weather events such as drought will very likely increase in the near future (IPCC 2012). The impact of drought events on susceptibility of trees to insect infestations and mortality is unclear (McDowell et al. 2011). In addition, within-species differences in tree responses to climate change and herbivory have received little attention. The variation in the adaptive capacity and plasticity of tree individuals to extreme weather events can depend on the respective provenance (Peuke et al. 2002; Kreyling et al. 2012; Thiel et al. 2012; Wellstein and Cianfaglione 2014). Therefore, studies that incorporate different tree provenances are highly important for a more precise understanding of tree responses to climate change as well as their biotic interactions.

The general effects of global warming on insects are relatively well documented (Robinet and Roques 2010). It is a fact that higher temperatures promote the development rates of insects as long as a certain threshold value is not exceeded (Bale et al. 2002; Netherer and Schopf 2010). Beyond increases in insect population densities, alterations in insect size, genetic composition, voltinism, duration of life cycles or exploitation of host plants could also be induced by higher temperatures (Bale et al. 2002).

Extreme weather events such as drought will also influence pest species dynamics and the interaction between insect herbivores and host plants. Tree resistance to bark beetle infestations, for example, is reduced under drought (Berg et al. 2006). Insect calamities might follow drought events and may cause greater damages to already stressed plants (Huberty and Denno 2004; Rouault et al. 2006; Netherer and Schopf 2010). However, the combined effect of warming and drought on the interaction between insect herbivores and plant species is poorly examined (Jamieson et al. 2012).

In the context of climate change, leaf palatability and resistance of host plants may influence insect herbivores indirectly. It is known that drought can change the nutritional quality of host trees (Rouault et al. 2006; Netherer and Schopf 2010). An example is the increase of nitrogen in leaves of woody plants due to drought (Mattson and Haack 1987a). Thus, folivorous species are more often observed in moderately water-stressed trees due to higher leaf nitrogen contents (Mattson and

Haack 1987b; Larsson 1989). Focusing on warming, Zvereva and Kozlov (2006) did not find alterations in leaf nitrogen content under elevated temperature in their meta-analysis. Additionally, missing alterations in leaf nitrogen content could be detected in tree species due to elevated temperature and the insect leaf consumption was also not affected (Williams et al. 2000).

Plants guard against herbivory with allelochemicals (Stamp and Yang 1996) or leaf traits such as trichomes (i.e., leaf hairs) (Hanley et al. 2007; Agrawal et al. 2009). It is well known that climatic factors such as warming or drought are able to change plant secondary chemistry (Mattson and Haack 1987a; Llusia and Peñuelas 1998; Zvereva and Kozlov 2006; Bidart-Bouzat and Imeh-Nathaniel 2008; Gutbrodt et al. 2011; Tharayil et al. 2011) and thus can influence the leaf palatability. Beyond leaf allelochemical contents and trichome densities, the specific leaf area (i.e., the ratio of leaf area to leaf mass) provides information about the leaf nitrogen content and thus suitability of leaves to insect herbivores. In environments with resource stress, the specific leaf area of plants tends to be small (Cornelissen et al. 2003), and therefore, leaf nitrogen content compared to leaf carbon content is low, whereby leaf suitability for insects decreases (Bresinsky et al. 2008). Furthermore, a small specific leaf area value displays a long leaf lifespan as well as investments in leaf defense, particularly in leaf traits (Cornelissen et al. 2003).

Evidence exists that the specific leaf area as well as the trichome density vary between provenances of oak species such as *Quercus robur* L. (Nikolic and Orlovic 2002) and *Q. suber* L. (Ramírez-Valiente et al. 2009). Moreover, the oak leaf palatability can be influenced by the physical characteristics of the leaves of the host plant species (Foss and Rieske 2003). In view of the impact of warming and drought on leaf characteristics of oak species, Arend et al. (2011) revealed a decrease of the whole-tree leaf area of different provenances of *Q. petraea* Liebl., *Q. pubescens* Willd., and *Q. robur* induced by drought, whereas warming did not impact this parameter. Günthardt-Goerg et al. (2013) detected a reduction of the mean single leaf dry mass and area of *Q. petraea*, *Q. pubescens*, and *Q. robur* provenances when subjected to drought, while warming lead to an increase of both parameters.

Climate change will also likely result in species shifts (Lenoir et al. 2008), whereby higher mean temperatures could promote an area expansion of warmth-adapted plant (Peñuelas and Boada 2003) and insect species (Parmesan et al. 1999; Ayres and Lombardero 2000; Bale et al. 2002). Area expansion will likely take place earlier for insects than for plants (Rouault et al. 2006; Vanhanen et al. 2007). The risk of infestations and mass propagations of pest insects could increase in the near future following global warming and drought (Logan et al. 2003). Certainly, the response of insect herbivores to warming and drought will vary among insect species as well as among generalist versus specialist species (Koricheva et al. 1998; Huberty and Denno 2004; Gutbrodt et al. 2011). For instance, *Lymantria dispar* L. (gypsy moth, Lepidoptera, Lymantriidae) is a well-known generalist forest pest across Europe, Asia, and in the USA. A potential range shift of *Lymantria* species to the north is predicted by model studies (Vanhanen et al. 2007). The food spectrum of the generalist species contains 270-450 host plants with *Quercus* species belonging to their first degree food plants (Wellenstein 1978). Focusing on *Quercus* species, Cocco et al. (2010) monitored over 24 years (1980-2004) population dynamics and the frequency of *L. dispar* infestations in Sardinian forestland dominated by *Q. suber*, *Q. ilex* L., and *Q. pubescens*. A relative frequency of *L. dispar* mass propagations of 7-8 years could be found. Considering plantings of drought-tolerant oak species, it is noteworthy that Dorow et al. (2012) detected a rapid acceptance of the planted Mediterranean species *Q. pubescens*, *Q. ilex*, and *Q. frainetto* Ten. by *L. dispar* at German sites dominated by *Q. robur*. Moreover, in feeding experiments, a preference for the deciduous species *Q. robur*, *Q. pubescens*, and *Q. frainetto* compared to the evergreen *Q. ilex* was found.

In this study, we investigate the effects of warming and drought as well as their interaction on the leaf palatability of *Q. pubescens* seedlings of four different European provenances (Bulgaria, Germany, Hungary, and Italy). To test for alterations in leaf palatability, we carried out a palatability experiment with the generalist forest pest *L. dispar*. Beyond the impact of the climatic manipulations, we focus on differences in leaf traits of the selected tree provenances in order to determine their influence on leaf consumption of *L. dispar*. We chose *Q. pubescens*, because global warming could also promote an area expansion of *Q. pubescens*, and therefore plantings with this drought-tolerant species in Germany might be beneficial for forestry (Sayer 2000; Glatzer and Schramm 2010).

We hypothesize that the climatic manipulations warming and drought influence the leaf palatability (H1), and that the four *Q. pubescens* provenances differ in leaf palatability (H2). Furthermore, we assume that leaf palatability is related to 'density of trichomes' and 'specific leaf area', whereas leaves with high 'density of trichomes' and small 'specific leaf area' values are less palatable (H3).

Materials and methods

Experimental site

The tree provenance trial was established in Bayreuth, Germany, in the Ecological-Botanical Garden of the University of Bayreuth (49°55’19” N, 11°34’55” E) within the framework of the EVENT-experiment series (Beierkuhnlein et al. 2011; Jentsch et al. 2011) and the FORKAST project (Wellstein and Beierkuhnlein 2011). The long-term mean annual temperature at this site is 8.2°C and the long-term mean annual precipitation is 724 mm with a precipitation peak in December/January and July/August (data: German Weather Service).

Plant material

We selected *Q. pubescens* (see Wellstein and Spada 2014) provenances from Bulgaria (BU), Germany (GER), Hungary (HU), and Italy (IT) (for further informations about the selected provenances see Wellstein and Cianfaglione 2014). The geographic origin and the corresponding climatic conditions of the selected *Q. pubescens* provenances are provided in Table 1 and Fig. 1.

Table 1 Origin of the *Q. pubescens* provenances used in the palatability experiment and climatic information (MAT = mean annual temperature, MAP = mean annual precipitation), climatic data for the period 1950-2000 from WorldClim (Hijmans et al. 2005) (table reproduced from Wellstein and Cianfaglione 2014).

Country/ Provenance	Latitude	Longitude	Altitude (m asl)	MAT (°C)	MAP (mm)
Bulgaria (BG)	N 43°12’41"	E 26°33’20"	247	10.6	620
Germany (GER)	N 47°36’51"	E 08°24’46"	530	9.1	950
Hungary (HU)	N 47°16’02"	E 17°35’01"	220	9.7	587
Italy (IT)	N 43°04’08"	E 13°07’55"	750	12.2	876

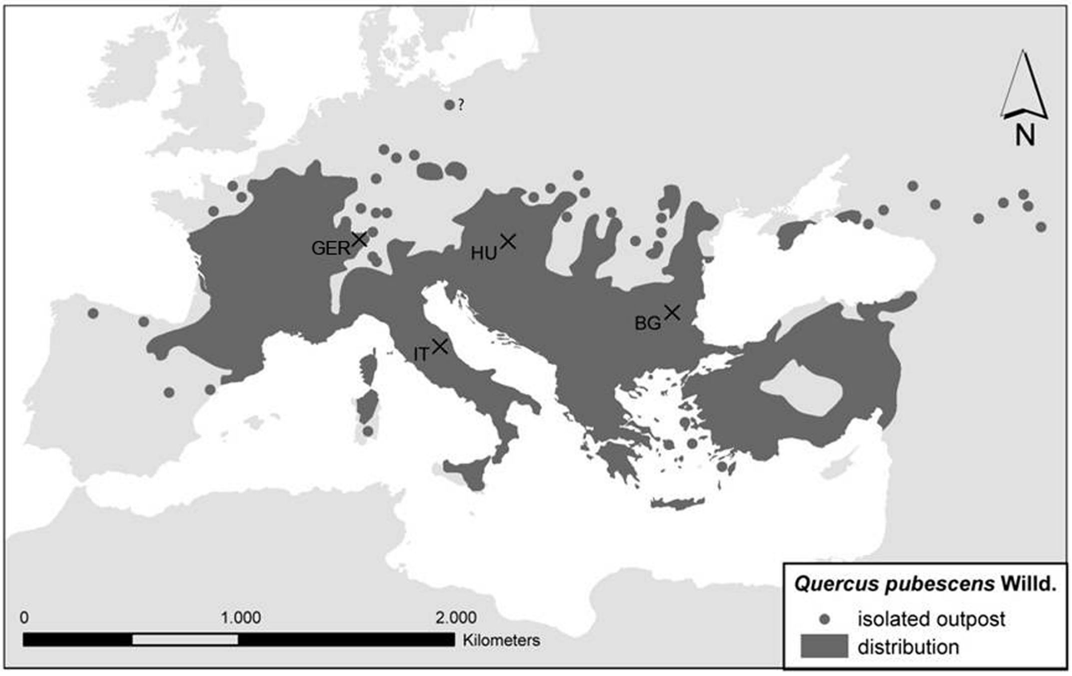

Fig. 1 The distribution of *Quercus pubescens* Willd. (Tutin et al. 1993) in Europe. In Wellstein and Spada (2014) the map is redrawn after Meusel et al. (1978) with added occurrences according to several authors. The northernmost outpost (marked by "?") might stem from plantation according to Ellenberg (1996). The origin of the selected provenances used in the experiment is indicated by a cross (*BG* Bulgaria, *GER* Germany, *HU* Hungary, *IT* Italy) (figure reproduced from Wellstein and Spada 2014).

Experimental design of climatic manipulations

In Bayreuth, the *Q. pubescens* seedlings were exposed to the fully crossed twofold factorial combination of (1) a continuous warming manipulation (warming and control) and (2) a drought manipulation (drought and control). This resulted in four climatic manipulations (control, warming, drought, warming combined with drought) and subsequently, four experimental units. The plants were exposed to the warming treatment continuously over the whole period of the experiment from 31 March to 20 October 2011 (204 days). The drought manipulation took place from 31 March to 6 June 2011 (67 days). The provenances (Bulgaria, Germany, Hungary, and Italy) were nested within each experimental unit. Each provenance was further replicated with six plants per climatic treatment (nested replicates). The available plants were assigned randomly to the climatic treatments. The air temperature

(°C) at plant height and soil moisture (%) at a depth of 5 cm were measured hourly in each experimental unit over the course of the experiment (Fig. 2; air temperature data were only available until mid July 2011 and soil moisture data were only available until the end of July 2011). The air temperature was measured using two thermistors (B57863-S302-F40, EPCOS) per experimental unit, which were connected to a dl2 datalogger (Delta). For the soil moisture measurements, three Ech2o EC-5 moisture sensors (Decagon Devices Inc., Pullman, Washington, USA) were used per experimental unit. Further details about the experimental setup and the climatic manipulations (warming and drought) are provided in Wellstein and Cianfaglione (2014).

The leaf phenology was documented twice a week according to the BBCH (Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry) code (Meier 2001) recording stages 7 (bud burst), 15 (leaf unfolding), and 19 (leaf fully developed). The phenology was recorded in order to select an appropriate drought date and to determine the leaf age at the start of the palatability experiment. The leaves were all in the same phenological stage, i.e., fully developed (19), ranging between 107 and 152 days since bud break, with 70 % of the leaves exhibiting an age of 152 days. No leaf sample reached the stage of senescence by 20 September 2011 when the palatability experiment was conducted.

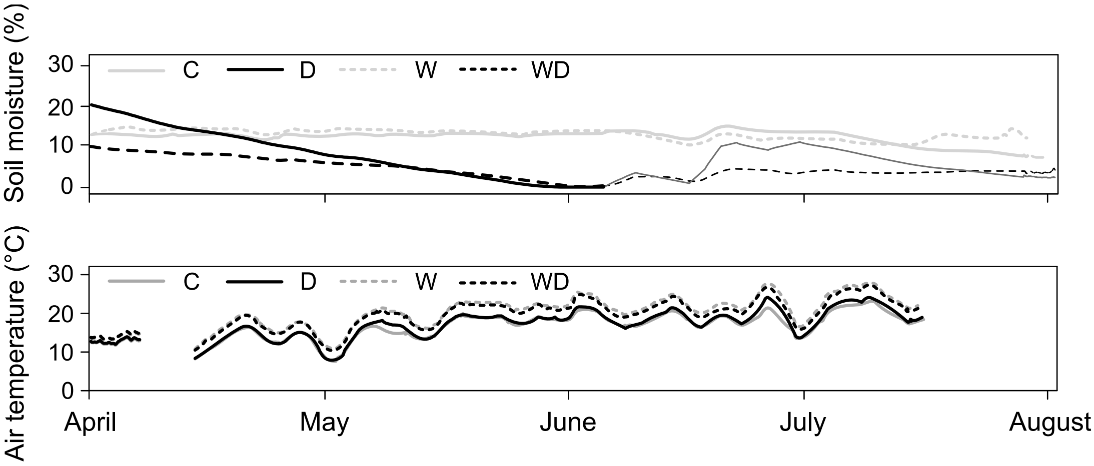

Fig. 2 Soil moisture (%) and air temperature (°C) at plant height for climatic treatments (C – control, D – drought, W – warming, WD – warming combined with drought) over the course of the experiment in 2011. The drought manipulation took place from 31 March to 6 June 2011. The end of drought is reflected by the change in line thickness of the soil moisture curve of the drought and warming combined with drought manipulation.

Rearing the caterpillars of *L. dispar*

L. dispar eggs were used from New Jersey Standard Strain for the palatability experiment and were provided by the USDA - APHIS Otis Method Development Center, Massachusetts, USA. At time of delivery (6 September 2011), the insect herbivores were in the diapause stage. The rearing of the caterpillars followed in a laboratory at the University of Bayreuth. The eggs were divided into petri dishes (8 cm) and covered with a cap to avoid desiccation and possible escape of the caterpillars. The petri dishes were stored in a climate chamber at 22 °C with a day-night-rhythm of 16:8 h. Five days after delivery the *L. dispar* caterpillars hatched out of the eggs. The hatched caterpillars were collected daily and kept in aluminum boxes (7 x 4 x 4 cm), which were perforated for air circulation and covered with a transparent cap, in a climate chamber. The caterpillars were fed with a wheat germ diet (2 g per cup) according to Bell et al. (1981). Every second day, the caterpillars were relocated into new aluminum boxes for cleaning and restocking the wheat germ diet. After ten days, the *L. dispar* caterpillars reached the second larval stage.

Palatability experiment

The palatability experiment took place on 20 and 21 September 2011. We used *L. dispar* caterpillars of the second larval stage for six replicates of each combination of provenance and treatment (4 provenances x 6 replicates x 4 climates = 96 plants). Some *Q. pubescens* seedlings died before the onset of the growing season. Therefore, only 74 plants in total were available for the analyses. We sampled one fully developed leaf per plant and randomly sampled two leaves per plant in some cases in order to compensate for the loss of individuals. The leaf samples for the palatability experiment were cut on 20 September 2011, sprayed with deionized water to keep them turgescent and stored in plastic bags in a cool box for transport. In the laboratory, the leaf samples were stored in a refrigerator at 4 °C until the start of the experiment. The palatability experiment started a few hours after leaf sampling.

Two tissue samples per leaf were taken with a hole punch (10 mm diameter) beyond the leaf-veins if possible, as *L. dispar* caterpillars prefer leaf tissue beyond the leaf-veins (Schowalter 2006). One leaf sample was for the caterpillar, and the other one was used as a reference sample to determine by how much the weight of the sample changes through the drying process. The fresh weight of both samples was recorded. Afterward, one sample was fed. The dry leaf sample weight as well as the weight of the leaf remains after the palatability experiment were recorded after being dried at 70 °C for 2 days. Based on the ratio of fresh and dry weight of the unscathed reference leaf samples, we calculated a leaf specific conversion factor to determine the consumed leaf dry weight (see formula below).

Each *L. dispar* caterpillar was isolated in one petri dish with a cap for 24 h without food and weighed prior to starting the palatability experiment. We placed one caterpillar on each leaf sample, which was also placed on the margin of a moist filter paper (ca. 8 cm^2), in a petri dish (4 cm diameter). All caterpillars were randomly distributed across the ***Q.*** *pubescens* leaf samples. The experiment duration was 24 h in the climate chamber (22° C, day-night-rhythm 16:8 h). The live weights of the caterpillars were then recorded. Caterpillars were killed using isopropyl (alcohol), dried at 70 °C for 2 days for dry weight measurement.

In order to estimate the caterpillar dry weight using live weight, we determined the dry to live weight ratio from 90 randomly selected caterpillars of the same egg strain. The 90 caterpillars were weighed (live weight), then killed using isopropyl (alcohol), dried at 70 °C for 2 days, and weighed again. We defined the factor ***F*** as the ratio of the mean dry to the mean live weight over the sample of the 90 caterpillars. The factor ***F*** evaluated to 0.1983.

Response parameters

Consumed leaf dry material

'Consumed leaf dry material' X_c was calculated as:

$$X_c = \frac{x_{lf} x_{l2d}}{x_{l2f}} - x_{ld},$$

where x_{lf} and x_{ld} denote the leaf sample fed to the caterpillar, whereby x_{lf} being the initial fresh weight and x_{ld} the dry weight of the leaf remains. The fresh and dry weight of the second leaf sample for the dry weight determination are denoted by x_{l2f} (fresh) and x_{l2d} (dry). As explained above, the ratio x_{l2d}/x_{l2f} determines by how much the weight of this particular leaf changes during drying. The above term $x_{lf}\,(x_{l2d}/x_{l2f})$ is thus an estimate of the full dry weight of the sample fed to the caterpillar, which could never be recorded. Subtracting from this the measured dry weight of the leaf remains x_{ld} results in X_c, the dry weight of the amount actually consumed.

Leaf traits

At the end of the drought manipulation period (6 June 2011), one fully developed leaf per individual was sampled in order to measure the ’specific leaf area’ and the ’density of trichomes’. The ’density of trichomes’ was measured on the abaxial leaf surface, which has a higher density of trichomes compared to the adaxial surface. ’Specific leaf area’ measurements followed the standard protocol according to Cornelissen et al. (2003). ’Density of trichomes’ was measured by microscopic counting over a counting area of 8.73 mm^2 per leaf.

Statistical analysis

Analysis of covariance (ANCOVA) was applied to test the main and interactive effects of the provenance (levels: Bulgaria, Germany, Hungary, Italy) and treatment factors (levels: warming, drought, warming and drought, control) on the parameter ’consumed leaf dry material’ with ’herbivore initial dry weight’ as a covariate (Raubenheimer and Simpson 1992; Horton and Redak 1993). The ’herbivore initial dry weight’ covariate was used in order to avoid an influence of differences in initial fitness of herbivores. The interaction between provenance and replicate number of leaves as well as between treatment and replicate number of leaves was included in the ANCOVA model due to the multiple sampling of the *Q. pubescens* leaves per individuals. In case of significant main effects of the ANCOVA, post hoc comparisons (Tukey’s test) were performed according to Hothorn et al. (2008). In order to test for dependencies between the leaf consumption of the *L. dispar* caterpillars and the leaf traits of the four provenances, we applied least-squares regressions between the parameters ’consumed leaf dry material’ and ’density of trichomes’ as well ’specific leaf area’. If necessary, the data were log-transformed to improve the normality of residuals and the homogeneity of variances prior to analysis (Faraway 2006). The covariate ’herbivore initial dry weight’ prior to starting the palatability experiment was included in the regression models. The level of significance was set to $p < 0.05$. All statistical analyses were conducted with the software R 2.13.1 (R Development Core Team 2011) and the additional package nlme.

Results

Influence of climatic manipulations and different provenances on leaf palatability

Surprisingly, climatic manipulations did not affect the leaf palatability (F = 0.3; p = 0.854). However, we detected a significant difference in the 'consumed leaf dry material' among the Bulgarian (BG), German (GER), Hungarian (HU), and Italian (IT) provenances (F = 3.7; p = 0.017; Tukey's post hoc test: GER-BG: p = 0.220; HU-BG: p = 0.044; IT-BG: p = 0.016; HU-GER: p = 0.708; IT-GER: p = 0.506; IT-HU: p = 0.998). The leaves of the Bulgarian *Q. pubenscens* individuals were preferred by the caterpillars compared to the plants from Hungary and Italy (Fig. 3). There was no interaction between the factors provenance and treatment (F = 1.5; p = 0.197).

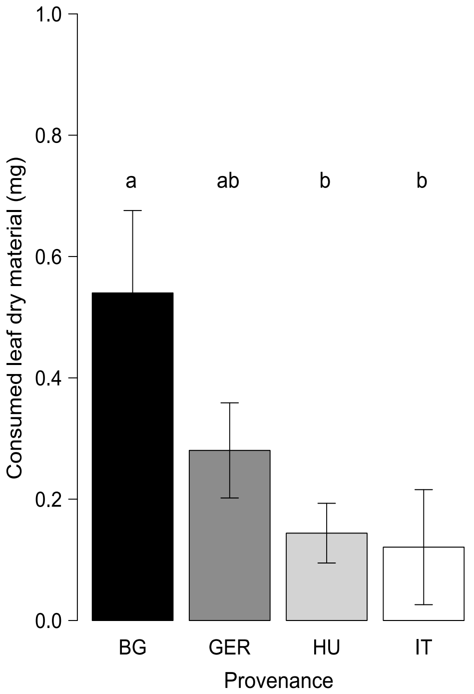

Fig. 3 Consumed leaf dry material (mg) of the four *Quercus pubescens* provenances. Mean values and standard errors are shown. The lower case letters represent significant differences as revealed by the post-hoc test. Category 'a' differs from 'b', whereas 'ab' does not differ significantly from the others. *BG* Bulgaria, *GER* Germany, *HU* Hungary, *IT* Italy.

Leaf traits

The 'density of trichomes' was significantly correlated with the 'consumed leaf dry material' (R^2 = 0.17; p = 0.017), whereas the leaf consumption of the insect herbivores decreased with an increase in 'trichome density' (Fig. 4). The 'specific leaf area' showed no correlation with the 'consumed leaf dry material' (R^2 = 0.02; p = 0.686).

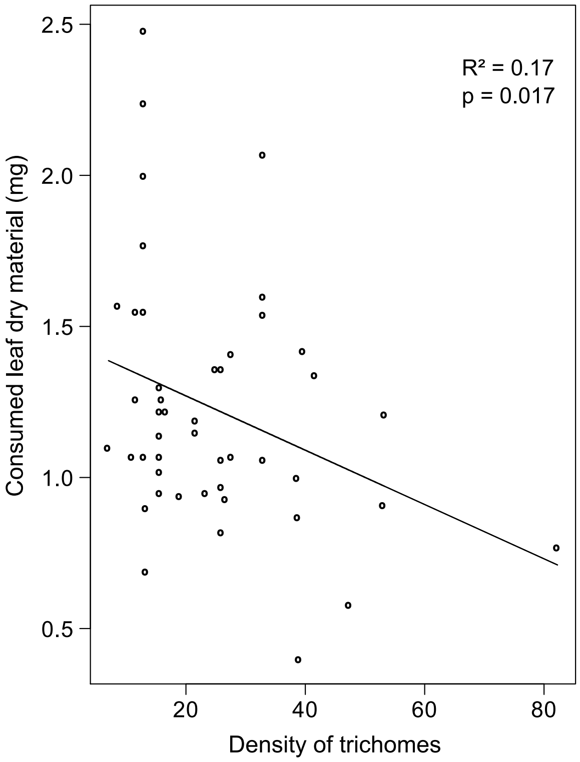

Fig. 4 Consumed leaf dry material (mg) of the four *Quercus pubescens* provenances (Bulgaria, Germany, Hungary, and Italy) as a function of density of trichomes. Consumed leaf dry material values are transformed. Linear regressions are shown, where significant ($p < 0.05$).

Discussion

Warming and drought do not influence leaf palatability

Surprisingly, we detected no significant climatic manipulation effect on the palatability of *Q. pubescens* leaves. Neither warming nor drought or the combination of both influenced the leaf consumption of the *L. dispar* caterpillars. Therefore, the hypothesis H1, which assumed an influence of those, has to be rejected. Our results are in discordance to the general opinion that climate change will affect the leaf palatability and thus the leaf consumption of chewing insects (e.g. Rouault et al. 2006; Jactel et al. 2012; Jamieson et al. 2012). Especially in the case of insects, such as *L. dispar* caterpillars, that live on leaves an impact might be expected due to the results of Jactel et al. (2012). They found a higher impact of insect pests living on leaves compared to insect pests living in wood on tree species under drought stress in their meta-analysis.

Apart from this, *Q. pubescens* is deemed to be heat and drought adapted. Our climatic manipulations were based on forecasted climate events and trends in Europe (IPCC 2012) and represent a millennium drought for the provenance from the southernmost region. The determination of the millennium drought followed Jentsch et al. (2007) using a Gumbel distribution (Gumbel 1958). Nevertheless, it is possible that the strength of our simulated warming and drought manipulation was insufficient to achieve an alteration in the leaf quality of pubescent oak. This assumption is supported by the results of another study on the very same *Q. pubescens* provenances in the same experiment which yielded no significant impact of warming and drought on the nitrogen content of *Q. pubescens* leaves from the four European provenances (Wellstein and Weigel, unpublished data). Similar results in terms of leaf nitrogen content under drought conditions were found by Gutbrodt et al. (2011) and for elevated temperature by Zvereva and Kozlov (2006).

Leaf palatability is linked to geographic origin

We detected a preferential consumption of the Bulgarian *Q. pubescens* leaves by the *L. dispar* caterpillars. This approves our second hypothesis (H2), in which differences between the four provenances were expected. Wellstein and Weigel (unpublished data) showed that the Bulgarian provenance of *Q. pubescens* displayed the lowest 'density of trichomes' compared to the Italian and Hungarian *Q. pubescens* provenances. The findings of Wellstein and Weigel (unpublished data) coupled with our detection of a significant correlation between 'consumed leaf dry material' and 'density of trichomes' most likely explain the caterpillars' preference of the Bulgarian provenance. Therefore, our hypothesis H3 which stated a relation between both quantities is supported by the findings for this leaf trait. Similar results for the correlation between the density of trichomes and the leaf consumption were found by several studies (e.g. Dimock and Tingey 1988; Ågren and Schemske 1993; Fordyce and Agrawal 2001). In summary, our findings suggest differences in investments in leaf traits such as trichomes among the European *Q. pubescens* provenances, whereas the Bulgarian *Q. pubescens* provenance seems to be least protected against the insect herbivore *L. dispar*.

Regarding the 'specific leaf area', Wellstein and Weigel (unpublished data) showed that *Q. pubescens* had a smaller 'specific leaf area' in response to warming compared to ambient temperature conditions. However, we could not find a correlation between the 'consumed leaf dry material' of *L. dispar* caterpillars and the 'specific leaf area' of the pre-exposed leaves of *Q. pubescens*. Thus, hypothesis H3, which stated a relation between both quantities, is rejected for this leaf trait. A preference for leaves with a larger 'specific leaf area' by the *L. dispar* caterpillars would be expected, as leaves with a larger 'specific leaf area' value are preferred by insect herbivores due to higher nitrogen than carbon contents (Bresinsky et al. 2008). Yet, the similar leaf nitrogen contents in all climatic treatments may be an explanation for the lacking leaf size preference of the caterpillars and thus explain the missing correlation between 'consumed leaf dry material' and 'specific leaf area'.

Conclusions

The climatic manipulations (warming and drought) did not affect the leaf consumption of *L. dispar* caterpillars. Thus, it is possible that the attractiveness of *Q. pubescens* within its distribution range will remain stable under a changing climate. Inferred from the effect of the 'density of trichomes' on the leaf palatability, the variability of this leaf trait is a more likely factor modulating the attractiveness of pubescent oak within its distribution range. We thus conclude that leaf traits deserve further attention in plant-insect herbivore interactions in the context of climate change.

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Manuscript 3: Different reactions of central and marginal provenances of *Fagus sylvatica* to experimental drought

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Abstract

Climate extremes are expected to increase in frequency and magnitude as a consequence of global warming, threatening the functioning, services and goods of forest ecosystems. Across Europe, the ecologically and economically important tree species *Fagus sylvatica* is expected to suffer particularly under such conditions. The regional introduction of provenances from drier and warmer climates is one option to adapt beech forest ecosystems to these adverse effects of climate change. Marginal populations from the drought-prone southern and north-eastern edges of the species' distribution come into focus in search of suitable candidates for Central European deciduous forests. Here, we test three marginal provenances (Spain, Bulgaria and Poland) and three provenances from the centre of the distribution range (Germany) for their response to drought in two different soil types (sand, loam) in a full factorial common garden experiment in Landau, Germany. Drought impacted all growth parameters negatively (leaf damage +22 % (percentage points), height -40 % and diameter increment -41 %), and the sandy substrate exacerbated this effect. However, provenances differed in their response to drought and soil type. Evidence for a local adaptation to summer drought was detected, especially in terms of mortality rates. The Bulgarian and Spanish provenance showed a stable performance under drought conditions (BG -27 % in diameter increment; ES -32 %), compared to the Polish (-48 %) or the most sensitive German provenances (-57 %), yet for Bulgaria on a low level of total increment. This may indicate a trade-off between drought tolerance and growth. Therefore, a sole focus on drought-resistant marginal provenances seems to not be conducive, as they might be less adapted to other climatic factors, e.g. frost, as well. However, intermixed with local Central European provenances, these may act as functional insurance in future drought-prone forest stands.

Keywords: Extreme events, Local adaptation, Phenotypic plasticity, Plant-climate interactions, Provenance trial, Within-species diversity

Introduction

Alongside an increase in average temperature, global warming is expected to trigger an increase in magnitude and frequency of climatic extremes such as prolonged drought events (IPCC 2012). This will especially affect the functioning of ecosystems with long-living organisms, such as forests, and may lead to a declining provision of forest goods and services (Allen et al. 2010, Lindner et al. 2010). The velocity of these changes in ecosystem functioning may exceed the natural dispersal rates of trees and the speed of evolutionary adaptation processes (Bertrand et al. 2011, Chen et al. 2011).

Fagus sylvatica is a naturally dominant and economically important, but drought-susceptible tree species in Central Europe that deserves special attention in the face of these changing conditions (Fotelli et al. 2009). Even though *F. sylvatica* is considered a high-risk species in the face of climate change (Ohlemuller et al. 2006, Scherrer et al. 2011), the area stocked with *F. sylvatica* constantly increases in Central Europe/Germany due to forest conversion from coniferous to mixed stands. According to Polley et al. (2009), beech is the species in Germany with the highest increase in stocked area between 2002 and 2007 (83,000 ha). Drought events most probably determine the southern edge of the distribution range (Jump et al. 2006). In particular at lower elevations, growth and distribution of *F. sylvatica* are expected to decline at its southern limits (Matyas et al. 2009, Hlasny et al. 2011, Jezik et al. 2011) and on xeric sites in Central Europe (Czucz et al. 2011). The observed recent decline in beech forest productivity in France could be linked to low water availability, especially in early summer (Lebourgeois et al. 2005, Charru et al. 2010). Furthermore, Piovesan et al. (2008) showed that basal area increment of European beech stands decreased with decreasing water availability in the Apennine Mountains in Italy. The exceptional 2003 drought period over Central Europe generated distinct negative effects on the performance of *F. sylvatica* (Czajkowski et al. 2005, Leuzinger et al. 2005, Betsch et al. 2011). Moreover, it seems that *F. sylvatica* loses its competitive advantage to less drought-sensitive species, e.g. *Quercus petraea*, under water-limited conditions in southern and south-eastern part of its distribution range (Bonn 2000, Fotelli et al. 2001, Friedrichs et al. 2009, Clark et al. 2011, Scharnweber et al. 2011), whereas positive impacts of climate change on competition may prevail on the northern distribution edge in Sweden (Bolte et al. 2010). Drought periods also increase pathogen and fungi susceptibility of *F. sylvatica*, as shown by Jung (2009) for the post-2003 years in a Bavarian beech stand. Therefore, Rennenberg et al. (2004) and Gessler et al. (2007) consider the focus on *F. sylvatica* in the ongoing forest conversion as very risky.

However, *F. sylvatica*, with its wide geographic distribution, covers a broad range of climatic conditions (see Kölling 2007). The introduction of *F. sylvatica* provenances from warmer, more drought-prone regions, especially from the edges of its distribution range to Central Europe might therefore be one potential adaptation tool to dampen the adverse effects of future climatic environments (Bolte et al. 2009, Kreyling et al. 2011). The genetic composition of forest trees determines the phenotypic plasticity and with this their adaptive capacity to environmental stressors such as drought (Schaberg et al. 2008, Matyas et al. 2009). In Central Europe, *F. sylvatica* exhibits a high genetic diversity within populations (Konnert 1995, Vornam et al. 2004), whereas on a continental scale, the genetic differentiation between populations becomes more distinct (Comps et al. 1990, Magri et al. 2006). These differences in genetic configuration between populations, most likely, display differences in adaptive capacity between provenances. In numerous provenance trials over the last decade, a contrasting performance of provenances from different geographic origins has been demonstrated (e.g. von Wuehlisch et al. 1995). Furthermore, it was shown that different provenances exhibit specific responses to abiotic stressors such as late frosts (Kreyling et al. 2012) or drought (Schraml und Rennenberg 2000, Peuke et al. 2006). In a European-wide provenance trial network, macroclimatic adaptation could be detected: the performance of different provenances was negatively correlated with climatic distance (absolute differences in certain climatic parameters) between test site and origin of the provenance (Matyas et al. 2009). Czajkowski and Bolte (2006) found a significantly higher transpiration and therefore higher water stress under drought conditions for German and West Polish provenances than for provenances from the more continental Central Polish eastern distribution edge of *F. sylvatica*. In the extraordinary dry year 2003, NW-Greek beech populations only experienced mild drought stress compared to the reported damages in beech forests in Central Europe, despite comparable climatic conditions (Fotelli et al. 2009), thereby indicating a local adaptation of Greek populations to water shortages. Therefore, such marginal populations from the dry edges of the current distribution range of *F. sylvatica* may come into focus in search of drought-resistant ecotypes (Rose et al. 2009, Kreyling et al. 2011). The susceptibility to climatic stressors such as drought, however, strongly depends on the interaction with additional biotic or abiotic factors (Messaoud and Chen 2011). The impact of drought and water stress on the performance of *F. sylvatica* depends on air ozone concentration (Pollastrini et al. 2010), carbon dioxide concentrations (Penuelas et al. 2008), light availability (Czajkowski et al. 2005, Lof et al. 2005) and soil chemistry (Weitner et al. 2007). Evidence suggests that, in general, drought reduces nutrient availability and uptake in forest soils (Rennenberg et al. 2009, Kreuzwieser and Gessler 2010). The interactive effects of soil nutrient balance and water availability on fine roots, microorganisms and soil nutrient cycling processes are poorly understood (Kreuzwieser and Gessler 2010). However, especially for *F. sylvatica* which grows on various soil types throughout its distribution range (Ellenberg and Leuschner 2010), the occurrence of drought conditions or water stress is a complex interactive mechanism between climatic and edaphic factors (Gaertner et al. 2008).

Considering the reported negative impacts of drought on the performance of *F. sylvatica* and the intraspecific variability in drought response, it is important to know whether certain provenances of *F. sylvatica* are less susceptible to these negative effects and whether site factors such as soil type influence specific responses. This knowledge is crucial to assess the potential of selective transplanting of drought-resistant provenances as a tool to dampen negative impacts of such climate extremes in the course of climate change.

Here, seedlings of six provenances of *F. sylvatica*, three from the centre of its distribution range and three from the margins (SE, SW, NE), were exposed to drought in two different soil types (with contrasting nutrient availability and field capacity) in a full factorial common garden experiment. We hypothesized that (1) provenances differ in the extent of leaf damage, height and diameter growth and (2a) drought and (2b) sandy soil substrate negatively influence these performance indicators. We further assumed that (3) marginal populations are less drought sensitive, and we expected (4) that soil conditions and drought interactions significantly affect plant performance.

Material and Methods

Experimental site

The provenance trial (EVENT 3-Landau) is a subproject of the EVENT-Experiment series in Bayreuth, Germany (Beierkuhnlein et al. 2011). It was established in 2010 in the vicinity of the University of Koblenz-Landau, at the Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Siebeldingen (49°13'03" N, 8°02'47" E, 202 m a.s.l.). The mean annual temperature at the site is 10.2 °C and the mean annual precipitation is 643 mm, which is distributed bimodally with two major peaks in Mai/June and November/December (data: German Weather Service). Annual average temperature is expected to increase by 3.7 K by the end of the century (2080uxs compared to 1950-2000), and precipitation is projected to decrease by about 50 mm per year, with a major decrease in summer (JJA) precipitation (-60 mm), according to the A1B scenario of the MPI-ECHAM5-model (data: WorldClim).

Plant material

Seeds of six provenances of *F. sylvatica* were obtained in autumn 2009 and brought to germination at the *Bavarian Institute for Forest Seeding and Planting* (ASP) in Teisendorf, Germany, in spring 2010. All six provenances stem from autochthonous populations (Fig. 1, Table 1). The provenances were chosen to represent the centre of the distribution range (DE1, DE2, DE3) and the south-eastern (BG) and south-western (ES) edges, where drought limits the distribution of the species, and the north-eastern edge (PL), where besides summer precipitation winter conditions and late frost are important limiting factors (Table 1, Fig. 1). In January 2011, the seedlings were transported to Siebeldingen and in March 2011, they were planted in 12-litre pots with two different soil types. Individuals were selected randomly for each provenance and treatment from all living plants at planting date. Mean plant height at the start of the experiment was 22.1 cm (±6.6 cm SD), mean plant diameter 2 cm above the root collar 4.7 mm (4.4–5.3 mm) with a standard deviation of ±0.8 mm SD (0.6–0.8 mm).

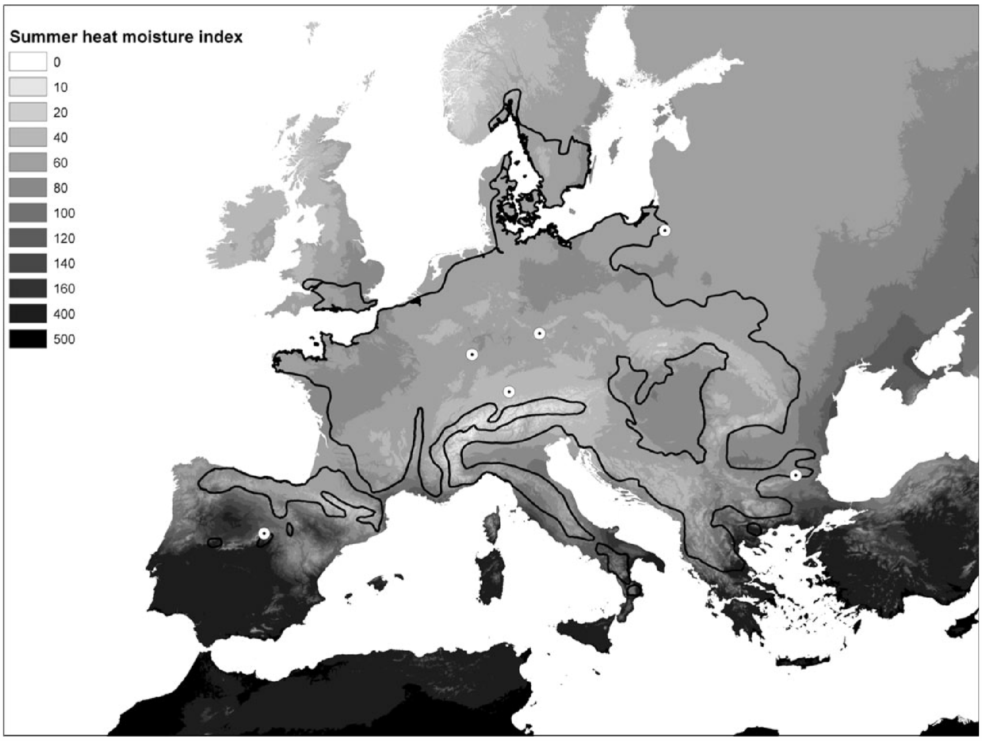

Fig. 1 Geographic origins of provenances used in the experiment. Grey scales display the Summer Heat Moisture index based on WorldClim data (Hijmans et al. 2005). Distribution range of *F. sylvatica* is outlined according to EUFORGEN.

Table 1 Site information for the provenances used in the experiment. Mean temperature of the warmest month (MWT), summer precipitation (P(5-9)) and Summer Heat Moisture index derived from WorldClim (Hijmans et al. 2005).

Code	Location	Country	Latitude	Longitude	Elevation (m a.s.l.)	SHMI	MWT (°C)	P (5-9) (mm)
BG	Kotel	Bulgaria	N 42°51'59"	E 26°26'40"	600	61	19.2	313
ES	Montejo de la Sierra	Spain	N 41°07'12"	W 03°30'36"	1,350	80	17.7	212
DE1	Hengstberg	Germany	N 50°08'00"	E 12°11'00"	569	47	15.9	341
DE2	Johanniskreuz	Germany	N 49°18'00"	E 07°50'00"	570	42	16.0	381
DE3	Kempten	Germany	N 47°44'48"	E 10°08'54"	803	26	15.3	580
PL	Mragowo	Poland	N 53°52'00"	E 21°20'00"	137	51	18.0	350

Experimental set-up

The potted individuals were exposed to a fully crossed experimental design consisting of a precipitation treatment (drought and control) and two different soil types (sandy loam, loamy sand). Each treatment combination (drought with sandy loam, drought with loamy sand, control with sandy loam and control with loamy sand) was replicated with nine individuals. For two provenances (Spain, Poland) the number of replicates had to be reduced to 8 and 7, respectively, due to mice damage, resulting in 204 plants overall. The experiment was set up completely randomized in a concrete bed filled with sand. All pots were put on plastic saucers.

On April 13, the experiment was covered by a rainout shelter constructed of a steel frame (GlasMetall Riemer GmbH, Rahden-Sielhorst, Germany) and covered with a transparent polyethylene sheet (0.2 mm, SPR 5; Hermann Meyer KG, Rellingen, Germany). The lower edge of the rainout shelter was 80 cm above ground. The shelter permitted the penetration of nearly 90 % of the photosynthetically active radiation. Additionally, as seedlings of *F. sylvatica* are sensitive to direct radiation, a shading canvas (Quadra 105ME, 105 g, DM-Folien GmbH, Reutlingen, Germany) was attached to the inside of the polyethylene sheet, resulting in a penetration of about 55 % of the photosynthetically active radiation. As the shade-tolerant species European beech has shown to yield highest growth rates when exposed to medium light levels (e.g. Schall et al. 2012) most likely no limitation by light availability occurred in our experiment. From the planting date until the rainout shelter was set up, the plants received the ambient precipitation and were additionally watered with groundwater (March 14, 18, April 1, 4, 8 and 11). After the rainout shelter was set up, and the plants were watered with groundwater on April the 18, 28 and 30, so that a sufficient water supply and root growth was guaranteed. From May 2, on all plants were exposed to the control precipitation regime which simulated the local daily 40-year average precipitation. The application was done twice a week with groundwater.

The drought manipulation consisted of a 36-day period without irrigation. It started on May 9 end was completed when 20 % of the plants showed strong drought damage (76-100 % of the leaves damaged), on June 13. Approximately 12 days after the start of the treatment, soil moisture dropped below the permanent wilting point (pF = 4.2; Fig. 2) for the loamy sand substrate and about 1 week later for the sandy loam substrate. In the re-watering phase after the drought period, the plants were exposed to the control precipitation treatment again. Additionally they received the amount of water which was given to the control plants over the 36-day drought period, evenly distributed over 1 week, so that at the end of the experiment, both the individuals in the control as well as in the drought treatment received the same amount of water.

The volumetric soil water content was measured with dielectric permittivity sensors (EC-5 soil water sensor, Decagon Devices, Inc., Pullman, USA). Altogether 10 sensors with two data loggers (ECH2O, Decagon Devices, Inc. Pullman, USA) were installed in the pots in 5-10 cm depth, three for the two soil types each in the drought treatment and two each in the control treatment.

The soil treatment consisted of two different soil types. The first one was a sandy loam substrate from a local forest topsoil dugout, from now on called *loam* or *loamy substrate*. The second soil type was a loamy sand substrate, consisting of a mixture between the loam and arenaceous quartz sand from a local sand pit (50 % each), from now on referred to as *sand* or *sandy substrate* (Table 2).

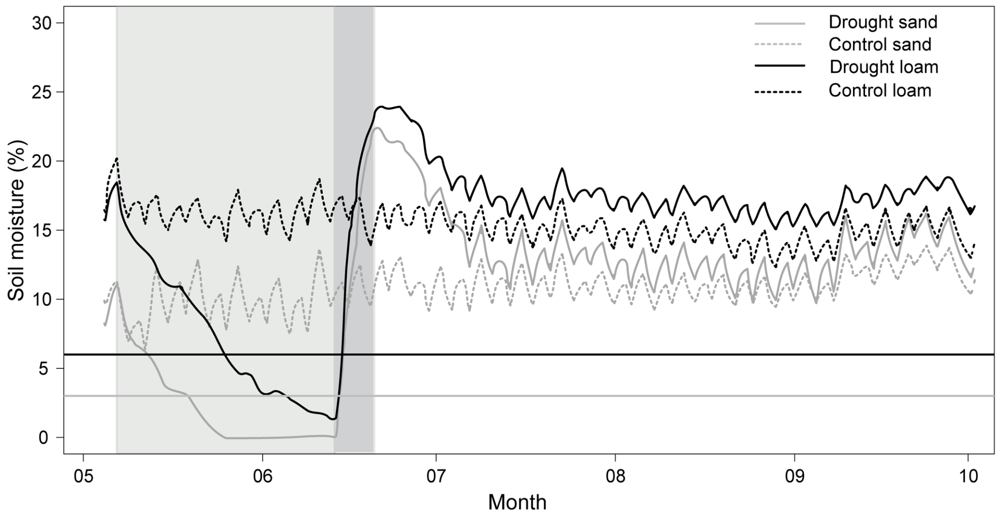

Fig. 2 Soil moisture for all four treatment combinations. The *light grey area* shows the artificial drought period, the *dark grey area* shows the re-watering phase. The *two horizontal lines* depict the approximated permanent wilting points for the loamy (*black*) and the sandy substrate (*grey*).

Table 2 Nutrient content and pH-values for the two soil types used in the experiment

	K (mg/kg)	Mg (mg/kg)	P (mg/kg)	NO_3 (mg/kg)	NH_4 (mg/kg)	C_{org} (%)	pH-CAT	pH-water	N (%)
Loamy sand	54.8	121	11.1	14.1	2.02	0.56	6.3	8.5	<
Sandy loam	118	267	48.3	31.0	3.98	1.92	4.7	7.6	0.14

Response Parameters

Short-term response to the treatments was determined by visual estimates by assigning the average percentage of damaged leaves per plant to six classes (0, 1-5, 6-25, 26-50, 51-75, 76-100 %). A leave was counted as "damaged", as soon as it exhibited necrotic tissue. The share of necrotic tissue of the whole leave was not taken into consideration. This assessment took place at the last day of the drought treatment. For each individual, mid points of the six classes were used for further statistical analyses.

Long-term effects of the treatments were determined by measuring height and diameter shortly after planting time in April and after the growing period at the end of September. Increment was calculated by subtracting the initial values from the values at the end of the growing period. The statistic were conducted both for absolute and relative increment values. As the initial plant dimensions were very similar over the provenances and thus the relative values yielded similar results, we concentrate on absolute values in this study. Dead individuals were counted, and the percentage of dead individuals per treatment, soil type and provenance was calculated. Furthermore, the Summer Heat Moisture index (Wang et al. 2006) was calculated ((mean temperature of warmest month)/(mean annual summer (May-September) precipitation/1,000)) for each geographic origin of the provenances (data: WorldClim; Hijmans et al. 2005).

Statistics

Analyses of variance (ANOVA) combined with linear models were applied to test for the main and interactive effects of the three factors provenance, drought and soil on height and diameter increment and on leaf damage. Data was square root transformed in order to improve homogeneity of variance and normality of residuals prior to analysis if necessary. In case of significant effects, post hoc comparisons were run (Tukey's HSD).

To test for the long-term impacts of short-term damage, an ordinary least-squares regression was applied between the reduction of height and diameter increment in the drought treatment compared to control (in %) and the percentage of damaged leaves per plant averaged per provenance. Generalized linear models were used to determine the effects of treatment, soil and provenance on mortality, least-squares regressions to correlate mortality with increment rates.

Least-squares regressions were also used to assess the correlation between the Summer Heat Moisture index (SHM) at the geographic origins of the provenances and increment reduction (drought compared to control in %), leaf damage (%) and mortality (%) in order to detect local adaptations to drought events.

All statistical analyses were conducted with the software R.2.13.0 (R Development Core Team 2010).

Results

Leaf damage

The provenances differed in the percentage of damaged leaves per plant (ANOVA: $F = 2.5$, $p = 0.035$; Fig. 3a). The German provenance from Kempten (DE3) showed the highest share of damaged leaves (23 %), whereas the Bulgarian provenance exhibited a significantly lower percentage of damaged leaves (6.5 %). The drought treatment increased the percentage of damaged leaves significantly from 4 % in the control treatment to 26 % on average for plants under the drought treatment (ANOVA: $F = 30.9$, $p < 0.001$; Fig. 3b). Plants grown in sand showed a significantly higher share of damaged leaves (22.1 %) than plants in loam (7.8 %) (ANOVA: $F = 16.1$, $p < 0.001$; Fig. 3c).

Soil type significantly impacted the drought effect on leaf damage (ANOVA: soil x drought: $F = 9.0$, $p = 0.003$; Fig. 3d). In the loamy substrate, drought caused 4.7 times (*D* 12.8 %, *C* 2.7 %) more damage compared to control, whereas in sand, drought caused 7.2 times (*D* 39.1 %, *C* 5.4 %) more damage than the control treatment. Provenance-specific reactions to drought or soil type could not be detected (ANOVA: provenance x drought: $F = 1.3$, $p = 0.283$; provenance x soil: $F = 2.3$, $p = 0.128$).

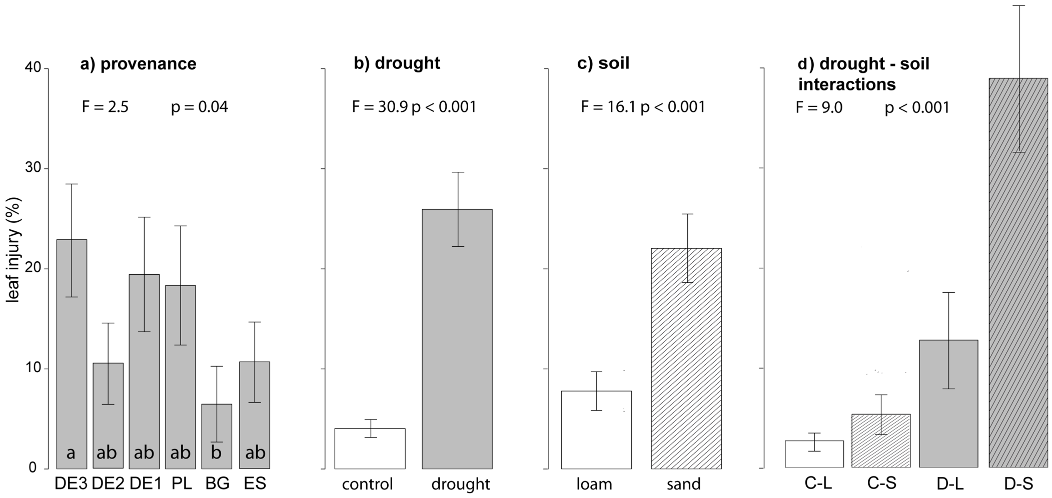

Fig. 3 Leaf damage of *F. sylvatica* at the end of the drought manipulation (June 13) in % in response to **a** provenance, **b** drought and **c** soil type. All significant main and interaction effects (**d**) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI.

Height increment

Provenances differed in terms of height increment (ANOVA: $F = 18.5$, $p < 0.001$; Fig. 4a). The Bulgarian and the German provenance from Johanniskreuz (DE2) showed significantly less increment than the other provenances (BG 5.1 cm, DE2 5.7 cm). The German provenance Hengstberg (DE1) performed best with 14.9 cm height increment. The drought treatment reduced the height increment significantly from 13.5 cm under control conditions to 8.1 cm on average for plants exposed to drought conditions (ANOVA: $F = 32.9$, $p < 0.001$; Fig. 4b). Soil did not influence increment in height (ANOVA: $F = 2.1$, $p = 0.149$; Fig. 4c).

The provenances showed different height increment patterns across the two soil types (ANOVA: provenance x soil: $F = 2.6$, $p = 0.026$; Fig. 4d). The Bulgarian provenance reacted positively to sandy soil, whereas all other provenances showed no or a negative reaction to the sand compared to the loam.

Provenance-specific reactions to drought or interactive effects of drought and soil could not be detected (ANOVA: provenance x drought: $F = 1.4$, $p = 0.235$; drought x soil: $F = 2.3$, $p = 0.132$).

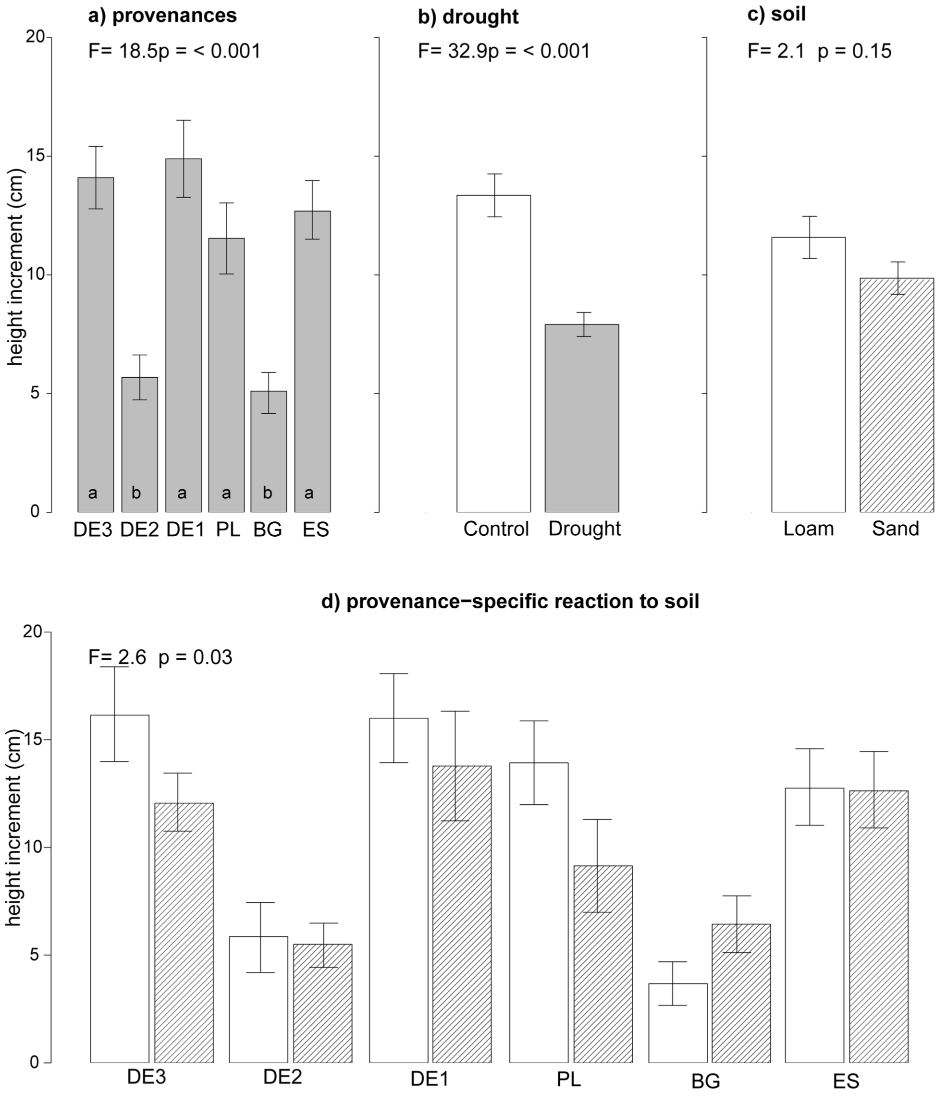

Fig. 4 Height increment of *F. sylvatica* in response to **a** provenance, **b** drought and **c** soil type. All significant main and interaction effects (**d**) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI.

Diameter increment

The Bulgarian provenance (BG) grew significantly worse (2.0 mm) than all other provenances in terms of diameter (ANOVA: $F = 3.1$, $p = 0.011$, Fig. 5a), while all others showed similar growth rates (2.6-2.9 mm). The drought treatment reduced the diameter increment significantly from 3.4 mm under control conditions to 2.0 mm on average for plants exposed to drought conditions (ANOVA: $F = 89.7$, $p < 0.001$, Fig. 5b). The sandy substrate also had negative impacts on secondary growth, reducing increment from 3.1 mm in loamy substrate to 2.2 mm in sandy substrate (ANOVA: $F = 37.3$, $p < 0.001$, Fig. 5c).

The two soil types triggered a provenance-specific reaction in terms of diameter increment (ANOVA: provenance x soil: $F = 2.6$, $p = 0.027$; Fig. 5d). The Bulgarian and Spanish provenances responded with a relatively stable diameter increment to sandy substrate compared to the loam, whereas all other provenances showed higher growth rates on loam. The German provenance Hengstberg (DE1) exhibited a notably strong decline in mean diameter increment on sand (even if not significantly different from reactions of other German and Polish provenances). Furthermore, the provenances showed a different tolerance to drought with regard to diameter increment (ANOVA: provenance x drought: $F = 2.7$, $p = 0.020$, Fig. 5e). Only a minor decrease could be detected for the Bulgarian provenance, while the German provenances Hengstberg (DE1) and Kempten (DE3) showed strong cutbacks in diameter increment in response to drought. Soil type significantly impacted diameter increment in response to drought (ANOVA: drought x soil: $F = 7.7$, $p = 0.006$). The sand fostered the negative impacts of the drought period on diameter increment (control 3.1 mm; drought 1.3 mm), whereas in the loamy soil, the drought impact was more moderate (control 3.6 mm; drought 2.6 mm).

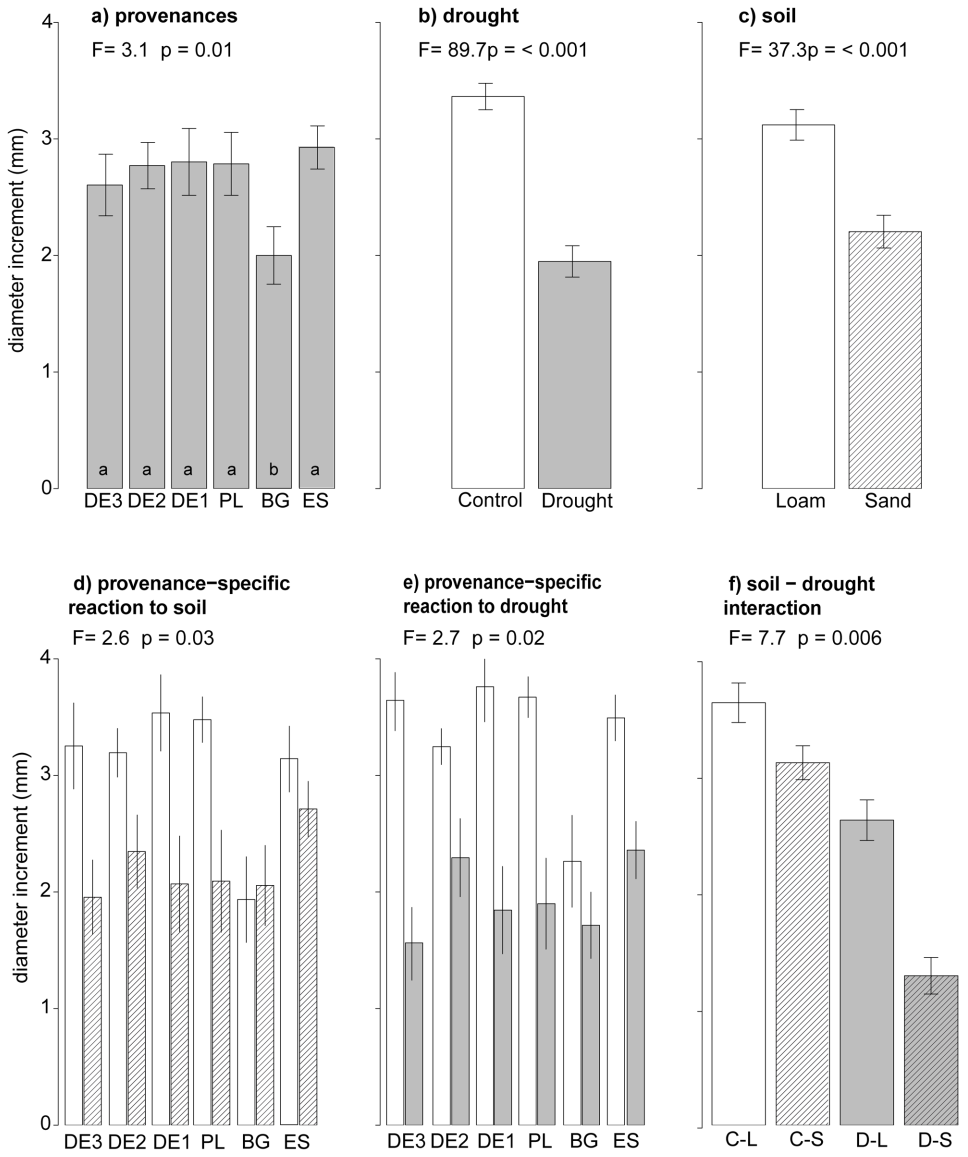

Fig. 5 Diameter increment of *F. sylvatica* in response to **a** provenance, **b** drought and **c** soil type. All significant main and interaction effects (**d-f**) of the three-factorial ANOVA are displayed. Provenances are arranged with increasing SHMI.

Mortality

Mortality occurred solely within the drought treatment, and within the drought treatment mainly in the sandy substrate (drought/loam = 7.8 %, drought/sand =

25.5 %; ANOVA: $p = 0.012$). Provenance did not impact mortality significantly (ANOVA: $p = 0.097$), even though mortality correlated significantly with summer drought conditions at the places of origin of the provenances (see Results, Local adaptation). Mortality tended to increase (non-significantly) with diameter increment reduction (adjusted $r^2 = 0.39$, $p = 0.110$) in the drought treatment compared to control.

Correlation of leaf damage with increment

The average percentage of damaged leaves per provenance was strongly correlated to a reduction in diameter increment (adjusted $r^2 = 0.99$, $p < 0.001$). The reduction in height increment exhibited a non-significant trend towards an increasing reduction with increasing leaf damage (adjusted $r^2 = 0.42$, $p = 0.097$).

Local adaptation

The SHM at the geographic origin of the provenances did not influence the reaction to drought with regard to height increment (adjusted $r^2 = 0.01$, Fig. 6a). However, even if non-significant, provenances from origins which are more prone to summer drought events (higher SHM) tended to be more drought tolerant in terms of diameter increment and leaf damage, as SMH at the geographic origins explained 25 % of the variance in diameter increment reduction (Fig. 6b), 31 % of the variance in leaf damage (Fig. 6c). A significant correlation ($p = 0.041$) could be detected between the SMH index and mortality (Fig. 6d). Here the Summer Heat Moisture index at the geographic origin explains 69 % of the variance in mortality.

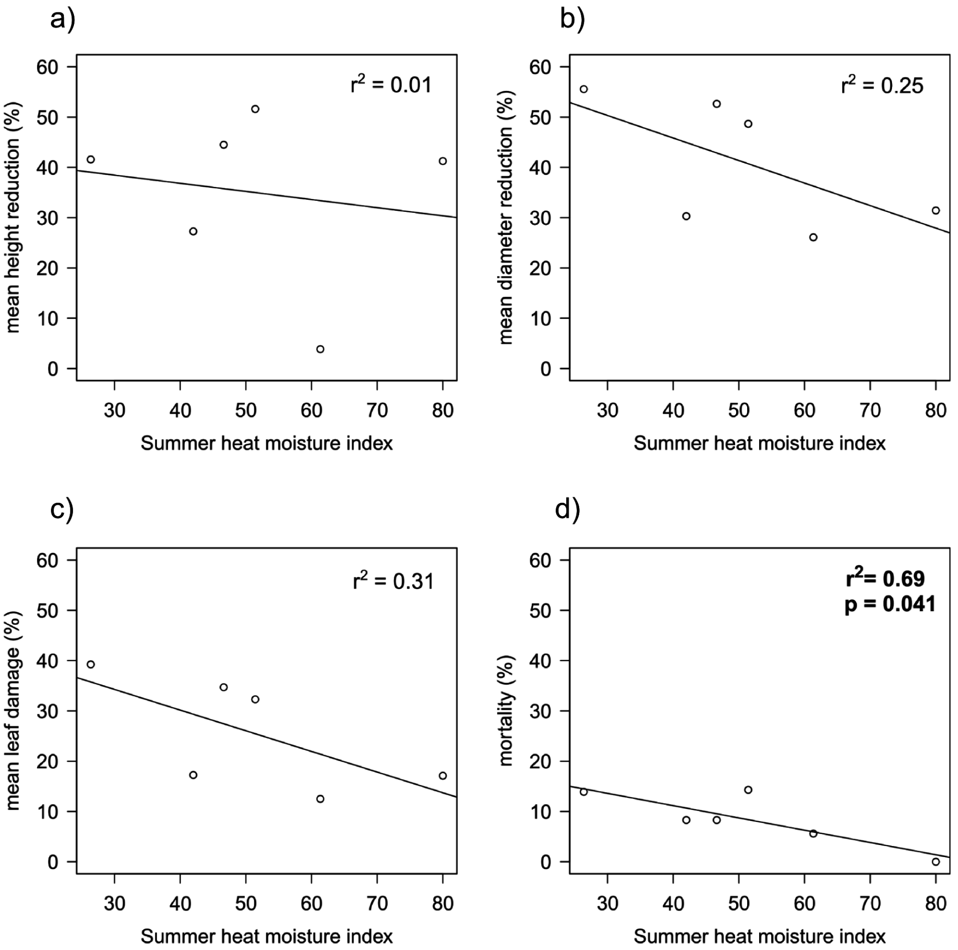

Fig. 6 Correlation between the Summer Heat Moisture index at the origin of the provenances and the mean reduction in **a** height and **b** diameter increment, **c** mean leaf damage per provenance and **d** mortality per provenance.

Discussion

Intra-specific variation

The current distribution of *F. sylvatica* is a result of multiple glacial periods. *F. sylvatica* survived the last glacial maximum in several refuge areas in Europe (Magri et al. 2006). Most probably, refuge areas in the south-western and Dinaric Alps played an important role in the re-colonization of Central and Western Europe, whereas the Mediterranean populations seem to have survived in several geographically distinct refuges on the Iberian and Italian peninsulas and in the Balkan (Magri et al. 2006, Magri 2008, Brus 2010). In particular, the Balkan (and Italian) populations are genetically distinct from the other European populations (Magri 2008). This post-glacial migration pattern and genetic differentiation, most probably, also resulted in phenotypic variation between provenances. Distinct performances of European beech provenances grown in common garden experiments are well known and often demonstrated (e.g. von Wuehlisch et al. 1995, Nielsen and Jorgensen 2003, Giannini and von Wuehlisch, 2009). The differences between provenances in all of the examined parameters in this study are consistent with these previous findings and likely express the differences in genetic make-up.

In particular, the Bulgarian provenance showed a distinct performance in all parameters. Bulgarian beech populations are genetically distinct from Central European populations (Magri et al. 2006), which may explain the significantly lower increment. Surprisingly, one German provenance (DE2) performed significantly worse in terms of height increment than the other two German provenances (DE1, DE3), despite a most likely similar post-glacial history and assumingly comparable genetic make-up. Yet, in Central Europe, genetic diversity is higher within populations than between populations (Konnert 1995). Furthermore, the silvicultural history of particular beech stands is often hard to be reconstructed. In addition, local soil conditions and small-scale rainfall patterns may be also responsible.

Effects of drought and soil on tree performance

Drought adversely impacted all measured parameter significantly: it increased the amount of leaf damage and decreased height and diameter increment. This is in compliance with numerous studies reporting on the drought sensitivity of *F. sylvatica* (Rennenberg et al. 2004, Lebourgeois et al. 2005, Jump et al. 2006, Piovesan et al. 2008, Friedrichs et al. 2009, Betsch et al. 2011, Hlasny et al. 2011, Jezik et al. 2011, Scherrer et al. 2011).

As expected, the sandy substrate with lower nutrient availability and water storage capacity resulted in more severe negative impacts on the performance of plants, compared with loamy substrate. The drought impact on leaf injury rates and diameter increment was more severe for plants grown in the sandy substrate, which shows the importance of the interaction between precipitation rates and soil properties (Gaertner et al. 2008). The soil moisture in the sandy substrate dropped below the approximated permanent wilting point 1 week before this point was reached in the loamy substrate. Plants in the sandy substrate therefore experienced ~ 24 days of water stress, whereas plants grown in the loamy substrate only ~ 17 days. The reduced nutrient uptake due to lacking water absorption during the drought might furthermore have weaker impacts in nutrient-richer soils than in poorer soils. Apart from nutrient uptake, evidence suggests that also nutrient availability of the soil is reduced due to drought conditions (Rennenberg et al. 2009, Kreuzwieser and Gessler 2010).

In this study, we did not investigate biomass allocation to above- and belowground components of the tree seedlings. In particular, at a seedling age, the ability of European beech to plastically react to environmental/drought conditions in terms of reallocation of resources is quite high, as Schall et al. (2012) demonstrated. European beech seedlings increased the investment into belowground biomass as a reaction to drought. According to the balanced-growth hypothesis (Hunt 1975, Shipley and Meziane 2002) plants reallocate resources to the organ that exploits the limiting resource, in our case the root system. Such potential reallocation effect of a drought treatment might lead to overestimation of growth reduction, as it might mask a constant biomass production, when just observing above ground growth. When trees get older, this morphological plasticity decreases and physiological acclimatization processes becomes more important (Schall et al. 2012). Therefore, a transfer of results from seedlings to mature trees should be backed by more research, e.g. in long-term provenance trials.

Provenance-specific reactions and local adaptation to drought and soil

Drought sensitivity in terms of diameter increment varied between the provenances in our experiment. The Bulgarian provenance (BG) showed a weak performance under the control treatment, yet the smallest reduction of diameter increment due to the drought treatment. The increment reduction between drought and control in other provenances (DE1, DE3, PL) amounted to 50 %. However, their absolute increment values under drought conditions were still higher than for the BG provenance. This behaviour of provenances correlates strongly with leaf injury rates ($r^2 = 0.99$). The interactive effect of provenance and drought treatment was not significant in this case, yet this is due to the fact that leaf injury almost exclusively occurred under the drought treatment. Given the relatively small number of provenances in our trial, significant correlations between the drought response of increment and leaf damage with the climate at the origin of the provenances could not be established. Yet, the reactions of both parameters tended towards a correlation with the long-term Summer Heat Moisture index at the origin of the provenances. This trend is supported by previous findings by Czajkowski and Bolte (2006) who demonstrate a correlation between the drier, continental climate at the origin of a beech provenance from central Poland with low water stress sensitivity and the moister climate of provenances from Germany and West Poland with higher drought stress sensitivity. It is further in compliance with the results of Nielsen and Jorgensen (2003) who found a significant interaction between soil water content and diameter increment for 14 provenances in a common garden experiment in Denmark. However, these authors found a greater adaptability of southern provenances to changing water conditions. In our experiment, the Bulgarian provenance showed a low but stable increment over the treatments while most northern provenances (except DE2 Johanniskreuz) performed at similar low levels under drought conditions but showed a much more positive response to favourable conditions than the Bulgarian one. Similar to the Bulgarian provenance, the Spanish one is not very responsive to drought in terms of leaf damage under dry conditions and showed the lowest mortality rates, however, in contrast to the Bulgarian provenance on a higher increment level and more notable reductions in increment. Surprisingly, the performance of the German provenance from Johanniskreuz (DE2) resembles more the southern provenances (than the other two German and the Polish provenances, which are geographically closer) with low leaf injury rates, low height growth and lower drought-induced growth reductions. Nielsen and Jorgensen (2003) show in their study that northern provenances display a low but stable growth level and southern ones a more plastic response.

The results of our study and the results of Nielsen and Jorgensen (2003) might be superficially interpreted as inconsistent. On closer inspection, however, the mere geographic division of provenances into north and south might not reflect the actual climatic, and site conditions populations were exposed to in past and present at their places of origin. The southern provenances used by Nielsen and Jorgensen (2003) do not stem from the very edges of the distribution range, except one from Mount Etna, Sicily (IT), and the places of origin of these southern provenances have relatively high precipitation rates, whereas the northern provenances come from regions with relatively continental climate with low precipitation sums (e.g. eastern Germany and Central Poland). They might be even more exposed to dry conditions at their origins. The same might be applicable for the concept of geographically marginal and central populations, as this concept excludes micro- and meso-climatic effects, and small-scale differences in soil and site conditions. Even in the centre of the distribution range, very unfavourable and “marginal” sites can be found, whereas on the geographic margins, favourable growth conditions can occur on small scale, e.g. at the windward side of small mountain ranges with orographic rainfall patterns.

In this study, the Summer Heat Moisture index of the ES provenance is even higher than for BG, still the drought tolerance in term of stable growth is not as high as in BG, which may be due to edaphic reasons (Gaertner et al. 2008). This could mean that the actual drought severity, derived from precipitation and soil characteristics, might be lower at the origin of the Spanish provenance (ES). The behaviour of the German provenance (DE2) is also similar to the marginal provenance from BG, although it stems from the centre of the distribution range with climatic conditions comparable to the other German provenances. DE2 stems from the Palatinate Forest, a German mountain range with lower Triassic sandstone formations. The sandy soil there might create much drier conditions than what the other two German provenances experience at their point of origin. Furthermore, information on slope, exposition, soil depth and substrate at the exact location of seed sampling (i.e. of single mother trees) might improve explanatory power in future analyses.

Additionally, these intricate findings might indicate a complex pattern of within and between population variations, developed from the interplay of post-glacial history, local climatic adaptation, local growing conditions and human silvicultural practices. Herbette et al. (2010) and Wortemann et al. (2011) claim that phenotypic variability in drought tolerance, here measured in cavitation resistance, is mainly caused by phenotypic plasticity and not by genotypic differences between populations. Yet, our data point towards some degree of inherited local adaptation to climate conditions at the origins of the provenances even when considering our small selection of provenances. Accordingly, marginal beech populations which face more adverse conditions are under stronger genetic selection (Wortemann et al. 2011) than populations on favourable sites. In our study, the Bulgarian provenance (BG) shows the most distinct provenance effect and provenance-specific reaction. It stems from the very south-eastern distribution edge of *F. sylvatica*, where summer drought is most probably the range-limiting factor (Jump et al. 2006). This again emphasizes the importance of marginal beech populations in the search of drought-resistant ecotypes (Rose et al. 2009).

A trade-off between survival under drought conditions and high increment under favourable conditions has been shown for four broad-leaved species in Kenya (Kondoh et al. 2006). Accordingly, *F. sylvatica* displays the lowest mortality under shade, yet the lowest growth rate under light compared to other deciduous trees (Petritan et al. 2007). Likewise, a continental and a Mediterranean *Pinus sylvestris* provenance did not differ under dry conditions, whereas under wetter conditions the continental provenance showed higher root biomass allocation and seedling recruitment rates (Richter et al. 2012). This confirms previous findings, that high phenotypic plasticity appears beneficial in highly variable environments.

The drought manipulation in our experiment can be considered as relatively mild. Only few individuals died due to the treatment. Provenance did not impact the mortality rate significantly in general. However, there is a clear and significant correlation between summer drought and mortality, with lower mortality rates of the southern marginal provenances due to drought treatment (BG = 11.1 %, ES = 0 %, all provenances 15.3 %), providing evidence for an adaptation to local climate conditions. Given the above-mentioned potential trade-off mechanisms, this slight trend towards higher mortality with stronger diameter growth reduction could point towards higher survival rates of less plastically responding provenances. A stronger genetic selection in drought-prone ecotypes (Wortemann et al. 2011) could lead to lower genetic diversity (Kawecki 2008) and thus low phenotypic plasticity (Schaberg et al. 2008, Matyas et al. 2009), though Kawecki (2008) also reports that for some marginal species, no genetic depauperation for ecologically relevant traits could be detected. Therefore, marginal populations could potentially secure ecosystem persistence under very extreme climatic events, yet might increase the risk of lower growth rates under favourable conditions.

Furthermore, the Bulgarian, Spanish and the German provenance from Johanniskreuz (DE2) showed relatively stable responses in height and diameter increment in the sandy substrate compared to the loamy substrate. This might indicate that these provenances are not able to exploit the improved water and nutrient condition in the loam. It could also indicate a local adaptation to certain soil characteristics, which was, e.g. found for *Pinus nigra* (Varelides et al. 2001). Still, such a potential trade-off between stress-tolerance and high performance under more favorable conditions is yet to be supported by more evidence in future provenance trials.

At the north-eastern edge of the distribution range, the SHM is not as high as at the southern edge. The Polish provenance (PL) originates from the ecotone between beech dominated forest and boreal forests. Beech dominates on moraine loamy soil, while Scots pine dominates on drier and sandier soils (Bolte et al. 2007). In particular, at the north-eastern margin where continental climate conditions are becoming more prevailing, correlations with single macro-climatic factors fail to explain distribution margins of *F. sylvatica* (Bolte et al. 2007). There, winter conditions and frost are at least as important as drought. Giving the clear response to drought in our experiment, it seems that a strong selection towards drought-resistant ecotypes does not take place.

As mentioned above, below-ground biomass production was not determined in this study, yet there might be differences in plasticity of biomass allocation between the provenances (Richter et al. 2012), which deserve more attention in future studies.

Conclusion and implications for research and forest management

Provenances of *F. sylvatica* differ in increment and exhibit differences in their sensitivity to drought. This intraspecific variability can probably be used to adapt forest ecosystems to future climate conditions. Yet, the impact of the interplay between climatic and soil and site characteristics on the emergence of drought conditions have to be accounted for as provenances react specifically to contrasting soil types. Therefore, the concept of marginality should be extended from a geographic to a more site-related concept; yet, especially marginal populations from the drought-prone southern edges of the distribution range are potential targets in the search for drought-resistant ecotypes. However, a potential trade-off between high phenotypic plasticity with high performance rates under favourable conditions, and stress-tolerance and survival under drought conditions and its impact on forest growth requires future research. Long-term provenance trials with a greater number of central and marginal (maybe also from "marginal" sites in the centre of the distribution range) provenances, where the performance of trees can be monitored beyond the seedling age, could contribute in approaching these open questions. Differing from existing trials, a hypothesis-based selection of provenances and the option for climate manipulations in later ontogenetic stages should be applied. Furthermore, a single drought manipulation does not reflect a change in long-term mean values and changes in frequency of extreme events and there long-term impacts. Therefore, a network of several climatically different trial sites, including marginal sites, is needed.

Furthermore, more multi-factorial experiments could address the problem that resistance to one abiotic factor such as drought does not necessarily imply resistance to other climatic or abiotic factors such as frost (Kreyling et al. 2012) or forest pests. However, the establishment of multi-factorial, multi-site and long-term trials and experiments will take longer than there is time to start adaptive actions in forest management with regard to the velocity of climate change. Evidence suggests that the selection of a single best drought-adapted provenance for transplantations is not reasonable, considering the limited knowledge of the long-term effects. Nonetheless, marginal and drought-adapted ecotypes might be intermixed, together with local provenances and ecotypes adapted to other abiotic factors, in order to secure yield and stability under more extreme climatic conditions in the future. Forest management should therefore aim at increasing the genetic diversity of forest stands to secure populations against breakdown due to climatic extremes.

Acknowledgments

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Manuscript 4: Recurrent mild drought events increase resistance toward extreme drought stress

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Abstract

The frequency and magnitude of extreme weather events such as drought are expected to increase in the future. At present, plant responses to recurrent extreme events have been sparsely examined and the role of stress history on subsequent stress response has been widely neglected. In a long-term field experiment, we investigated the response of grassland and heath communities to a very severe drought event, which exceeded the duration of projected drought scenarios. During the preceding 6 years, the plant communities experienced scenarios of varying water supply, including annually recurring drought, heavy rain, regular watering, and natural drought periods. Single species and plant communities that were regularly watered in the preceding years revealed highest tissue die-back under a very severe drought when compared to plants that experienced mild or severe drought stress before. Contrary to expectations, the root to shoot ratio did not increase due to previous recurrent drought occurrences. Furthermore, pre-exposure effects on *Vaccinium myrtillus* and *Plantago lanceolat*a tissue die-back and reproductive biomass (*P. lanceolata*) were altered by community composition. Recurrent mild drought stress seems to improve drought resistance of plant communities and species. Potential reasons could be epigenetic changes or soil biotic legacies. Morphological legacies such as altered root to shoot ratio did not play a role in our study. Imprinting events which trigger this ecological stress memory do not have to be extreme themselves. Thresholds, longevity of effects, and the role of biodiversity shown by the importance of community composition require further attention.

Keywords: drought memory, EVENT-experiment, legacy, precipitation change, pulse pressure, resilience, resistance

Introduction

The frequency and magnitude of extreme weather events such as drought, heat waves, and heavy rain are expected to increase in the future (IPCC 2012). Understanding the impact of extreme events on plant performance is an important research goal in ecology and is increasingly investigated in experimental studies (Gutschick and BassiriRad 2003; Schroter 2005; Jentsch and others 2007; Suttle and others 2007; Knapp and others 2008; Kreyling and others 2008a; Beierkuhnlein and others 2011; Jentsch and others 2011; Walter and others 2011; Kreyling and others 2013). Extreme weather events may cause stronger effects on plants, plant communities, and ecosystems than gradual warming. How powerful a single event can be was demonstrated in the heat wave year 2003 (Schär and others 2004), in which plant gross primary production was reduced by approximately 30 % in Europe (Ciais and others 2005). However, so far, research has mostly focused on the impact of single extreme events (van Peer and others 2004; Gallé and others 2007; Kreyling and others 2008a; Noormets and others 2008).

We argue that climate change impact research should be extended to studying recurrent events and interacting pulse pressures, in order to gain insights on the resilience of plant species, communities, and ecosystems. The duration and magnitude of the first event and the point in time when the next extreme event occurs can have an influence on the respective plant individual and its ability to recover between and after the two events. Walter and others (2011) found a higher percentage of living biomass in the grass *Arrhenatherum elatius* after two pulsed drought events compared to individuals which were subjected to only one pulsed drought event over one growing season. An improved photoprotection in the double-stressed grasses was indicated by reduced maximum quantum efficiency, which was caused by reductions of maximum fluorescence (Maxwell and Johnson 2000). An ecological stress memory in double-stressed *A. elatius* seems to exist and enables the plant to acclimate within its lifespan (Walter and others 2011). Accumulating evidence suggests that plants subjected to recurrent extreme events are able to deal better with subsequent extremes by epigenetic changes or accumulation of signaling proteins or transcription factors, for instance (Bruce and others 2007; Boyko and Kovalchuk 2011). Meisner and others (2013) detected legacy effects in soil biota induced by drought in an inoculation experiment with soil previously exposed to drought and then planted with native and exotic plant species. In particular, this legacy of drought in the soil biota influenced the exotic plant species positively and the natives negatively, whereas the opposite was found for controls, i.e. when the soil was not treated with drought before planting. Meisner and others (2013) suggest that this legacy effect might be associated with alterations in the soil biota and their soil processes. Soil previously exposed to drought showed a higher inorganic nitrogen availability compared to soil without drought immediately before planting.

However, positive effects are not guaranteed. The efficacy of the stress memory effect may depend on the plant species, life forms, and threshold effects due to the magnitude and frequency of extreme weather events. Zavalloni and others (2008), for instance, did not find an increased drought resistance in grasslands, which experienced mild short droughts and warming over 3 years followed by a prolonged drought. Moreover, hints exist that perennial species such as trees may not positively 'remember' a previous drought period when exposed to another drought several years later. *Quercus ilex*, for example, showed a reduction in resprouting and survival after a second drought event in 1995 compared to the first drought in 1985 (Lloret and others 2004). For *Pinus edulis* a higher mortality rate was found after a recurrent drought event in 2002 in comparison to a preceding drought in 1996 (Mueller and others 2005).

A plant's response to extreme events can further depend on their neighboring species (van Peer and others 2004; Wang and others 2007; Saccone and others 2009; van Ruijven and Berendse 2010; Kreyling and others 2011; Otieno and others 2012; Arfin Khan and others 2014; Grant and others unpublished data). For instance, the dwarf shrub *Vaccinium myrtillus* revealed a stronger reduction in biomass production due to drought when growing together with another dwarf shrub and two grasses than when growing only with the other dwarf shrub (Kreyling and others 2008a). Moreover, shifts in flower phenology due to drought were found for *Calluna vulgaris* in a plant community with another dwarf shrub and two grasses compared to a plant community with only another dwarf shrub (Jentsch and others 2009). Novoplansky and Goldberg (2001) detected a lower survival time in *Scleropogon brevifolius* plants under drought conditions if *Sporobolus airoides* plants were located in its' neighborhood. On the other hand, *S. airoides* survival time was independent from plant neighbors. These examples indicate an influence of the plant neighborhood or community composition on drought response. Therefore, an effect of community composition on plant species' ecological stress memory seems

conceivable.
The aim of our study was to examine the role of preceding drought occurrences for the response of plants in different plant community compositions (temperate grassland and heath communities of varying species and functional group number) and monocultures to a very severe drought event. All plants had previously been involved in a 6-year long-term field experiment, which included annually recurrent experimental and natural drought periods (EVENT-I in Bayreuth, Germany; Jentsch and others 2007, 2011).

We hypothesized that (i) a 6-year long pre-exposure to different drought occurrences influences the stress resistance of plant communities and species during a very severe drought event, with better performance of plants formerly subjected to drought. Furthermore, we expected that (ii) this ecological stress memory effect of single species is altered by plant community composition.

Methods

Experimental Site

The EVENT-I experiment (Jentsch and others 2007) was established in the Ecological Botanical Garden of the University of Bayreuth, Germany (49°55’19”N, 11°34’55”E, 365 m a.s.l.) in 2005. The long-term mean annual temperature at the site is 8.2°C and the long-term mean annual precipitation is 724 mm (1971–2000) with a precipitation peak in December/January and June/July (data: German Weather Service). The previously homogenized and drained soil consisted of loamy sand (82 % sand, 13 % silt, 5 % clay) with a pH = 4.5 in the upper (0-20 cm) and pH = 6.2 in the lower (20-80 cm) soil layer (measured in 1 M KCl).

Experimental Design of Pre-exposure Manipulations

From 2005-2010, the experiment was carried out in a two-factorial design: (1) precipitation manipulations (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) (Table 1) and (2) plant community composition (grassland and heath in different community compositions) (Table 2).

Table 1 Overview of pre-exposures (2005-2010). In 2011 all plots underwent the same very severe drought manipulation.

Year	Ambient control	Drought	Heavy rain	Regular watering
2005	Ambient precipitation	Drought 09.06.-10.07.	170 mm 27.06.-10.07.	Ambient precipitation
2006	Ambient precipitation	Drought 24.05.-24.06.	170 mm 10.06.-23.06.	Roof artifact control (irrigated with ambient precipitation below roof) 24.05.-24-06.
2007	Ambient precipitation	Drought 20.05.-20.06.	170 mm 08.06.-21.06.	Historical average precipitation below roofs twice per week 01.04.-24.09.
2008	Ambient precipitation	Drought 19.05.-30.06.	260 mm 10.06.-30.06.	Regular watering 01.04.-30.09.
2009	Ambient precipitation	Drought 19.05.-29.06.	260 mm 09.06.-29.06.	Regular watering 01.04.-30.09.
2010	Ambient precipitation	Drought 11.05.-21.06.	260 mm 01.06.-21.06.	Regular watering 01.04.-30.09.
2011	Drought 17.05.-28.08.	Drought 17.05.-28.08.	Drought 17.05.-28.08.	Drought 17.05.-28.08.
Regular watering: mean weekly amount of precipitation of the past 30 years over the growing season (only addition if natural amount not reaching historical mean per week)				

Table 2 Experimental heath plant communities, grassland plant communities, and monocultures in the EVENT-I experiment (Jentsch and others 2007).

Abbreviation	Vegetation type	Description	Species
H4-	Heath	Four species, two functional groups (dwarf shrub, grass)	*Calluna vulgaris*, *Vaccinium myrtillus*, *Agrostis stolonifera*, *Deschampsia flexuosa*
H4-	Heath	Two species, one functional group (dwarf shrub)	*Calluna vulgaris*, *Vaccinium myrtillus*
G4-	Grassland	Four species, two functional groups (grass, forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Geranium pratense*
G4+	Grassland	Four species, three functional groups (grass, forb, leguminous forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Lotus corniculatus*
M-	Monoculture	monocultures of four species within one plot (each monoculture 1 m^2, established 10-2006, only for ambient control and drought)	*Calluna vulgaris*, *Vaccinium myrtillus*, *Arrhenatherum elatius*, *Holcus lanatus*
H = Heath; G = Grassland; M = Monoculture; 2/4 = Number of species; - = without legume; + = with legume.			

Intensity of ‘drought’ and ‘heavy rain’ was based on the local 100-year and 1000-year extreme event in each category. The 100-year (for 2005-2007) and 1000-year extreme events (for 2008-2010) were calculated based on the precipitation data of the growing season (April to September) from 1961 to 2000 (data: German Weather Service) using Gumbel I distributions (Gumbel 1958). Further specifics of the climatic manipulations are described in Table 1 and Jentsch and others (2011). The plots of the ‘drought’ manipulation were covered with rain-out shelters (steel frames: Hochtunnel, E & R Stolte GmbH, Germany) that permitted nearly 90 % penetration of photosynthetically active radiation (transparent plastic sheets: 0.2 mm polyethylene, SPR5, Hermann Meyer KG, Germany) during the simulated drought events. The ‘heavy rain’ and ‘ambient control’ plots were exposed to the ambient weather conditions of Bayreuth without rain-out shelters. In 2005, the ‘regular watering’ plots were treated identically as the ‘ambient control’. In 2006, the ‘regular watering’ plots were covered during the same time as the ‘drought’ exposure and received the rainfall amount of the ‘ambient control’ to reveal roof artifacts, resulting in no significant roof artifact effects (Kreyling and others 2008b). In 2007, the regularly watered plots were covered during the whole growing season with rain-out shelters alike the ones used for the drought exposure. During this period, these plots received the weekly long-term precipitation sum with one part irrigated at the beginning of the week and the second 3-4 days later ensuring continuous water supply. ‘Regular watering’ started in 2008 (without using rain-out shelters). The ‘regular watering’ plots received at least the long-term (1971-2000) precipitation sum per week during the growing season. If natural rainfall was less than the long-term average sum for the same week, the missing amount was added by irrigation. If weekly rainfall exceeded the long-term sum, it was not subtracted from the next irrigation.

The weekly precipitation sums for the pre-exposures and the volumetric soil water content (vol.%) in the years 2005 to 2010 are provided in the appendix (Fig. S1). Natural drought periods in the four different pre-exposures during the growing seasons 2008-2010 are provided in Table 3 (continuous soil moisture data for the growing seasons 2005-2007 are not available).

Table 3 Number of days and the maximum number of consecutive drought days with volumetric soil water content less or equal to the permanent wilting point (7 vol.%) during the growing seasons 2008-2010 for the pre-exposures ‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’.

Days with volumetric soil water content $\leq$ PWP (7 vol.%) during growing season				
Year	Ambient control	Drought	Heavy rain	Regular watering
2008	11	33	8	0
2009	0	32	5	0
2010	15	14	12	2
Max. number of consecutive drought days with volumetric soil water content $\leq$ PWP (7 vol.%) during growing season				
2008	8	33	5	0
2009	0	22	5	0
2010	10	10	8	2

Plant community compositions were established in combinations with an increasing number of plant functional groups (Table 2). For heath, there were combinations of two species of one functional group (H2-) and four species of two functional groups (H4-). For grassland, four species of two (G4-) or three functional groups (G4+) were combined. Additionally, there were monocultures (M-) for ‘ambient control’ and 'drought' of key species of heath (*C. vulgaris* and *V. myrtillus*) and grassland (*A. elatius* and *Holcus lanatus*). The total setup consisted of five replicates of each factorial combination, 75 plots of two by two meters in size and 10 plots of two by two meters in size divided into four small plots for the four monoculture target species (each monoculture 1 m^2). The factors were applied in a split-plot design with the different community compositions blocked and randomly assigned within each weather manipulation (Jentsch and others 2007). The originally planted species composition of the year 2005 was maintained by periodical weeding.

Very Severe Drought Manipulation

In the year 2011, we conducted a very severe drought event exceeding projected climate change scenarios, which lasted for 57 % (104 days) of the growing season (April-September) for all pre-exposures. We covered the whole experimental site by a steel frame (Haygrove Tunnels Ltd., Ledbury, United Kingdom) and a transparent polyethylene sheet (0.18 mm, UV M 42, folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany; total area 50 m length x 31.5 m width; 3.75 m height). The sheet edges of the rain-out shelters were at a height of 1.05 m on the long sides and 2.55 m on the front/back side. The polyethylene sheet permitted nearly 90 % penetration of photosynthetically active radiation. Prior to starting the drought manipulation, the plants received a watering treatment of 46.6 mm divided into two applications (11th/13th of May 2011) to adjust all plants to the same initial condition. The amount of the watering treatment (46.6 mm) was calculated out of the difference of long-term average precipitation sum to natural rainfall sum from April 2011 (the start of the growing season to the start of the very severe drought).

The drought manipulation took place from 17th of May until 28th of August 2011 (104 days). Volumetric soil water content (vol.%) was measured weekly in each plot in a depth of 10 cm (Field Scout TDR Soil Moisture Meter, Spectrum Technologies Inc., Plainfield) over the course of the experiment (Fig. 1). The volumetric soil water content of all pre-exposed plots dropped below the permanent wilting point (7 vol.%) on 26th of May 2011 and stayed there for the rest of the drought manipulation.

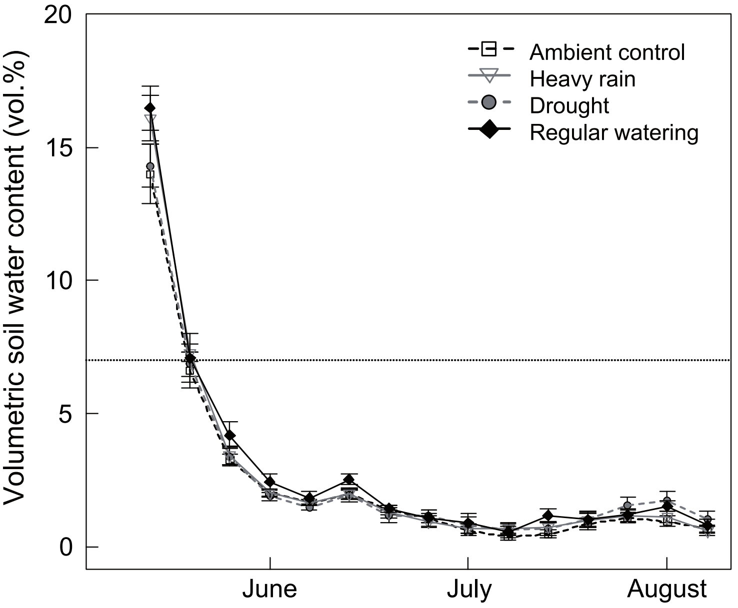

Fig. 1 Volumetric soil water content (vol.%) (weekly mean) over the course of the very severe drought experiment in the year 2011. The permanent wilting point (pF = 4.2 = 7 vol.%) is indicated by the *dotted black line*.

Response Parameters

Aboveground Biomass, Tissue Die-Back, and the Ratio of Reproductive to Total Biomass

We harvested the aboveground biomass in the heath and grassland plant communities biweekly in different subplots for each single plot (harvest 1 and 2: 17th/30th of May, harvest 3 and 4: 14th/27th of June, harvest 5 and 6: 11th/25th of July, harvest 7 and 8: 8th/22th of August 2011) and three times in the monocultures (harvest 2: 30thh of May, harvest 4: 27th of June, harvest 7: 8th of August 2011) during the 104 days of drought. During each harvest, plant material in a subplot of 20 cm x 40 cm within each plot was cut at the surface of the soil. In the heath plant communities (H4- and H2-) subplots were required to encompass one individual of *C. vulgaris* and *V. myrtillus*, respectively, in the 20 cm x 40 cm frame. Subsequently, the plant material (entire aboveground biomass) was sorted into three groups per species: vegetative, reproductive (seeds and flowers), and dead parts of the plant. The parts of plant would be defined as vegetative/reproductive (alive), if the plant material or parts of the plant material were still green. Dead parts of the plant were defined

as brown and wilted that lost its chlorophyll. The woody part of the dwarf shrubs was also separated into dead and alive based on visual evaluation of the transport system. All plant material was dried at 60°C for 72 h before weighing.
Tissue die-back (TD) was calculated as

$$TD = \frac{dead\,biomass\;(g)}{total\,biomass\;(g)} \times 100 \tag{1}$$

The ratio of reproductive to total biomass (RR) was calculated as

$$RR = \frac{reproductive\,biomass\;(g)}{total\,biomass\;(g)} \tag{2}$$

Belowground Biomass and Root to Shoot Ratio

To estimate the belowground biomass, the roots were collected with a root core (4 cm diameter and 14 cm length; corresponding to 0.00126 m^2 surface area). The main rooting zone was within the upper 20 cm. Hardly any roots penetrated the soil deeper than 20 cm. Root core samples were taken from harvest 2-8 in all four plant communities. Due to sampling problems, some data sets are missing, in particular the second and eighth harvest of the heath plant community H2- as well as the second harvest of the heath plant community H4-. Three root core samples (corresponding to 0.00378 m^2 surface area in total) per subplot were combined to one mixed sample at each sampling date after harvesting the 20 cm x 40 cm (corresponding to 0.08 m^2 surface area) aboveground biomass sample. In the heath plant communities, the root core was positioned 2 cm beside the root beginnings of a *C. vulgaris* individual, 2 cm beside the root beginnings of a *V. myrtillus* individual, and one root core in the middle between both individuals. In the grassland plant communities, three root cores were randomly positioned. After harvesting, the root samples were washed, dried at 60°C for 72 h, and the remaining stones/sand were removed before weighing.
The root to shoot ratio (RSR) was calculated out of:

$$RSR = \frac{belowground\,biomass\;(g)/0.00378\,m^2}{aboveground\,biomass\;(g)/0.08\,m^2} \tag{3}$$

Aboveground biomass represents the sum of the vegetative, reproductive, and dead parts of plant.

Statistical Analysis

Analysis of variance (repeated measure ANOVA) combined with linear mixed effect models were applied to test for the main effect of the factor 'pre-exposure' (levels: 'ambient control', 'drought', 'heavy rain', and 'regular watering') for aboveground biomass (per plant community and per single species), tissue die-back (per plant community and per single species), reproductive biomass (seeds and flowers; only for the species analyses), ratio of reproductive to total biomass (only for the species analyses), belowground biomass (only for the plant community analyses), and root to shoot ratio (only for the plant community analyses). We analyzed the first harvest separately from the other seven harvests to test for pre-exposure effects before the beginning of the very severe drought (i.e. simple lag effects, not stress memory effects). No harvest one was available for the monocultures as well as for the response parameters belowground biomass and root to shoot ratio of the plant communities. Sometimes the sample weights of the response parameters reproductive biomass and ratio of reproductive to total biomass of the plant species within the entire harvest one were zero and thus no analysis could be performed. The 'harvest date', the 'plot number', and the 'repetition number' were included as random factors in the mixed effect models from harvest 2-8, thus taking the split-plot design and the repeated measurements into account. The 'harvest date' was included as a random factor in the mixed models, because of no significant interaction of this factor with the factor 'pre-exposure' in a pre-analysis. The mixed effect models of harvest one included the random factor 'repetition number'. The pre-exposure 'regular watering' does not exist in the plant community with a legume species (G4+) and in the monoculture (M-), and 'heavy rain' is also missing in the monoculture (M-). Each experimental plant community, each monoculture, and each species were separately analyzed in order to achieve full factorial combinations for all analyzed subsets. The *A. elatius* monoculture had to be taken out of the species analysis, because of insufficient plant replicates in the pre-exposures. If necessary, the data were square root-, log-, power-, or (asin(sqrt(y)/100))-transformed to improve the normality of residuals and the homogeneity of variances prior to analysis (Faraway 2006). In case of a significant 'pre-exposure' effect of the linear mixed effect model, post hoc comparisons with the Tukey's test were performed according to Hothorn and others (2008). The level of significance was set to $P < 0.05$. All statistical analyses were conducted with the software R 2.13.1 (R Development Core Team 2011) and the additional packages nlme (Pinheiro and others 2012) and multcomp

(Hothorn and others 2008).

Results

Lag Effects of the Pre-exposures Before the Beginning of the very severe Drought

No significant pre-exposure effects were found in any of the eight different community cases (four communities, two response parameters) before the beginning of the very severe drought:

Tissue Die-Back of Plant Communities

Regarding the first harvest, no pre-exposure effect was found in tissue die-back for any plant community before the beginning of the very severe drought (first harvest; see appendix Fig. S2; Table S1).

Aboveground Biomass of Plant Communities

No pre-exposure effect could be detected in aboveground biomass for any plant community before the beginning of the very severe drought (first harvest; see appendix Fig. S3; Table S2).

Tissue Die-Back of Plant Species

None of the plant species showed a pre-exposure effect in tissue die-back before the beginning of the very severe drought event (first harvest; see appendix Fig. S4; Table S3).

Aboveground Biomass of Plant Species

Plantago lanceolata revealed a significant pre-exposure effect in the grassland plant community with a legume species (G4+), whereby plants pre-exposed to 'heavy rain' showed a higher amount of aboveground biomass than plants previously exposed to 'drought' (first harvest; see appendix Fig. S5; Table S4).

Furthermore, no pre-exposure effect could be detected for *C. vulgaris*, *V. myrtillus*, *A. elatius*, and *H. lanatus* in aboveground biomass before the beginning the very severe drought (first harvest; see appendix Fig. S5; Table S4).

Reproductive Biomass of Plant Species

None of the five plant species, where an analysis was possible, exhibited a pre-exposure effect in reproductive biomass and in the ratio of reproductive to total biomass before the beginning of the very severe drought (first harvest; see appendix Tables S3 and S4).

Only one significant pre-exposure effect was found for one plant species out of 28 different species cases before the beginning of the very severe drought (five species, four community combinations, four response parameters; not all combinations were available).

Pre-Exposure Effects on Plant Communities in the Face of a Very Severe Drought

Tissue Die-Back of Plant Communities

Tissue die-back due to the very severe drought differed between the pre-exposures (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) in all plant communities within harvest 2-8 (Fig. 2, top panel; Table 4). The plants subjected to the pre-exposure ‘regular watering’ were most negatively affected by the very severe drought event (Fig. 2, top panel). The plants of heath plant communities (H4- and H2-) and of the grassland community without a legume species (G4-), which had in previous years been exposed to ‘ambient control’, ‘drought’, and ‘heavy rain’ conditions revealed no significant differences between the pre-exposures (Fig. 2, top panel). In contrast, the plants of the ‘ambient control’ pre-exposure in the grassland plant community containing a legume species (G4+) showed a significantly higher tissue die-back due to the very severe drought in comparison to the plants previously subjected to ‘drought’ and ‘heavy rain’. Generally, the species of the heath plant communities (H4- and H2-) were less affected by the very severe drought and had a lower tissue die-back than the grassland plant communities (G4- and G4+).

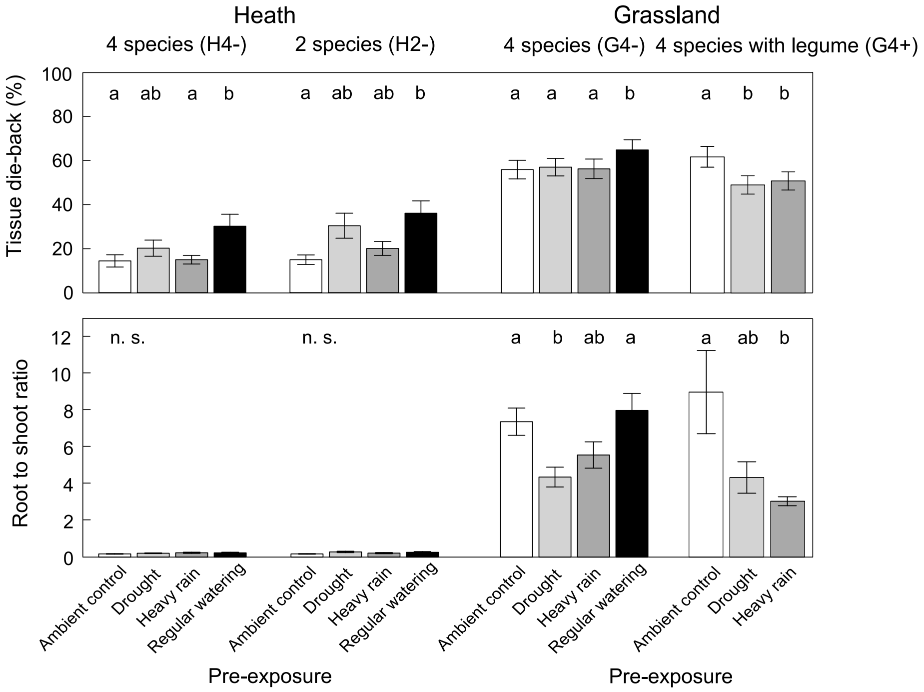

Fig. 2 Tissue die-back (*top panel*) and root to shoot ratio (*bottom panel*) of heath and grassland plant communities from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean percentage over all sampling dates and the standard error. *n. s.* not significant.

Table 4 ANOVA results for the effects of pre-exposure ('ambient control', 'drought', 'heavy rain', and 'regular watering') on tissue die-back and root to shoot ratio of the different experimental heath (H4- and H2-) and grassland (G4- and G4+) plant communities from harvest 2-8. Significant values are shown in bold ($P < 0.05$).

Factor	Pre-exposure			
Parameter	Tissue die-back (%)		Root to shoot ratio	
Plant community	F	P	F	P
H4-	4.5	**0.005**	1.0	0.391
H2-	4.6	**0.004**	2.1	0.109
G4-	4.0	**0.009**	6.1	**0.001**
G4+	6.5	**0.002**	4.8	**0.011**

Aboveground Biomass of Plant Communities

Significant pre-exposure effects were found for grassland plant communities (G4- and G4+) in the aboveground biomass during the very severe drought (harvest 2-

8; see Fig. 3, top panel; appendix Table S5). Grassland plant communities after pre-exposure to 'heavy rain' produced higher amounts of aboveground biomass compared to 'ambient control' (both G4- and G4+). The grassland plant community without a legume species (G4-) produced higher amounts of aboveground biomass also after pre-exposure to 'drought' compared to 'ambient control'.

The heath plant communities (H4- and H2-) did not show a significant pre-exposure effect in the aboveground biomass when subjected to a very severe drought (see Fig. 3, top panel; appendix Table S5).

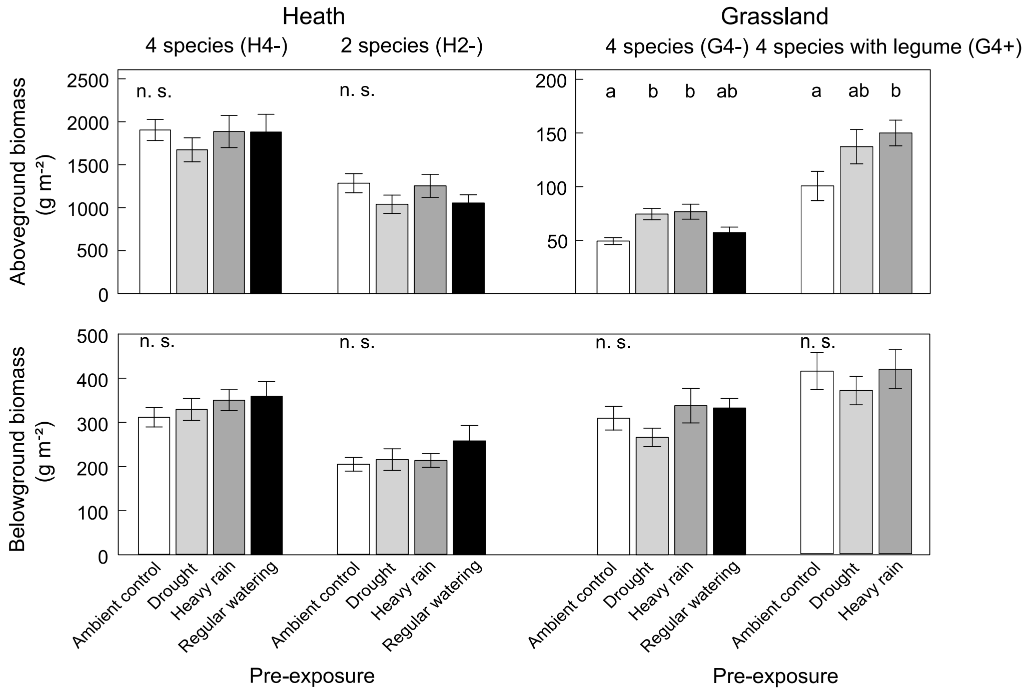

Fig. 3 Aboveground biomass (g m^{-2}) (*top panel*) and belowground biomass (g m^{-2}) (*bottom panel*) of heath and grassland plant communities from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean over all sampling dates and the standard error. *n. s.* not significant.

Belowground Biomass of Plant Communities

Belowground biomass was not affected by any pre-exposure in neither grassland nor heath plant communities (Fig. 3, bottom panel; appendix Table S5).

Root to Shoot Ratio of Plant Communities

The root to shoot ratio during the very severe drought manipulation of grassland plant communities (both G4- and G4+) was affected by the different pre-exposures (Table 4). The plants in the grassland plant community without a legume species (G4-) reached a higher root to shoot ratio, if in previous years exposed to 'regular watering' or 'ambient control' conditions than if previously exposed to annually recurrent 'drought' (Fig. 2, bottom panel). In the grassland plant community with a legume species (G4+) we found a higher root to shoot ratio of the plants in the 'ambient control' than in the 'heavy rain' pre-exposed plots (Fig. 2, bottom panel). For heath plant communities (H4- and H2-), root to shoot ratio was not affected by pre-exposure during the very severe drought (Fig. 2, bottom panel; Table 4).

Pre-Exposure Effects on Plant Species in the Face of a Very Severe Drought

Tissue Die-Back of Plant Species

Tissue die-back revealed pre-exposure effects for some of the target species in the different plant communities and monocultures (analysis of harvest 2-8; Table 5). *C. vulgaris* (in H4- and H2-), *V. myrtillus* (in H2-), *H. lanatus* (in G4-), and *P. lanceolata* (in G4-) showed a reduced drought resistance when subjected to 'regular watering' in previous years (Fig. 4). However, there was no difference in tissue die-back after pre-exposure to 'ambient control' conditions, 'drought' or 'heavy rain' for *C. vulgaris* (in H4- and H2-), *H. lanatus* (in G4-), and *P. lanceolata* (in G4-). Regarding the four monocultures, only the *V. myrtillus* monoculture showed a significant pre-exposure effect (Fig. 4).

Regarding the plant community composition effect, *V. myrtillus* growing together with *C. vulgaris* (H2-) was less affected by tissue die-back than *V. myrtillus* in the monoculture (M-) (Fig. 4) comparing 'ambient control' conditions with annually recurrent 'drought' in each plant community composition, respectively (analysis of harvest 2-8). Likewise, the response of *P. lanceolata* to the very severe drought was modified by plant community composition. Growing in the plant community without a legume species (G4-), *P. lanceolata* exhibited no significant difference between 'ambient control' and 'heavy rain' pre-exposure, but tended toward a lower tissue die-back when previously exposed to 'ambient control' conditions. The opposite was found in the plant community containing a legume species (G4+), where *P. lance-*

olata showed a lower tissue die-back in the 'heavy rain' compared to the 'ambient control' pre-exposed plants (harvest 2-8; Fig. 4).

Table 5 ANOVA results for the effects of pre-exposure ('ambient control', 'drought', 'heavy rain', and 'regular watering') on tissue die-back and reproductive biomass (seeds and flowers) of the heath and grassland plant species from harvest 2-8. Empty fields indicate that no analysis was performed. Significant values are shown in bold ($P < 0.05$).

Factor	Pre-exposure				
Parameter		Tissue die-back (%)		Reproductive biomass	
Plant community	Species	F	P	F	P
H4-	*Calluna vulgaris*	3.2	**0.027**	1.6	0.192
	Vaccinium myrtillus	2.6	0.053	1.2	0.330
H2-	*Calluna vulgaris*	4.0	**0.010**	0.9	0.439
	Vaccinium myrtillus	4.8	**0.003**	0.7	0.566
G4-	*Arrhenatherum elatius*	0.5	0.656	0.8	0.492
	Holcus lanatus	4.2	**0.007**	3.2	**0.025**
	Plantago lanceolata	5.0	**0.003**	3.2	**0.027**
G4+	*Arrhenatherum elatius*	4.8	**0.011**	1.0	0.390
	Holcus lanatus	2.9	0.060	4.4	**0.015**
	Plantago lanceolata	6.7	**0.002**	15.1	**< 0.0001**
M-	*Calluna vulgaris*	1.7	0.205	1.4	0.252
	Vaccinium myrtillus	8.4	**0.008**	1.8	0.189
	Arrhenatherum elatius				
	Holcus lanatus	0.0	0.846	2.8	0.107

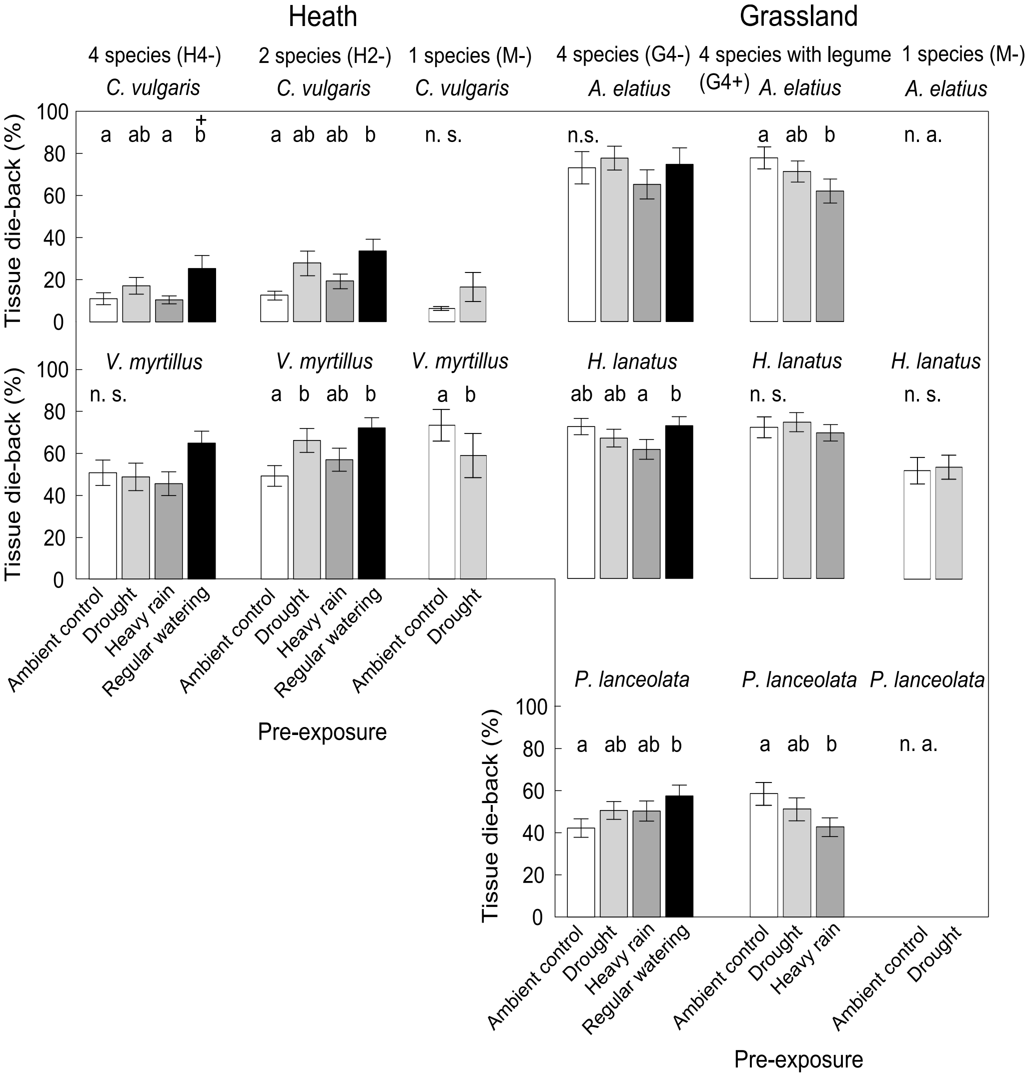

Fig. 4 Tissue die-back of plant species in heath and grassland communities from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean percentage over all sampling dates and the standard error. *n. a.* not available; *n. s.* not significant; "+" marginal significant (post hoc test for regular watering versus heavy rain $P = 0.055$).

Aboveground Biomass of Plant Species

The aboveground biomass of *P. lanceolata* in both grassland plant communities (G4- and G4+) showed significant pre-exposure effects when subjected to a very severe drought (analysis of harvest 2-8; see appendix Table S6). In the absence of a legume species (G4-), *P. lanceolata* produced more aboveground biomass under very severe drought conditions when previously exposed to ‘drought’ compared to ‘ambient control’ and ‘regular watering’. On the other hand, in the presence of a legume species (G4+), *P. lanceolata* produced more aboveground biomass under very severe drought conditions when pre-exposed to ‘heavy rain’ compared to ‘ambient control’ and ‘drought’ (see appendix Fig. S6; Table S6). Again, the response of *P. lanceolata* differed between the two plant community compositions G4- and G4+ for the parameter aboveground biomass. As already mentioned above, the case of *P. lanceolata* in the presence of a legume species was the only one with a significant pre-exposure effect also before the beginning of the very severe drought.

C. vulgaris, *V. myrtillus*, *A. elatius*, and *H. lanatus* did not reveal any pre-exposure effect in the aboveground biomass during the very severe drought (see appendix Fig. S6; Table S6).

Reproductive Biomass of Plant Species

Significant pre-exposure effects were found in the production of reproductive biomass (seeds and flowers) during the very severe drought for *H. lanatus* and *P. lanceolata* in grassland plant communities (G4- and G4+) (harvest 2-8; Table 5). *H. lanatus* produced more reproductive biomass (Fig. 5) and had a higher ratio of reproductive to total biomass (see appendix Fig. S7; Table S6) after ‘heavy rain’ than after ‘regular watering’ (in G4-) as well as after ‘heavy rain’ than after ‘drought’ pre-exposure (in G4+). *P. lanceolata* produced more reproductive biomass if previously pre-exposed to ‘heavy rain’ in the plant community containing a legume species (G4+). However, this was not the case in the plant community without a legume species (G4-), where *P. lanceolata* showed no differences between the ‘heavy rain’ and the other pre-exposures (Fig. 5). Thus, *P. lanceolata* revealed a differentiated response in reproductive biomass with regard to the plant community composition during the very severe drought. Furthermore, *P. lanceolata* did not exhibit a significant pre-exposure effect in the ratio of reproductive to total biomass as *H. lanatus* did (see appendix Table S6).

C. vulgaris, *V. myrtillus*, and *A. elatius* were not affected by previous exposure in reproductive biomass and in the ratio of reproductive to total biomass during the very severe drought (harvest 2-8; Table 5; appendix Table S6).

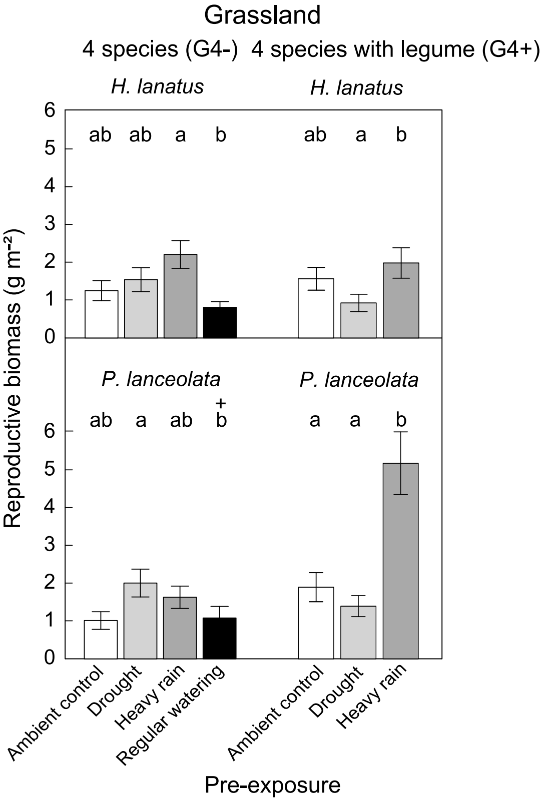

Fig. 5 Reproductive biomass (g m^{-2}) (seeds and flowers) of *Holcus lanatus* and *Plantago lanceolata* from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean over all sampling dates and the standard error. "+" marginal significant (post hoc test for regular watering versus drought *P* = 0.058).

Discussion

Recurrent Mild Drought Stress Triggers Ecological Stress Memory

Surprisingly, plant communities and species with recurrent mild drought stress over several years showed a higher drought resistance than plants without drought experiences for 3 years in the face of a very severe drought event. The higher drought resistance revealed itself in the form of lower tissue die-back (dead over total biomass). This effect was apparent in all plant communities being pre-exposed to 'regular watering' (Fig. 2, top panel). Focusing on the differences, the heath communities showed a lower relative tissue die-back than the grassland communities in the face of the very severe drought. The conservative life strategy of heath (longevity, lignification) compared to grassland (Larcher 2003) and thus their ability to resist might lead to the better performance. An ecological stress memory (Walter and others 2013) could be an explanation for the relatively better performance of the plants when pre-exposed to 'ambient control' conditions, recurrent 'drought', or 'heavy rain' events. Since plants pre-exposed to 'regular watering' can be expected to have experienced natural drought periods before 2007 (see method section), the length of the observed ecological stress memory appears to be shorter than 4 years. Moreover, our results are strengthened by the missing pre-exposure effects (except for *P. lanceolata* in aboveground biomass) before the beginning of the very severe drought, implying that the observed pre-exposure effects are no simple carry-overs but rather true stress memory effects.

Our finding suggests that the events which trigger drought resistance do not have to be extreme themselves. Plants pre-exposed to 'ambient control' conditions and annually recurrent 'heavy rain' events experienced 26 and 25 days with volumetric soil water content dropping below the permanent wilting point over the 3 years before the very severe drought. By contrast, plants pre-exposed to 'regular watering' experienced only two such days over the same period. Thus, the variability and recurrence of water stress might lead to a higher drought resistance of the plants as compared to permanently favoring conditions in respect of the very severe drought in 2011.

However, an influence of recurrent mild or extreme drought stress could not be found when considering other parameters than tissue die-back such as total aboveground biomass (Fig. 3, top panel; appendix Fig. S3, S5, and S6). The growth performance of the plant communities and species with different pre-exposures was mostly similar. However, tissue die-back did suggest that plant adaptations to recurrent drought exist. In the cases considered, the way the biomass is distributed among living and dead components has apparently a greater responsiveness than the total amount of biomass itself.

Increasing root to shoot ratio and root dynamics into deeper soil layers are known as strategies to deal with drought stress (Kalapos and others 1996; Kahmen and others 2005; Newman and others 2006; Ehdaie and others 2012). Based on these findings, increasing root to shoot ratio was suggested as a mechanistic explanation for ecological stress memory (Walter and others 2013). However, our results for root to shoot ratio did not show an increase in belowground biomass in the pre-stressed plants. Moreover, the belowground biomass of all plant communities did clearly not exhibit any differences driven by pre-exposure in previous years

(Fig. 3, bottom panel). Thus, no adaptation of the root system to recurrent drought events over subsequent years was observed in any plant community (Fig. 2, bottom panel). Yet, our findings for belowground biomass are restricted to the uppermost 14 cm of the soil and thus cannot provide insights into root adaptations to recurrent drought in deeper soil layers. In accordance with our results, Kreyling and others (2008b) as well as Gilgen and Buchmann (2009) also found no alterations in plant belowground biomass in reaction to recurrent drought events. In our study, the experienced weather extremes and climatic variability in years prior to the very severe drought have probably improved the drought resistance of the plant communities without affecting the root to shoot ratio. The higher root to shoot ratio of the plants pre-exposed to 'regular watering' in the grassland community without a legume species (G4-) and of plants pre-exposed to 'ambient control' conditions in both grassland communities (G4- and G4+) (Fig. 2, bottom panel) may imply that shifts in root to shoot ratio are rather short-term responses of less well adapted plants. Here, a direct stress reaction to the very severe drought conditions might explain the plant root performance instead of an ecological stress memory. This assumption is strengthened by root to shoot ratio results from September 2010 (79 days after the drought manipulation in 2010; root depth of 15 cm), where grassland communities (G4- and G4+) did not show a significant pre-exposure effect (data not shown). However, root samples from September 2010 were collected with a different method compared to 2011. Thus, a direct comparison between the findings of 2010 and 2011 cannot be provided.

Other mechanisms for the explanation of the observed ecological stress memory include the accumulation of signaling proteins or transcription factors, which could promote a rapid response to subsequent recurrent stresses (see Conrath and others 2006; Bruce and others 2007). Moreover, epigenetic changes in terms of modification of DNA activity by methylation, histone modification or alterations in genome stability and chromatin organization due to the environmental stress are possible explanations for the plant response in our study and thus for the ecological stress memory (Madlung 2004; Bruce and others 2007; Boyko and Kovalchuk 2011). Furthermore, soil biotic legacy effects induced by preceding drought occurrences before planting were found by Meisner and others (2013). This was associated with an increase of the inorganic nitrogen availability owing to preceding drought. Soil biotic legacy effects might therefore be an important aspect in understanding the mechanism of ecological stress memory considered in our study.

Influence of Community Composition on the Ecological Stress Memory of Plant Species

The ecological stress memory seems to be modified by community composition, i.e. the presence of neighbors for *V. myrtillus* and *P. lanceolata* (Fig. 4, 5, see appendix Fig. S5, S6). The respective plant neighborhood consisting of neighbors of the same plant species or of different plant species might play a role with regard to plant response to a subsequent very severe drought extreme. Especially, the presence of a legume species in the neighborhood influenced the response of *P. lanceolata* with regard to aboveground biomass (Fig. S5, S6), tissue die-back (Fig. 4), and reproductive biomass (Fig. 5). However, we cannot dismiss a simple carry-over effect from the previous years in this case, as aboveground biomass of *P. lanceolata* already showed a pre-exposure effect before the beginning of the very severe drought (Fig. S5). Importantly, however, no such carry-over by pre-exposure occurred in the community without a legume. The explanation is in accordance with findings in aboveground biomass within the EVENT-I experiment in previous years (2007-2010). Arfin Khan and others (2014) showed that *P. lanceolata* was facilitated by the legume species when exposed to ‘heavy rain’, but not under ‘drought’ conditions. The missing legume effect under drought exposure seems associated with a decreased N-uptake of the plant species than by a decline in legume N-fixation.

Plant neighbors have been shown in previous studies to affect plant performance under drought stress (e.g. Novoplansky and Goldberg 2001; Kreyling and others 2008a). Competition and facilitation under varying stress situations (Callaway 1997; Callaway and Walker 1997) might be relevant factors in the progress of the ecological stress memory of different plant species. For instance, Grant and others (unpublished data) show that an extreme drought event led to contrasting plant-plant interactions. While *A. elatius* was facilitated by neighboring plants under drought, the same drought event increased the competitive pressure of neighboring species on *Lotus corniculatus*.

However, our community compositions were fixed and not repeated with other plant species diversity level or rigorously tested for neighborhood effects by pairwise combinations of the grassland species. Therefore, the findings might be explained by sampling effects of the used plant species (e.g. the potential legume effect could also be a species identity effect by the one legume used here, *L. corniculatus*). Further experiments are required with other plant species combinations to confirm the generality of our results. Still our findings for *V. myrtillus* and potentially also for *P. lanceolata* partly confirm our stated expectation and are in accordance with the current knowledge about the influence of plant neighborhood and community composition under drought conditions. This finding of community composition altering the pre-exposure effects of at least some target species represents a new aspect in ecological stress memory research, where most studies up to now focused on the single plant level (Goh and others 2003; Molinier and others 2006; Whittle and others 2009; Cuk and others 2010; Walter and others 2011).

Conclusions

In summary, our findings suggest that already mild drought stress over several years seems to influence plant resistance and adaptation positively against extreme drought events. In particular, the lack of drought history in ‘regular watering’ over years reduced the drought resistance of plant species and communities. Interestingly, this study indicates that community composition and plant neighborhood might play a role in plant response and thus in alterations of this ecological stress memory. The complexity of plant-plant interactions under drought conditions has to be taken into account in further ecological stress memory research. By neglecting the competitive aspects within a plant community, experimental approaches might lead to false ecological implications concerning the ecological stress memory. Thereby, ecological stress memory findings on the single plant level have to be taken with caution. Additionally, thresholds and limited longevity of ecological stress memory effects need further research efforts. Finally, the ecological stress memory seems a supporting tool for heath and grassland plant communities and species to adapt to a changing and more extreme climate.

Acknowledgments

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Appendix

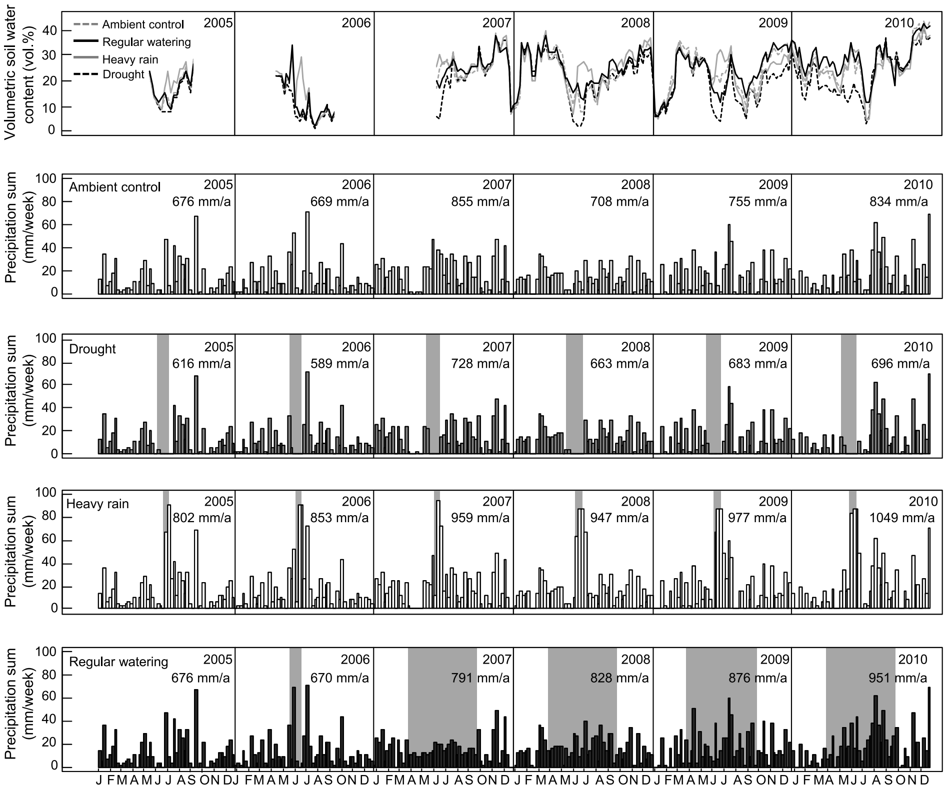

Fig. S1 Alterations in precipitation (weekly sums) and volumetric soil water content (vol.%) (weekly mean) for the pre-exposures ‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’ in the years 2005 to 2010 at the EVENT-I experimental site. The annual precipitation sum is provided for each year. Grey shaded areas indicate the duration of each pre-exposure manipulation.

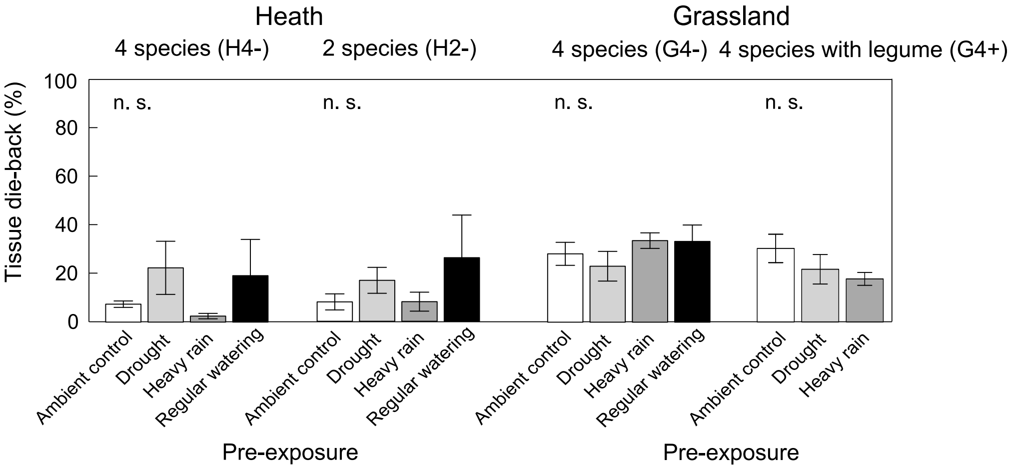

Fig. S2 Tissue die-back of heath and grassland plant communities from harvest one. Displayed are the mean percentage over all sampling dates and the standard error. *n. s.* not significant.

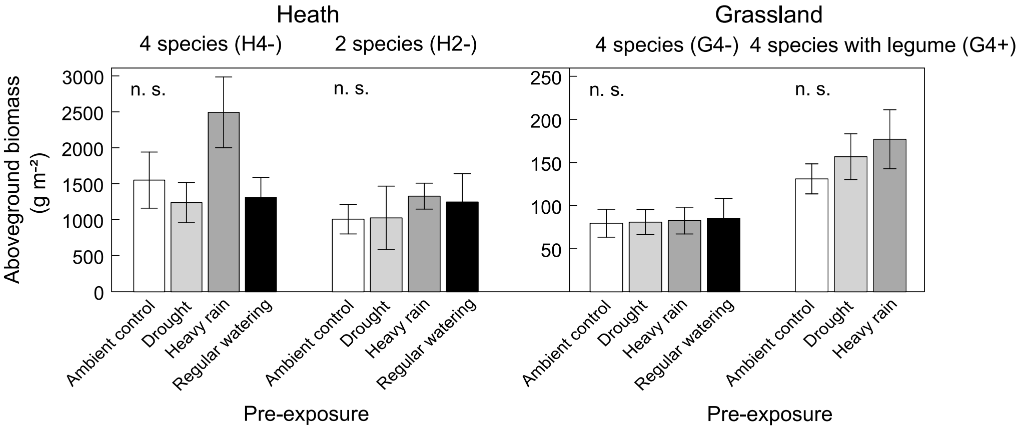

Fig. S3 Aboveground biomass (g m^{-2}) of heath and grassland plant communities from harvest one. Displayed are the mean over all sampling dates and the standard error. *n. s.* not significant.

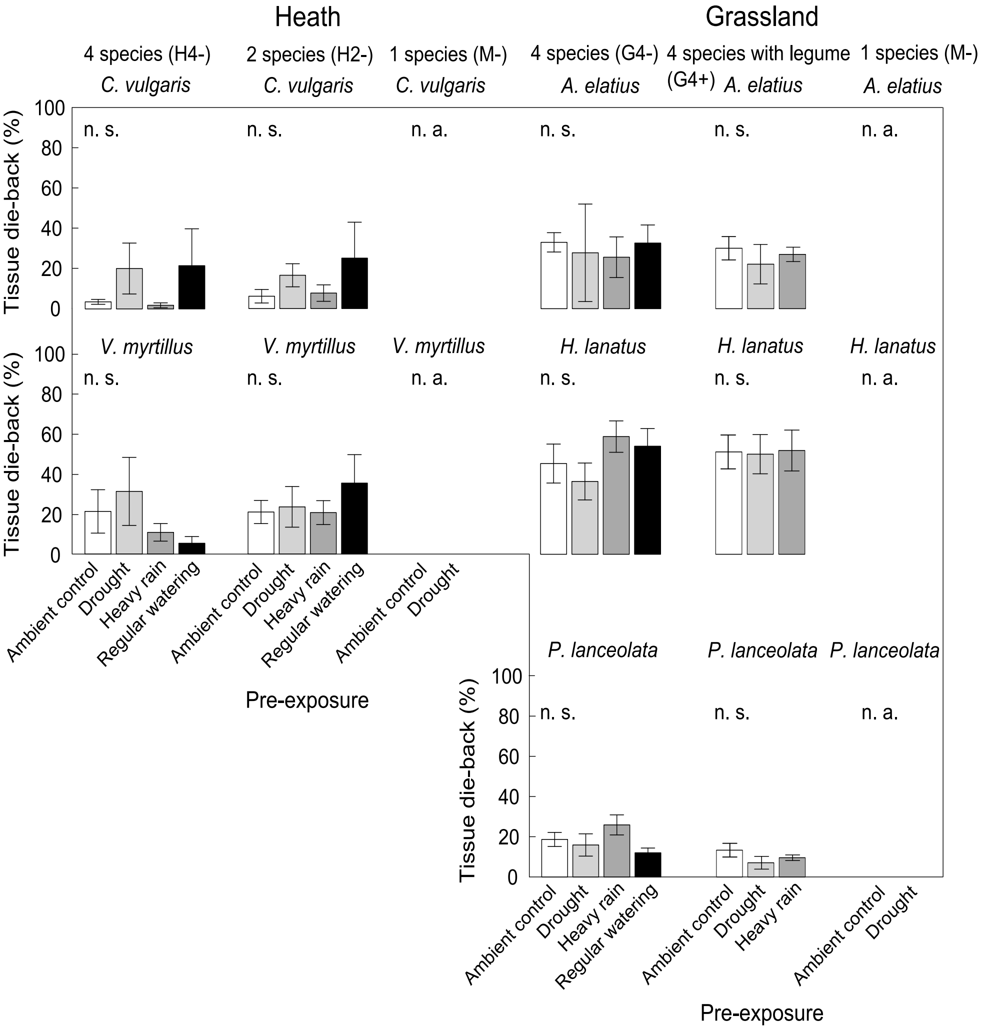

Fig. S4 Tissue die-back of plant species in heath and grassland communities from harvest one. Displayed are the mean percentage over all sampling dates and the standard error. *n. a.* not available; *n. s.* not significant.

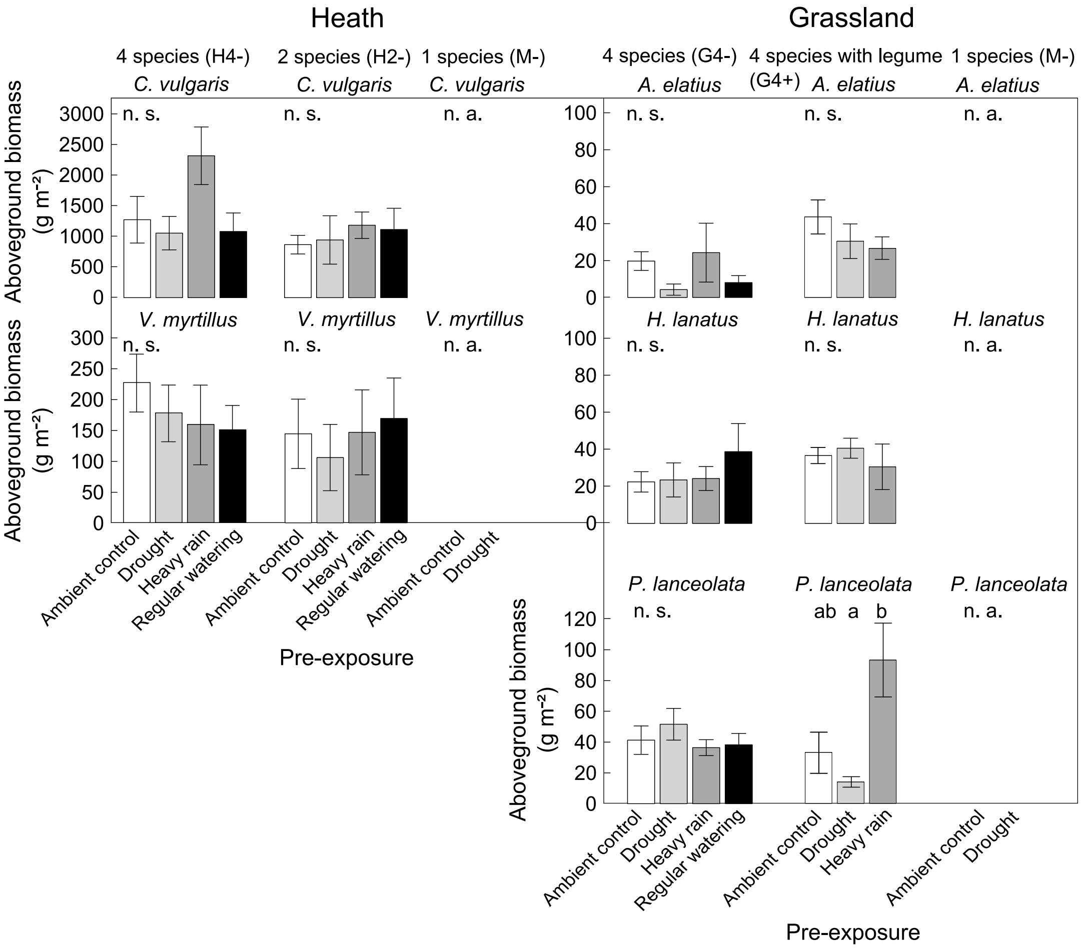

Fig. S5 Aboveground biomass (g m^{-2}) of plant species in heath and grassland communities from harvest one. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean over all sampling dates and the standard error. *n. a.* not available; *n. s.* not significant.

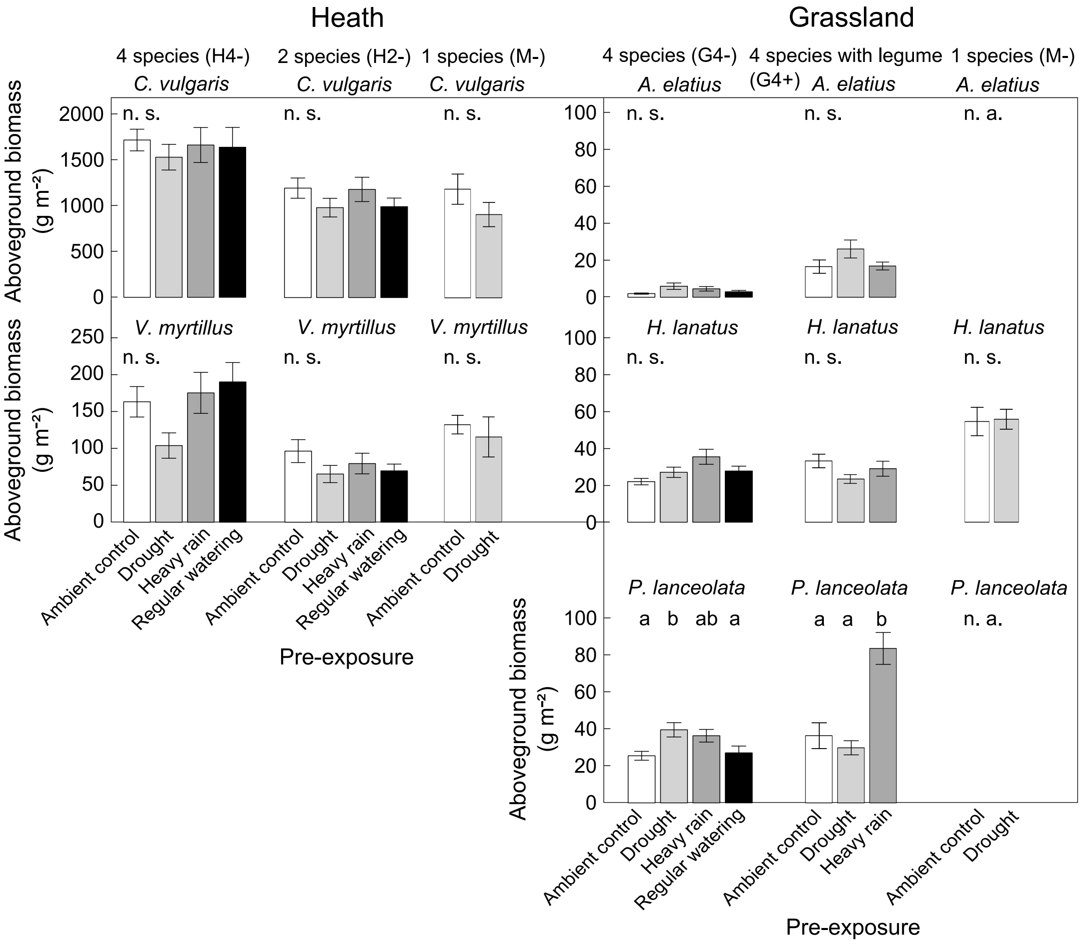

Fig. S6 Aboveground biomass (g m^{-2}) of plant species in heath and grassland communities from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean over all sampling dates and the standard error. *n. a.* not available; *n. s.* not significant.

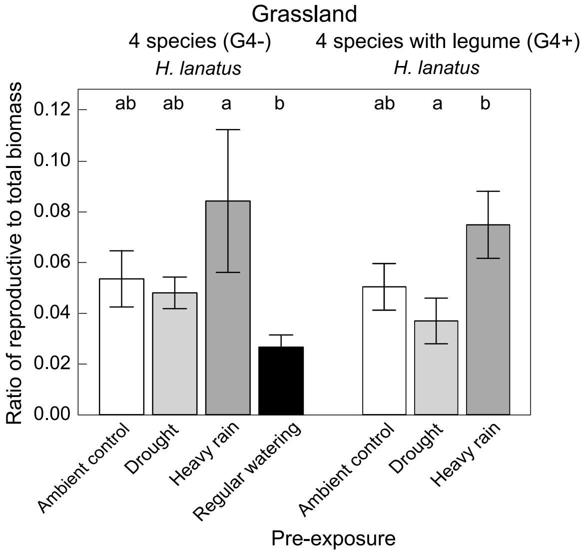

Fig. S7 Ratio of reproductive to total biomass of *Holcus lanatus* from harvest 2-8. The *lower case letters* represent significant differences as revealed by the post hoc test. Displayed are the mean over all sampling dates and the standard error.

Table S1 ANOVA results for the effects of pre-exposure ('ambient control', 'drought', 'heavy rain', and 'regular watering') on tissue die-back of the different experimental heath (H4- and H2-) and grassland (G4- and G4+) plant communities from harvest one.

Factor	Pre-exposure	
Parameter	Tissue die-back (%)	
Plant community	F	P
H4-	2.2	0.151
H2-	2.0	0.169
G4-	0.9	0.491
G4+	1.8	0.225

Table S2 ANOVA results for the effects of pre-exposure (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) on aboveground biomass of the different experimental heath (H4- and H2-) and grassland (G4- and G4+) plant communities from harvest one.

Factor	Pre-exposure	
Parameter	Aboveground biomass	
Plant community	F	P
H4-	2.8	0.098
H2-	0.4	0.772
G4-	0.0	0.997
G4+	0.9	0.448

Table S3 ANOVA results for the effects of pre-exposure (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) on tissue die-back and reproductive biomass (seeds and flowers) of the heath and grassland plant species from harvest one. - = no analysis could be performed.

Factor	Pre-exposure				
Parameter		Tissue die-back (%)		Reproductive biomass	
Plant community	Species	F	P	F	P
H4-	*Calluna vulgaris*	0.9	0.489	-	-
	Vaccinium myrtillus	1.3	0.342	-	-
H2-	*Calluna vulgaris*	1.4	0.294	-	-
	Vaccinium myrtillus	0.4	0.788	-	-
G4-	*Arrhenatherum elatius*	0.2	0.901	-	-
	Holcus lanatus	1.2	0.347	-	-
	Plantago lanceolata	2.1	0.158	0.5	0.712
G4+	*Arrhenatherum elatius*	0.3	0.724	1.4	0.296
	Holcus lanatus	0.0	0.962	1.0	0.410
	Plantago lanceolata	2.3	0.159	1.5	0.279

Table S4 ANOVA results for the effects of pre-exposure (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) on aboveground biomass and ratio of reproductive to total biomass of the heath and grassland plant species from harvest one. - = no analysis could be performed. Significant values are shown in bold ($P < 0.05$).

Factor	Pre-exposure				
Parameter		Aboveground biomass		Ratio of reproductive to total biomass	
Plant community	Species	F	P	F	P
H4-	*Calluna vulgaris*	3.0	0.080	-	-
	Vaccinium myrtillus	0.5	0.677	-	-
H2-	*Calluna vulgaris*	0.3	0.818	-	-
	Vaccinium myrtillus	0.3	0.858	-	-
G4-	*Arrhenatherum elatius*	2.0	0.192	-	-
	Holcus lanatus	0.5	0.677	-	-
	Plantago lanceolata	0.5	0.696	0.2	0.870
G4+	*Arrhenatherum elatius*	1.8	0.230	1.0	0.408
	Holcus lanatus	0.5	0.618	1.0	0.410
	Plantago lanceolata	7.0	**0.017**	0.7	0.513

Table S5 ANOVA results for the effects of pre-exposure (‘ambient control’, ‘drought’, ‘heavy rain’, and ‘regular watering’) on aboveground biomass and belowground biomass of the different experimental heath (H4- and H2-) and grassland (G4- and G4+) plant communities from harvest 2-8. Significant values are shown in bold ($P < 0.05$).

Factor	Pre-exposure			
Parameter	Aboveground biomass		Belowground biomass	
Plant community	F	P	F	P
H4-	0.5	0.693	0.8	0.491
H2-	1.5	0.220	0.5	0.669
G4-	6.3	**0.001**	1.7	0.167
G4+	4.5	**0.014**	0.4	0.689

Table S6 ANOVA results for the effects of pre-exposure ('ambient control', 'drought', 'heavy rain', and 'regular watering') on aboveground biomass and ratio of reproductive to total biomass of the heath and grassland plant species from harvest 2-8. - = no analysis could be performed. Significant values are shown in bold ($P < 0.05$).

Factor	Pre-exposure				
Parameter		Aboveground biomass		Ratio of reproductive to total biomass	
Plant community	Species	F	P	F	P
H4-	*Calluna vulgaris*	0.2	0.864	0.9	0.436
	Vaccinium myrtillus	2.6	0.057	0.9	0.433
H2-	*Calluna vulgaris*	1.1	0.354	0.7	0.579
	Vaccinium myrtillus	1.5	0.227	0.7	0.555
G4-	*Arrhenatherum elatius*	2.1	0.106	0.9	0.460
	Holcus lanatus	2.2	0.087	2.8	**0.044**
	Plantago lanceolata	5.0	**0.003**	1.6	0.182
G4+	*Arrhenatherum elatius*	2.2	0.112	0.9	0.405
	Holcus lanatus	1.8	0.174	4.8	**0.010**
	Plantago lanceolata	16.8	**< 0.0001**	2.5	0.089
M-	*Calluna vulgaris*	2.3	0.145	1.8	0.190
	Vaccinium myrtillus	1.6	0.213	2.7	0.111
	Arrhenatherum elatius	-	-	-	-
	Holcus lanatus	0.0	0.896	2.7	0.116

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8 Appendix

Further publications:

Juergen Kreyling, Constanze Buhk, **Sabrina Backhaus**, Martin Hallinger, Gerhard Huber, Lukas Huber, Anke Jentsch, Monika Konnert, Daniel Thiel, Martin Wilmking, Carl Beierkuhnlein (2014). Local adaptations to frost in marginal and central populations of the dominant forest tree *Fagus sylvatica* L. as affected by temperature and extreme drought in common garden experiments. Ecology and Evolution 4 (5): 594–605 (doi:10.1002/ece3.971).

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