

Plant responses to climatic extremes: within-species variation equals among-species variation

ANDREY V. MALYSHEV¹, MOHAMMED A. S. ARFIN KHAN^{2,3}, CARL BEIERKUHNLEIN⁴, MANUEL J. STEINBAUER⁴, HUGH A. L. HENRY⁵, ANKE JENTSCH², JÜRGEN DENGLER^{6,7}, EVELIN WILLNER⁸ and JUERGEN KREYLING¹

¹Experimental Plant Ecology, Institute of Botany and Landscape Ecology, Greifswald University, Soldmannstrasse 15, Greifswald 17487, Germany, ²Disturbance Ecology, BayCEER, University of Bayreuth, Bayreuth 95440, Germany, ³Department of Forestry and Environmental Science, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh, ⁴Biogeography, BayCEER, University of Bayreuth, Bayreuth 95440, Germany, ⁵Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada, ⁶Plant Ecology, BayCEER, University of Bayreuth, Bayreuth 95440, Germany, ⁷German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, Leipzig 04103, Germany, ⁸Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank, Satellite Collections North, Malchow/Poel 23999, Germany

Abstract

Within-species and among-species differences in growth responses to a changing climate have been well documented, yet the relative magnitude of within-species vs. among-species variation has remained largely unexplored. This missing comparison impedes our ability to make general predictions of biodiversity change and to project future species distributions using models. We present a direct comparison of among- versus within-species variation in response to three of the main stresses anticipated with climate change: drought, warming, and frost. Two earlier experiments had experimentally induced (i) summer drought and (ii) spring frost for four common European grass species and their ecotypes from across Europe. To supplement existing data, a third experiment was carried out, to compare variation among species from different functional groups to within-species variation. Here, we simulated (iii) winter warming plus frost for four grasses, two nonleguminous, and two leguminous forbs, in addition to eleven European ecotypes of the widespread grass *Arrhenatherum elatius*. For each experiment, we measured: (i) C/N ratio and biomass, (ii) chlorophyll content and biomass, and (iii) plant greenness, root ¹⁵N uptake, and live and dead tissue mass. Using coefficients of variation (CVs) for each experiment and response parameter, a total of 156 within- vs. among-species comparisons were conducted, comparing within-species variation in each of four species with among-species variation for each seed origin (five countries). Of the six significant differences, within-species CVs were higher than among-species CVs in four cases. Partitioning of variance within each treatment in two of the three experiments showed that within-species variability (ecotypes) could explain an additional 9% of response variation after accounting for the among-species variation. Our observation that within-species variation was generally as high as among-species variation emphasizes the importance of including both within- and among-species variability in ecological theory (e.g., the insurance hypothesis) and for practical applications (e.g., biodiversity conservation).

Keywords: disturbance, extreme climatic events, genetic diversity, inter-specific variation, intraspecific variation, niche models, provenance

Received 17 December 2014 and accepted 7 September 2015

Introduction

Ecological theory concerning biodiversity and species coexistence has been based largely on the species concept and has treated species as single, uniform entities across their distribution ranges (Valladares *et al.*, 2014). For example, efforts to describe, preserve and enhance biodiversity are often based on the insurance hypothesis (Walker *et al.*, 1995; Naeem & Li, 1997; Yachi & Loreau, 1999), which is defined as ‘any long-term effects of

biodiversity that contribute to maintain or enhance ecosystem function in the face of environmental fluctuation’ (Yachi & Loreau, 1999). Biodiversity insures ecosystem functioning in the context of environmental change or fluctuations; because of differences among species in disturbance tolerance and environmental adaptations, species that are less important or even redundant for ecosystem functioning in one environment might replace others and become key drivers of stability with environmental change (Walker *et al.*, 1999; Fig. 1a, b). The insurance hypothesis is often put forward as an argument for conserving species-rich systems (Yachi & Loreau, 1999). However, within-species

Correspondence: Andrey V. Malyshev, tel. 03834 864181, fax 03834 864096, e-mail: andrey.malyshev@uni-greifswald.de

genetic and phenotypic variation may be high as well, and at times equal to among-species variation (Hughes *et al.*, 2008; Poirier *et al.*, 2012), potentially being as strong of a buffer for maintaining community stability in the face of environmental change. In support of this statement, higher within-species genetic diversity has been shown to enhance the overall species diversity of a community (Whitlock *et al.*, 2007).

There is a disproportionately low amount of information regarding variation in traits within species relative to among species. There is evidence, however, that variation both within populations (Booth & Grime, 2003) and between populations (Beierkuhnlein *et al.*, 2011; Kreyling *et al.*, 2012) can be important for biodiversity conservation and ecosystem function (Jung *et al.*, 2010).

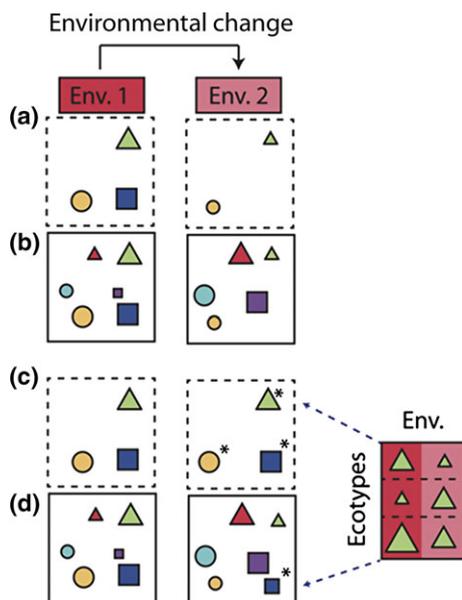


Fig. 1 The insurance hypothesis suggests that in species-poor communities (a) functioning is more likely to get lost when compared to species-rich communities (b). In species-rich communities, ecosystem functioning can be maintained despite environmental change, because species that are currently functionally redundant and poorly adapted may become important with environmental change (i.e., they might replace other species and take over their role in the system). However, if within-species variability of stress tolerance is as high as among-species variability, negative effects of environmental change may be buffered by (active, human induced or passive natural) introduction or the natural presence of better-adapted ecotypes. The latter is particularly important in species-poor communities (c) and less important in species-rich communities, where other species may maintain ecosystem functioning (d). Colors represent different species, symbols different functions, and the size of the symbol the quality of that function within the ecosystem under a particular environment. Asterisks represent newly introduced ecotypes.

Thus, if within-species differences are as great as among-species differences, the insurance hypothesis could be extended to differentiation within species, and the functional resilience of a community to environmental stress could be ensured through high ecotypic diversity (Fig. 1c, d); ecotypes here mean populations distinguished by morphological and physiological characters, and those that exhibit characters determined by genes restricted to the geographic regions in which they occur (Turrill, 1946). However, high genetic variation within a species is most likely for ecotypes exhibiting high spatial separation. Therefore, assisted gene flow (i.e., the translocation of locally adapted ecotypes) may be required to significantly increase the stability of an ecosystem in the context of current climate change (Kreyling *et al.*, 2011; Aitken & Whitlock, 2013). A number of strategies (e.g., predictive and composite provenancing) have been developed to optimize vegetation performance using seed sources of different provenances, ranging from simply increasing the genetic diversity within mixtures to determining ecotypes likely to be best adapted to projected climate changes (Breed *et al.*, 2012). As an example of predictive provenancing, using seed sources from the environmental optima of a species in the expanding range limit has been suggested to maximize survival (Atkins & Travis, 2010).

Adaptation to a local environment in plant species is common, yet the factors that make some species more apt to develop local adaptation are not known (Leimu & Fischer, 2008). Variation in local adaptation could also have important implications for species distribution modeling in response to climate change. Predicting range shifts in response to rapid climate change has become an important topic in ecology, and it commonly results in grim projections with respect to predicted range contractions (Thomas *et al.*, 2004; Thuiller *et al.*, 2005). Most approaches, however, fail to address genetic and phenotypic variation within species. Models of species range limits based on habitat suitability have indicated that incorporation of ecotype-specific responses (i.e., those of locally adapted populations within species - Hufford & Mazer, 2003) can result in different projections of species range changes as compared with species being treated as uniformly responding units (Oney *et al.*, 2013; Valladares *et al.*, 2014).

An 'extreme climatic event' is an episode or occurrence in which a period of statistical climate extremity alters ecosystem structure and/or function outside the bounds of what is considered typical or normal variability (Smith, 2011). Despite the emerging importance of extreme climatic events as a key component of climate change impacts (Jentsch *et al.*, 2007), empirical data comparing within-species variation in responses

to these events to variation among species are lacking. An overview of key recent studies exploring both within- and among-species variation in various measured traits shows that very few studies have explored the relative sizes of both variation types under stress in the same paper (Appendix S1). Rather, numerous studies have focused on either exploring within-species variation (e.g., Garamszegi & Moller, 2010) and its genetic causes (Ogura & Busch, 2015) or among-species variation (e.g., Grime *et al.*, 1997), which shows that both can vary greatly and need to be incorporated in documenting and generalizing plant traits and growth patterns. Increasing climatic variability is expected to increase the frequency of severe heat waves and the frequency and intensity of drought in many regions (Schär *et al.*, 2004; IPCC, 2013), and drought sensitivity is predicted to both change the competitive abilities of plant species and have important impacts at the ecosystem level (Jentsch *et al.*, 2011; Abeli *et al.*, 2014). In addition, an earlier onset of the growing season due to climate change may increase the risk of late frost damage in spring, despite a general air warming trend; this increased risk is expected to occur because the timing of late frost is expected to remain relatively stable (Augspurger, 2013), and the intensity and duration of frost events may not decrease within this century (Kodra *et al.*, 2011). Furthermore, in winter, warm spells can trigger de-acclimation of cold-acclimated plants within hours of warming, leaving plants susceptible to frost damage when freezing temperatures return (Kalberer *et al.*, 2006; Bokhorst *et al.*, 2009). Similar to drought, frost stress can play an important role in influencing plant community composition (Joseph & Henry, 2008), species distributions (Sakai & Weiser, 1973) and overall species diversity (Hettwer *et al.*, 2012). Comparisons of within- vs. among-species variation in responses to warming, drought and frost (the latter in either winter or early spring) are therefore relevant in the context of both community and individual plant stress responses to climate change, and they encompass most temperature-related stresses faced by plants.

Whether due to plastic responses, evolutionary change, or both, there is mounting evidence for local adaptation in plants (Franks *et al.*, 2014). These local adaptations may even be preserved in the presence of high within-species gene flow (Fitzpatrick *et al.*, 2015). Common European grass species express local adaptations to their climates of origin (Kreyling *et al.*, 2012). Genetic differences among populations within a species have been detected for *Arrhenatherum elatius*, which is a wide-spread and abundant grass species in Europe (Michalski *et al.*, 2010). Here, we compared variation in stress tolerance among and within species by analyzing

data from two previous experiments which had exposed ecotypes of four common European grass species stemming from five European countries to simulated summer drought as well as spring frost. We then conducted a winter warming plus frost experiment on four grasses, two nonleguminous forbs, two leguminous forbs, and 11 ecotypes of the grass *Arrhenatherum elatius* from different European countries (Ireland, Spain, Germany and Poland) to analyze how variation in responses among species from different functional groups sharing a common origin compare to within-species variation across Europe. We combined data from different experiments (across several years) and multiple stresses (drought, spring frost, winter warming plus frost), several species and also several response parameters in order to obtain results that are as general as possible rather than specific to single settings and species. Based on evidence for occurrences of both high among- and within-species variation to stress in different studies (Appendix S1), we hypothesized that among-species variation does not generally exceed within-species variation for the studied species under a range of stresses.

Materials and methods

Among- and within-species variation in stress responses were directly compared in three different experiments which all quantified plant growth performance under stressful conditions. In order to obtain results that were as general as possible, these three experiments focused on different stresses (drought, spring frost, winter warming plus frost) were carried out using different methodologies (field vs. laboratory experiments), and different parameters were measured (biomass, C/N ratio, chlorophyll, plant greenness, ¹⁵N uptake, etc.) in different species and ecotypes. Taken together, the results of the different experiments covered a broad range of conditions which all caused stress and allowed for a direct comparison of among- and within-species variation in stress responses. The drought experiment was carried out in 2009 and 2010, and the spring frost experiment was carried out in 2010; both were part of the EVENT common-garden experiments (Figure S1) (Jentsch & Beierkuhnlein, 2010). The location was in Bayreuth, Germany, on the property of the Ecological-Botanical Gardens of the University of Bayreuth, in proximity to EVENT 1 and EVENT 2 (49°55'19"N, 11°34'55"E). The long-term mean annual temperature for the site was 8.2 °C, and the long-term mean annual precipitation was 724 mm. The winter warming plus frost experiment was carried out in 2011 as a supplement to the other experiments. It broadened the range of the among-species group (including additional plant functional types) and added another stress. For all experiments, plants were obtained as seeds and grown under standardized conditions. All plant species used in the experiments are common grassland species with wide distributions across Europe. All species experience similar management intensity and strong temperature gradients along their

distribution ranges, which does not place bias on within-species variation for any particular species. Mean climate parameters and the distributions of all species used in all experiments are provided in Table 1 and Figure S2, respectively. Mean climate values and standard deviations were calculated using bioclimatic variables downloaded from Worldclim (Hijmans *et al.*, 2005), using a resolution of 10 arc-s. Adaptation to local climate was assumed, and regional climates were inferred from Worldclim data (Hijmans *et al.*, 2005).

Drought experiment

Four grasses from Central European managed grasslands were used (*Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Alopecurus pratensis* L., *Festuca pratensis* H., *Holcus lanatus* L.) (details on experimental design in Beierkuhnlein *et al.*, 2011). Besides local ecotypes of these four species from Germany (DE), we selected other European ecotypes of these grasses from environmentally distinct regions (Italy, IT; Hungary, HU; Bulgaria, BG; Sweden, SE; Table 2). For *A. elatius* and *F. pratensis*, ecotypes from all five target regions were available, while for *A. pratensis* and *H. lanatus*, there were only four. Therefore, within-species variation included five ecotypes for *Arrhenatherum elatius* and *Festuca pratensis* and four ecotypes for *Alopecurus pratensis* and *Holcus lanatus*. Among-species variation included four grass species for Bulgaria, Germany and Hungary and three grass species for Italy and Sweden (Table 2). Four treatments were created in a split-plot design from a replicated factorial combination of temperature (warming and control) and precipitation (drought and control). Twenty-one plants per ecotype were used in each treatment.

All seeds of the ecotypes were collected in the wild. Seeds from at least five mother plants per origin were combined to form a mixed sample for each ecotype. The target ecotypes were cultivated from seed simultaneously at the branch office of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Poel, Germany, from February 2009 to April 2009. Collections took place at supposedly autochthonous,

semi-natural stands. The seedlings were then transported to Bayreuth and individually planted into 4-L plastic pots filled with local forest topsoil. The soil substrate was sandy silt (pH = 7.27, total C = 1.89%, total N = 0.15%, plant-available NH_4^+ = 1.79 mg L⁻¹, plant-available NO_3^- = 22.50 mg L⁻¹). For the first two weeks after planting, the seedlings were watered generously with tap water to ensure growth. All individuals were then cut to a height of 7 cm in order to create comparable starting conditions and exposed to ambient precipitation until the start of the experimental treatments on May 25, 2009. The experiment ended in September 2010. The climate manipulations were performed twice, with the drought lasting 16–19 days in 2009, depending on the species-specific tolerance (drought ended when two-thirds of the individuals from one species showed severe senescence; see below for details), and 30 days for all species in 2010. Twenty-one replicates per treatment were used in 2009 and 15 from the 21 were used in 2010 (Figure S1).

Drought was induced by rain-out shelters constructed of a steel frame (GlasMetall Riemer GmbH, Rahden/Sielhorst, Germany) and covered with a transparent polyethylene sheet (0.2 mm, SPR5; Hermann Meyer KG, Rellingen, Germany). The lower edges of the rain-out shelters were at a height of 80 cm, and the shelters permitted nearly 90% penetration of photosynthetically active radiation. The control precipitation regime simulated the local daily 30-year average precipitation. The application was done under the shelters twice a week with collected rain water. The extreme drought treatment consisted of a period without precipitation. The drought treatment resulted in a dropping of soil moisture below the permanent wilting point of the soil approximately one week after the start of the treatment in both years. Experimental treatments were continued until a significant number of plants reached a defined state of senescence in 2009. The definition was based on a four-stage visual damage quantification key (0–3, where 0 stands for ‘completely undamaged’ and 3 stands for ‘totally dried out and brittle’). By the time, two-thirds of the individuals of one species had reached stages 2 or 3, or by the time one-third of the individuals had reached stage 3, the

Table 1 Biological information (Source: <http://www2.ufz.de/biolflor>) and mean climate parameter values and their respective standard deviation values from the distribution ranges of all species used in the experiments. *Arrhenatherum elatius*, *Festuca pratensis*, *Holcus lanatus*, and *Alopecurus pratensis* were used in the drought and warming experiment and the spring frost experiment, while the other species and *Arrhenatherum elatius* were used in the winter warming plus frost experiment

Species	Pollination		Annual mean temperature (°C)	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Annual precipitation (mm)
	type	Selfing				
<i>Alopecurus pratensis</i>	Wind	Self-incompatible	4.0 ± 4.6	22.6 ± 3.6	-13.0 ± 8.1	639 ± 219
<i>Arrhenatherum elatius</i>	Wind	± self-incompatible	8.3 ± 3.5	23.9 ± 3.8	-5.4 ± 5.3	684 ± 206
<i>Dactylis glomerata</i>	Wind	± self-incompatible	5.8 ± 4.8	24.2 ± 4.2	-11.0 ± 7.9	614 ± 219
<i>Festuca pratensis</i>	Wind	± self-incompatible	3.8 ± 4.1	23.1 ± 2.8	-14.1 ± 8.1	612 ± 209
<i>Geranium pratense</i>	Insect	Self-compatible	3.0 ± 3.9	23.1 ± 2.4	-15.7 ± 7.9	579 ± 154
<i>Holcus lanatus</i>	Wind	± self-incompatible	8.0 ± 4.0	23.7 ± 4.4	-5.8 ± 5.6	698 ± 256
<i>Lotus corniculatus</i>	Insect	Self-compatible	7.1 ± 4.4	24.0 ± 4.4	-8.2 ± 6.7	661 ± 244
<i>Plantago lanceolata</i>	Wind	± self-incompatible	6.0 ± 4.1	24.4 ± 4.0	-10.9 ± 7.5	600 ± 246
<i>Trifolium pratense</i>	Insect	Self-incompatible	4.3 ± 4.7	23.4 ± 3.7	-13.3 ± 8.4	608 ± 209

Table 2 Geography and climate of seed sources of species and ecotypes used in the drought and warming, spring frost, and winter warming plus frost experiments. In the winter warming plus frost experiment, the shading indicates distinct genetic groupings, as documented by Michalski *et al.* (2010), using pairwise genetic distance scores. Genetic diversity of ecotypes was measured by the proportion of polymorphic loci and by the mean pairwise Jaccard dissimilarity among individuals within ecotypes (*J*), based on amplified length polymorphism (AFLP). Responses of the local *A. elatius* ecotype (marked in bold) originating closest to the other local plant species were treated as part of among-species variation

Species	Origin	Mean maximum temperature of warmest month (°C)	Latitude	Longitude	Elevation (m a.s.l.)	Mean minimum temperature of coldest month (°C)	Annual precipitation (mm)
Species and ecotypes used in drought and warming experiment and in spring frost experiment							
<i>Arrhenatherum elatius</i>	Bulgaria	24.0	42°00'N	24°50'E	1008	-5.5	658
	Hungary	24.7	47°12'N	17°52'E	440	-5.4	621
	Sweden	20.9	59°51'N	17°38'E	20	-7.5	551
	Germany	22.6	49°17'N	09°58'E	460	-3.7	732
	Italy	30.0	44°55'N	09°44'E	110	-1.8	739
<i>Alopecurus pratensis</i>	Bulgaria	24.0	42°26'N	23°35'E	810	-5.8	593
	Hungary	24.7	47°12'N	17°52'E	440	-5.4	621
	Sweden	20.9	60°00'N	15°00'E	350	-9.8	738
	Germany	22.6	49°17'N	09°58'E	460	-3.7	732
<i>Festuca pratensis</i>	Bulgaria	25.5	42°19'N	23°45'E	710	-4.9	585
	Hungary	25.7	47°27'N	18°28'E	270	-4.7	571
	Sweden	20.9	60°00'N	15°00'E	350	-9.8	738
	Germany	22.6	49°17'N	09°58'E	460	-3.7	732
<i>Holcus lanatus</i>	Italy	20.7	44°33'N	09°27'E	1600	-3.2	981
	Bulgaria	27.7	42°31'N	24°48'E	330	-4.5	581
	Hungary	26.7	46°10'N	17°55'E	200	-4.3	675
	Germany	22.6	49°17'N	09°58'E	460	-3.7	732
	Italy	28.8	44°53'N	09°41'E	160	-2.0	758
Species and ecotypes used in winter warming plus frost experiment							
<i>Arrhenatherum elatius</i> (within-species diversity category)	Ireland 1	19.5	52°38'N	8°57'W	12	2.9	1011
	Ireland 2	18.6	52°30'N	8°51'W	42	1.6	1069
	Ireland 3	18.9	52°03'N	8°30'W	25	3.1	1300
	Germany 1	21.5	50°36'N	10°41'E	455	-5.2	673
	Germany 2	20.2	51°44'N	10°45'E	470	-4.2	820
	Germany 3	23.4	51°53'N	12°01'E	60	-2.2	493
	Poland 1	23.8	50°34'N	21°40'E	490	-7.3	564
	Germany 4	20.1	51°38'N	10°55'E	490	-4.1	791
	Germany 5	21.6	50°32'N	10°47'E	450	-5.0	672
	Spain 1	23.4	43°15'N	07°17'W	600	2.8	1050
	Spain 2	24.2	42°37'N	08°07'W	545	3.2	1321
	Spain 3	23.2	43°14'N	08°00'W	280	4.1	1175
	<i>A. elatius</i> , <i>H. lanatus</i> , <i>A. pratensis</i> , <i>G. pratense</i> P. lanceolata <i>L. corniculatus</i> , <i>T. pratense</i> , (among-species diversity category)	Germany	17.7	49°10'N	9°34'E	460	-2.5

drought was stopped. The drought treatment lasted 16 days for *H. lanatus*, 18 days for *A. pratensis* and *F. pratensis* and 19 days for *A. elatius*. However, a 30-day-long drought treatment was used for all species in 2010. The warming treatment was performed continuously throughout the whole experi-

ment. Warming was done passively via wind shelters and black floor covers, which increased the average temperature by 1.5 K compared with the temperature control treatment. All treatments were administered below identical shelters; therefore, the relative temperature and water addition differ-

ences were quantified for a single sheltered environment. The fourth treatment was a combination of extreme drought and warming. The additional warming increased the drought treatment effect by additionally reducing the soil moisture by approximately 1.5% on average.

Measured parameters for the drought experiment

Above-ground biomass was harvested at the end of June and the end of September in 2009 and 2010. Leaf C and N concentrations were measured in 2009 after the first drought. Nine replicates per ecotype per treatment were randomly selected (out of 21) for the C/N analysis. A sample for each replicate was taken from the above-ground dry biomass, including leaves and shoots, because N concentration varies among different plant parts. Selected samples were fine-milled and mixed well for C and N analysis. Samples (3 mg approx.) were analyzed using an elemental analyzer (EA 3000; Euro Vector, Italy). Relative plant C and N concentrations (%) were provided by this analysis.

Spring frost experiment

Some of the plants that had been used in the 2009 drought experiment (same ecotype stocks and same individuals) were overwintered outside in a sand-bed and under ambient conditions and used the next year in the spring frost experiment (details on experimental design in Kreyling *et al.*, 2012). As the mean response of all plants within an ecotype (with and without drought history) was used, no ecotypes had biased responses (prior treatment of each plant was used as a random factor to account for the variation in the statistical analyses). Three plants were used from each pretreatment (four treatments from 2009), resulting in 12 replicates per ecotype per treatment in 2010 for the spring frost experiments. From 26 to 27 May 2010, the plants were exposed to a simulated late frost event (based on local climate data) of -5°C (air temperature) for three hours by gradual cooling them inside a cooler truck. The control (nonfrozen) plants remained under ambient conditions.

Measured parameters for the spring frost experiment

The temporal pattern of chlorophyll content was monitored weekly over five weeks after the late frost manipulation using a SPAD-502 chlorophyll meter (Konica Minolta Sensing). Measurements were taken on six replicates per ecotype per treatment (three stemming from the drought pretreatment and three from the control of the 2009 drought experiment). Four leaves per plant and date were chosen randomly for the SPAD measurements, and an average was taken for the analyses. SPAD readings were calibrated to foliar chlorophyll content for 20 leaves per species. SPAD readings were conducted for these samples, and the leaves were then removed and kept at low temperature without freezing for the immediate determination of the fresh weight. Afterward, samples were frozen and kept at -29°C until chlorophyll extraction. For the latter, leaves were cooled in liquid nitrogen and ground in a ball mill

while frozen. The chlorophyll was dissolved in 1 mL cold methanol for 30 min and shaken every 10 min. Extracts were centrifuged for 5 min at 4°C and 14 000 rpm. The supernatant was stored in a freezer and warmed for 15 min at room temperature before the absorbance was measured at 645 and 663 nm using a spectrophotometer (DU-50, BECKMANN). The chlorophyll content per g fresh weight was calculated according to Porra *et al.* (1989) as:

$$[\text{chl}]_{\text{fwt}} \left[\frac{\text{mg}}{\text{g}} \right] = \frac{(8.02 \times E_{663\text{nm}} + 20.2 \times E_{645\text{nm}})}{1000} \times \frac{V(\text{MeOH} + \text{watercontent})[\text{mL}]}{\text{freshweight}[\text{g}]}$$

A linear regression between SPAD and chlorophyll content yielded significant correlations for all four species with r^2 values of 0.88 for *H. lanatus*, 0.70 for *A. pratensis*, 0.72 for *F. pratensis* and 0.68 for *A. elatius*. Above-ground biomass was harvested on July 6, 2010 as in the drought and warming experiment, dried for 48 h at 70°C and weighed.

Winter warming plus frost experiment

Ecotypes of *Arrhenatherum elatius* used in the winter warming plus frost experiment were chosen as genetically distinct seed lines (acquired from the seed bank at the Leibniz Institute of Plant Genetics and Crop Plant Research) based on previous genetic analyses which had been carried out on the same seed sources (Michalski *et al.*, 2010). Within-species variation was represented by 11 genetically distinct ecotypes of *A. elatius* selected from four European countries (Table 2). For this species, there is evidence of local adaptation in biomass production after spring frost at the continental scale, whereby ecotypes stemming from regions with a higher incidence of spring frost events were more resilient to spring frost damage (Kreyling *et al.*, 2012). Among-species variation was represented by four grasses (*Festuca pratensis*, *Holcus lanatus*, *Alopecurus pratensis*, *Arrhenatherum elatius*), two nonleguminous forbs (*Geranium pratense* L., *Plantago lanceolata* L.) and two leguminous forbs (*Lotus corniculatus* L., *Trifolium pratense* L.), all sharing the same seed origin (see Table 2).

Plants were cultivated from seed from the end of September to the end of November 2011 at the Leibniz Institute of Plant Genetics and Crop Plant Research. Seedlings were then transplanted into plastic pots (5 cm diameter \times 7 cm), using seed compost soil (Einheitserde Classic, Germany). NPK (Mg) liquid fertilizer (15+10+15 (+2)) was applied once at a concentration of 1 g L^{-1} (Hakaphos Blau, COMPO EXPERT, Germany).

During October and November, the plants were grown in a glasshouse, where night- and daytime temperatures averaged 6.4 and 20.0°C , respectively. Light was provided with 400-W lamps (approximately $600\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$), with a 10-h photoperiod. Plants were transferred to climate chambers at the end of November, and for two weeks, the day and night time temperatures were lowered to 10 and 6°C , respectively, photoperiod was decreased to 9 h, and PAR light intensity was $200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$. To complete plant cold acclimation, the photoperiod was lowered to 8 h for one month, with soil surface temperature averaging 0.0°C (minimum -6.2°C ; maxi-

mum +5.8 °C). Plants were kept at -1.5 °C prior to thaw treatments, which took place 12–23 February 2012.

On 12 February, all plants (six plants per ecotype and species per treatment) were assigned to one of three thaw treatments: 12 h at 4 °C (mild thaw), 2 days at 9 °C (moderate thaw) or 6 days at 9 °C (extended thaw). Here, we focused on the length of thaw on frost tolerance and not on the effect of frost itself (as compared to the other two experiments, where simply the presence or absence of drought and spring frost were the main stress factors). Freeze–thaw events are known for their ecological importance, and the control therefore included the same number of freeze–thaw events as the manipulations, while they differed in length. Regardless, the gradient of thaw durations was sufficient to generate significant differences in within- and among-species growth responses, which was the main objective. Potential changes in frost tolerance due to the respective thaw periods were assessed by quantifying the responses of the plants to a severe frost event. Frost was administered for 24 h right after the warm spell manipulations. Minimum chamber temperatures in the mild, moderate, and extended thaw treatments reached -11.9, -8.1, and -8.7 °C, respectively, while the respective mean temperatures were -7.2, -5.4, and -6.7 °C. The only appreciably lower minimum temperature was reached in the mild thaw treatment (i.e., the control) compared with the other two treatments (mean temperature was 1.5–2 °C lower and absolute minimum temperature was 3.2–3.7 °C lower than in the other treatments). Even though the mild thaw treatment reached lower minimum temperatures, it still resulted in plants having more than double the growth performance than plants which had experienced milder frost, but after the prolonged thaw/warming. After thawing, all plants were repotted (8 cm × 8 cm × 20 cm deep pots) and transferred to a glasshouse. Temperature was increased by 2 °C every 10 d to simulate spring, reaching ~14 °C on 14 March.

Measured parameters for the winter warming plus frost experiment

Above-ground biomass was harvested one month after the frost for a subset of plants ($n = 6$ per ecotype/species and warm spell treatment), with brown tissue assigned as dead tissue. Material was dried to a constant biomass at 60 °C and weighed. Percent greenness was quantified from digital pictures under standardized light conditions (a portable light-tight box - 20 cm × 20 cm × 60 cm, and artificial lighting) two weeks prior to the destructive harvest. Greenness calculations (Marchand *et al.*, 2004) used a transformation from the RGB-photos to the HSL color space. Threshold values of the HSL bands for 'greenness were determined with the remote sensing software ENVI 4.7 (Exelis Visual Information Solutions, Boulder, CL, USA) and ARCGIS 10 (Esri, Redlands, CA, USA). Processing and calculation of greenness percentage were performed with IMAGEMAGICK version 6.7.6-5 (ImageMagick Studio LLC, Landenberg, PA, USA).

A second set of plants was used for destructive analysis of root integrity. Root functional integrity was assessed immediately after thawing by measuring ^{15}N uptake ($n = 4$ per ecotype

and species). Plants and soil were first transferred into plastic cups (5 cm diameter × 10 cm deep). Twelve milliliters of 100 μM $^{15}\text{NH}_3^{15}\text{NO}_3$ solution was injected 1.5 cm deep into the soil in three aliquots, equidistant from the center. After 22 h of incubation at 20 °C, the plants were rinsed free of soil, washed with 50 mL of 5 mM KCl and 0.5 mM CaCl_2 , then rinsed with 200 mL of deionized water to remove ammonium passively adsorbed in the root cell walls via cation exchange (Epstein *et al.*, 1963). Roots were excised, and roots and shoots were oven dried separately at 60 °C for 48 h and fine-milled and analyzed using an elemental analyzer (see above). Leaf N uptake was quantified only for the mild and extended thaw treatments.

Parameters measured for all three experiments

Biomass was chosen as the central measured parameter because it encompasses the total sum of multiple parameter changes, such as nutrient uptake, height, growth rate, changes in physiology, etc., to show a net treatment effect on plant growth performance. Subordinate parameters were selected based on their suitability to assess the specific stress responses: C/N ratio is representative of plant nutrition differences and has been shown to change after drought stress (An *et al.*, 2005; Sardans *et al.*, 2008). Chlorophyll content and greenness are parameters that show abrupt change due to a sudden stress such as frost. Post-frost N uptake ability was used as an indication of root integrity.

Statistics

Overall treatment effects. Linear mixed effects models were used to test treatment effects on all plant species and ecotypes with respect to the measured parameters (C/N ratio, biomass, chlorophyll content, percent greenness, green leaf biomass, dead tissue biomass, ^{15}N uptake). For the drought experiment, the model was 'response~species*drought*warming + origin*drought*warming', using total yearly accumulated biomass, experimental unit (each treatment was replicated in three randomly assigned shelters), replication, and year (for biomass only) as random effects. For the spring frost experiment, the model was 'response ~ species*frost + origin*frost', with experimental unit, pretreatment (the previous year drought experiment), replication, and date of sampling (chlorophyll measurement only) as random effects.

For the winter warming plus frost experiment, one model was used for the species*treatment interaction and another for the ecotype*treatment interaction to show that both species and ecotypes had similar interactions with treatments. To investigate the effect of treatment independent of both ecotype and species, an additional model was implemented, where treatment levels comprised the fixed factor while species and ecotype identities were inserted as random effects. Homoscedasticity was checked with residual plots, and normality of residuals was tested with normal probability plots (Faraway, 2005). In the winter warming plus frost experiment, all data were square-root-transformed, while coefficients of variation (see below) were log-transformed to satisfy the normality assumption.

Within- vs. among-species variation. We compared variation in stress responses within and among species using coefficients of variations (CVs) (Valladares *et al.*, 2006). This method was comparable across the three experiments. Mean response values of every single ecotype within every treatment were used to calculate within-species coefficients of variation, and mean response values of every single species within every treatment were used to calculate among-species CV, for every parameter. The CV among individual plants within an ecotype yielded a single value. The mean of these values (one for each ecotype) was statistically compared against the mean of similarly calculated species CVs. In experiments that had more than one species for within-species variation (the drought and spring frost experiments), separate analyses were run for each species, comparing the variation within that one species with variation among other species (in each of the countries of seed origin). The analyses were run separately to compare variation within each species against among-species variation in each of the countries of seed origin and for each measured parameter for every treatment (e.g., spring frost experiment: 4 Species*5 countries*2 parameters*treatments = 80 *t*-tests). In each origin-specific within- vs. among-species comparison, the local ecotype of each species was included in among-species variation to preserve independence of samples. For example, ecotype 'Germany 1' of *A. elatius* (Table 2) was included in the among-species group (from Germany) for the winter warming plus frost experiment, because its seed source was closest to the seed sources of the among-species groups. This ensured that the variation in responses specific to *A. elatius* was also accounted for in the analysis of among-species variation and that independence of samples was maintained in among- vs. within-species variation analyses. Due to there being no warming effects on biomass and minimal warming effects on C/N ratio compared with drought (Appendix S2 and Figure S3), CV analyses were done for pooled control (C & W) and pooled drought (D & DW) treatments in the drought experiment. Here, temperature manipulation was used as a random factor in the CV analyses.

For the drought as well as spring frost experiments, variance partitioning was applied to disentangle the explanatory power of treatment from that of within- and among-species variability on the respective measured parameters. Explanatory power is quantified using adjusted R^2 as the goodness-of-fit measure. Joint and independent contributions are estimated by conducting multiple linear regressions with all possible sets of explanatory variables (explained in Legendre, 2008). In our experiments, variance partitioning was used to assess the independent contribution of ecotypes to variability of the respective measured parameters that goes beyond variability explained by species. Both species and ecotypes (their origin) were used as factors. Because ecotypes are nested in species, species cannot explain additional variability independently from ecotypes. In the midwinter warming plus frost experiment, partitioning of variation was not possible due to ecotypes being present only within one species. The analysis was conducted using R-package *vegan* version 2.0-10.

All statistical analyses were performed using R version 3.0.1 (R Core Team 2013) and additional packages *LMTEST* version

2.0-3 for fitting mixed models, *MULTCOMP* version 1.3-1 for post hoc comparisons, and *SCIPLOT* version 1.1-0 for graphical illustrations. Species distribution maps (Figure S2) were created from map scans (Meusel & Bräutigam, 1992) using *ARCGIS* version 10.2.2.

Results

Overall treatment effects on measured parameter values

All three extreme event simulations – 'spring frost', 'drought', and 'winter warming plus frost' – affected most measured parameters related to plant performance negatively (Figs 2a, 3a and 4a). Species- and ecotype-specific responses were inferred from interactions between species and treatments as well as from interactions between ecotypes (Appendix S2) and treatments. The mean differences among origins and their interactions with treatments represent the influence of ecotypic variation in shaping different species to respond similarly under specific climatic conditions. As both 'species' and 'ecotype' factors and their interactions with treatments were significant for biomass, chlorophyll content, dead tissue biomass and root N uptake (Appendix S2), responses were both species- and ecotype-specific.

Within- vs. among-species variation: differences in coefficients of variation

Within-species variation under different extreme events (summer drought and spring frost) matched and at times exceeded among-species variation in four common grass species across five European countries for all tested parameters (biomass, C/N ratio, chlorophyll content in the summer drought experiment as well as in the spring frost experiment, Table 3; Figs 2b and 3b). Similar results were obtained when among-species variation in response to winter warming plus frost was expanded to include multiple plant functional groups and additional response parameters (greenness, dead tissue biomass, ^{15}N uptake in the winter warming plus frost experiment, Table 3; Fig. 4b). In total, 156 comparisons of within- vs. among-species variation were made, each one representing the within-species variation for a specific species vs. among-species variation in a specific location (origin) for each response parameter and treatment. In six of these comparisons, within-species CVs significantly exceeded among-species CVs four times and was 32% higher on average (see relative effect sizes in Table 3). For the other two significant differences, among-species CVs were on average 40% higher than within-species CVs. For the drought and spring frost experiments, partitioning of variance

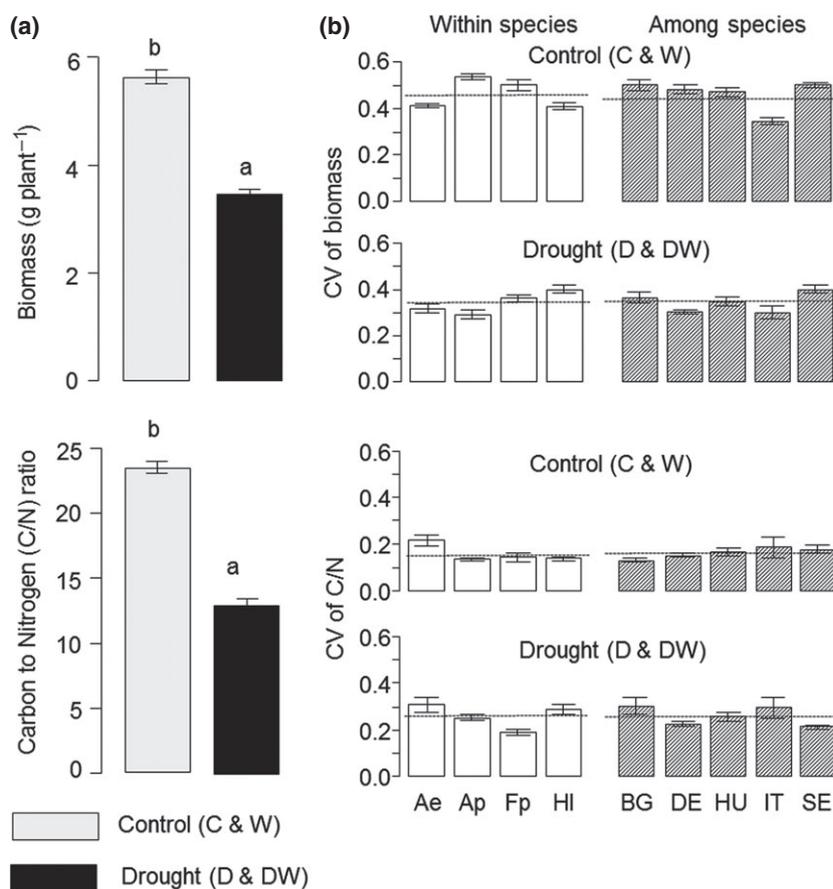


Fig. 2 Drought experiment. (a): Overall treatment effects on biomass and carbon to nitrogen ratio using pooled data from all species and ecotypes of the four grass species (see Table 2). Interactions between countries of origin/ecotypes, species, and treatments are presented in Appendix S2a. (b): Mean coefficients of variation for each species (4–5 ecotypes per species) represent within-species variation (Ae – *Arrhenatherum elatius*, Ap – *Alopecurus pratensis*, Fp – *Festuca pratensis*, HI – *Holcus lanatus*), while mean coefficients of variation for each origin (3–4 species stemming from each country) represent among-species variation (BG – Bulgaria, DE – Germany, HU – Hungary, IT – Italy, SE – Sweden) for each treatment. Error bars denote standard errors. Different letters indicate significant treatment differences in (a). Dashed lines indicate mean within- and among-species CVs in (b).

showed similar among- and within-species explanations of total variation, with a mean of 13% of variation explained by species (and thus ecotypes, which are nested in species), with an additional 9% of variation explained by ecotypes alone, having accounted for species-specific differences (Fig. 5).

Discussion

In our experiments, variation in within-species responses was generally as high as variation in among-species responses under a variety of environmental stressors, and across several species and functional groups. Previous studies comparing within- vs. among-species growth responses have focused primarily on functional trait values along environmental gradients under low or no stress, and in these studies, among-species variation has typically been high

relative to within-species variation (Albert *et al.*, 2010; Kichenin *et al.*, 2013). Only a few other studies have explored variation in stress responses (e.g., for frost stress, Annicchiarico & Iannucci, 2007; and for drought stress, Poirier *et al.*, 2012), and while they were in agreement with our findings, none of these studies included variation across functional groups to represent among-species variation. Therefore, our study is the first to demonstrate that, at least within common grasses, within-species variation at the continental scale results in ecotypes that react to climate extremes as differently as widely distributed common species (Figure S2) from a common origin. This strong influence of within-species variation has immediate theoretical implications within the scope of the insurance hypothesis and practical implications for species distribution modeling and the conservation of biodiversity.

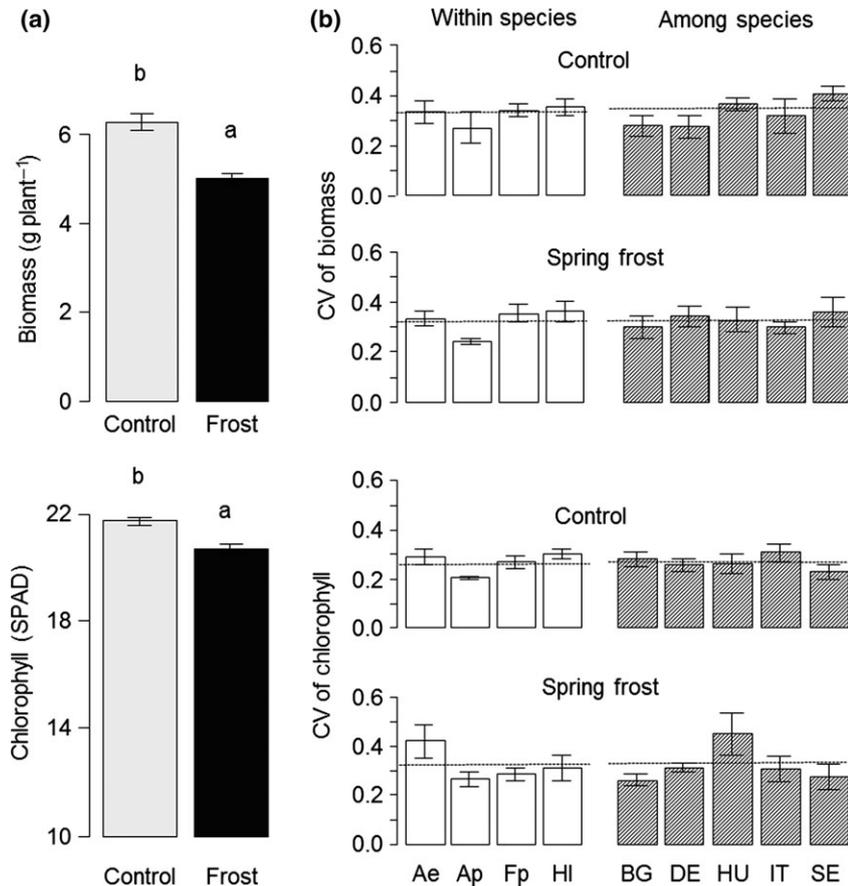


Fig. 3 Spring frost experiment. (a): Overall treatment effects on biomass and chlorophyll content using pooled data from all species and ecotypes of the four grass species (see Table 2). Interactions between countries of origin/ecotypes, species, and treatments are presented in Appendix S2b. (b): Mean coefficients of variation for each species (4–5 ecotypes per species) represent within-species variation (Ae – *Arrhenatherum elatius*, Ap – *Alopecurus pratensis*, Fp – *Festuca pratensis*, HI – *Holcus lanatus*), while mean coefficients of variation for each origin (3–4 species stemming from each country) represent among-species variation (BG – Bulgaria, DE – Germany, HU – Hungary, IT – Italy, SE – Sweden) for each treatment. Error bars denote standard errors. Different letters indicate significant treatment differences in (a). Dashed lines indicate mean within- and among-species CVs in (b).

Implications for the insurance hypothesis

Biodiversity encompasses more than species richness, and our results imply that diversity within species may be as important in insuring ecosystem integrity in times of increasing climatic perturbation as species richness. The mechanism behind within-species diversity (presence of diverse ecotypes) enhancing ecosystem integrity is likely to be higher genetic diversity. This may occur via an increase in population fitness, which in turn reduces the risk of species extinction. Higher genetic diversity has been shown to increase the fitness of individual populations by complementary resource use and niche differentiation (Reusch *et al.*, 2005), and via the creation of a stress-resilient portfolio effect, analogous to species-rich communities (Schindler *et al.*, 2010). Genetic differences were found within the *A. elatius* ecotypes

used in the winter warming plus frost experiment in an earlier study (Michalski *et al.*, 2010); however, a definite link between genetic differences among ecotypes and differences in responses was not established in the current study. Phenotypic plasticity is the capacity of a single genotype to produce different phenotypes in response to varying environmental conditions (Whitman & Agrawal, 2009), and it can also be responsible for among- and within-species variation. It was not explicitly quantified in this paper, but it is useful for projecting the ability of ecotypes and species to rapidly adapt to climate change. Most responses to climate change result from phenotypic plasticity and not new adaptations, according to one meta-analysis (Gienapp *et al.*, 2008). Adaptive plasticity can improve survival with environmental change (Chevin & Lande, 2010), thereby compensating for a lack of species and ecotypic

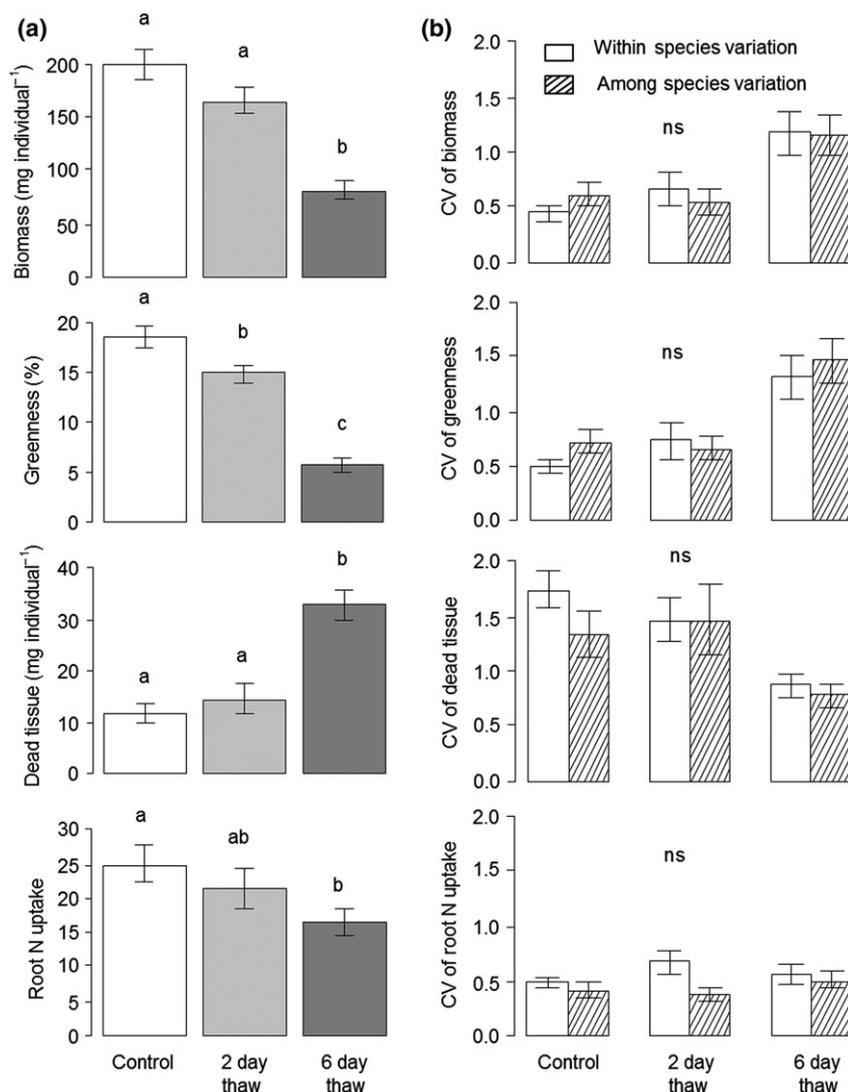


Fig. 4 Winter warming plus frost experiment. (a): Overall treatment effects on healthy and dead tissue biomass, greenness, and root ¹⁵N uptake following a 12-h thaw at 4 °C (Control), a 2-day thaw at 9 °C (2 day thaw treatment), or a 6 day at 9 °C (6 day thaw treatment) using pooled data from all species and ecotypes (see Table 2). Interactions between ecotypes, species, and treatments are presented in Appendix S2c. (b): Mean coefficients of variation for ecotypes vs. species (within- vs. among-species variation) for each parameter in each treatment (11 ecotypes vs. 8 species). Error bars denote standard errors. Different letters indicate significant treatment differences in (a).

diversity, facilitating survival in the context of climate change. High genetic variation within a species is most likely for ecotypes exhibiting high spatial separation, and therefore, the dispersal of ecotypes within a species range must also be considered. As mentioned previously, assisted migration of ecotypes may be required to significantly increase the stability of an ecosystem (Kreyling *et al.*, 2011). One can either increase the genetic diversity to enhance overall stress tolerance, or if one can accurately forecast the anticipated stress type and magnitude, predictive provenancing may help in selecting provenances

which vary less (with lower stress damage compared to present growth values). For a more detailed discussion of this topic, we suggest considering the debate on costs and limits of phenotypic plasticity (e.g., Murren *et al.*, 2015). The existing potential for maladapted hybrids and outbreeding depression, which can arise after genotypic mixing, can be overcome by following existing guidelines on the population selection process (Frankham *et al.*, 2011; Whitlock *et al.*, 2013). Furthermore, the chance of maladapted hybrids will likely be small in comparison with the negative consequences accompanying

Table 3 Relative effect size (% difference) for within- vs. among-species coefficients for each treatment in each experiment. Within-species variation in each species (3–4 ecotypes, see Table 2) is compared with among-species variation in each origin (3–4 species, see Table 2) for the drought experiment and the spring frost experiment. For each within- vs. among-species comparison, the local ecotype of each species was used as part of the among-species variation. CV = Coefficient of variation. Positive values (% difference) indicate that ecotypic variation is higher than among-species variation, negative values the reverse situation; NA: analysis not done due to unavailable local ecotypes for these species. In (a), treatments were pooled due to the nonsignificant/low effect size of warming on all plants in comparison with the drought effect. Control and warming treatments were pooled as control (C & W); drought and drought plus warming treatments were pooled as drought (D & DW). In 'C', additional species from different plant functional groups were added to among-species variation and additional ecotypes were added to within-species variation (see Table 2). Significant values (adjusted $P < 0.01$; according to Bonferroni correction, 4 or 5 multiple comparisons for each response parameter per species per treatment, therefore adjusted P is $< 0.05/4 = 0.013$ or $0.05/5 = 0.01$) are marked in bold

Parameter	Within species	Among species					
		Bulgaria ($n = 4$)	Germany ($n = 4$)	Hungary ($n = 4$)	Italy ($n = 3$)	Sweden ($n = 3$)	
(a) Drought experiment	CV of biomass	Control (C & W)					
		<i>Arrhenatherum elatius</i> ($n = 4$)	-22	-17	-15	18	-26
		<i>Alopecurus pratensis</i> ($n = 3$)	3	11	14	NA	7
		<i>Festuca pratensis</i> ($n = 4$)	-3	1	4	37	-1
	CV of C/N ratio	<i>Holcus lanatus</i> ($n = 3$)	-24	-21	-13	19	NA
		<i>Arrhenatherum elatius</i> ($n = 4$)	44	33	23	-7	22
		<i>Alopecurus pratensis</i> ($n = 3$)	9	-12	-31	NA	-40
		<i>Festuca pratensis</i> ($n = 4$)	14	-13	-18	-11	-44
	CV of biomass	<i>Holcus lanatus</i> ($n = 3$)	0.4	-13	-12	-34	NA
		Drought (D & DW)					
		<i>Arrhenatherum elatius</i> ($n = 4$)	-19	7	-10	13	-37
		<i>Alopecurus pratensis</i> ($n = 3$)	-16	-3	-19	NA	-53
	CV of C/N ratio	<i>Festuca pratensis</i> ($n = 4$)	-2	19	4	18	-14
		<i>Holcus lanatus</i> ($n = 3$)	2	30	11	27	NA
		<i>Arrhenatherum elatius</i> ($n = 4$)	-12	32	23	-6	36
		<i>Alopecurus pratensis</i> ($n = 3$)	-18	12	-9	NA	19
(b) Spring frost experiment	CV of biomass	<i>Festuca pratensis</i> ($n = 4$)	-56	-19	-43	-50	-12
		<i>Holcus lanatus</i> ($n = 3$)	-10	28	14	-7	NA
		Control					
		<i>Arrhenatherum elatius</i> ($n = 4$)	19	18	-16	13	-33
	CV of chlorophyll	<i>Alopecurus pratensis</i> ($n = 3$)	8	10	-46	NA	-83
		<i>Festuca pratensis</i> ($n = 4$)	16	23	-13	8	-21
		<i>Holcus lanatus</i> ($n = 3$)	25	20	0.1	1	NA
		<i>Arrhenatherum elatius</i> ($n = 4$)	-2	14	16	-14	21
	CV of biomass	<i>Alopecurus pratensis</i> ($n = 3$)	-37	-25	-25	NA	-17
		<i>Festuca pratensis</i> ($n = 4$)	-9	-1	-1	-12	21
		<i>Holcus lanatus</i> ($n = 3$)	8	19	9	-2	NA
		Spring frost					
	CV of chlorophyll	<i>Arrhenatherum elatius</i> ($n = 4$)	15	-7	-0.1	15	-13
		<i>Alopecurus pratensis</i> ($n = 3$)	-18	-49	-37	NA	-51
		<i>Festuca pratensis</i> ($n = 4$)	19	8	1	18	-8
		<i>Holcus lanatus</i> ($n = 3$)	13	-2	18	20	NA
CV of biomass	<i>Arrhenatherum elatius</i> ($n = 4$)	41	29	-26	29	36	
	<i>Alopecurus pratensis</i> ($n = 3$)	0	-21	-81	NA	5	
	<i>Festuca pratensis</i> ($n = 4$)	9	-16	-61	1	6	
	<i>Holcus lanatus</i> ($n = 3$)	24	2	-70	2	NA	
(c) Winter warming plus frost	Control		Germany ($n = 7$)				
	CV of biomass	<i>Arrhenatherum elatius</i> ($n = 10$)	NA	-43	NA	NA	NA
	CV of greenness	<i>Arrhenatherum elatius</i> ($n = 10$)	NA	23	NA	NA	NA
	CV of dead tissue	<i>Arrhenatherum elatius</i> ($n = 10$)	NA	-46	NA	NA	NA

Table 3 (continued)

Parameter	Within species	Among species				
		Bulgaria (n = 4)	Germany (n = 4)	Hungary (n = 4)	Italy (n = 3)	Sweden (n = 3)
CV of root ¹⁵ N	<i>Arrhenatherum elatius</i> (n = 10)	NA	16	NA	NA	NA
	2 day thaw					
CV of biomass	<i>Arrhenatherum elatius</i> (n = 10)	NA	19	NA	NA	NA
CV of greenness	<i>Arrhenatherum elatius</i> (n = 10)	NA	-1	NA	NA	NA
CV of dead tissue	<i>Arrhenatherum elatius</i> (n = 10)	NA	10	NA	NA	NA
CV of root ¹⁵ N	<i>Arrhenatherum elatius</i> (n = 10)	NA	43	NA	NA	NA
	6 day thaw					
CV of biomass	<i>Arrhenatherum elatius</i> (n = 10)	NA	1	NA	NA	NA
CV of greenness	<i>Arrhenatherum elatius</i> (n = 10)	NA	9	NA	NA	NA
CV of dead tissue	<i>Arrhenatherum elatius</i> (n = 10)	NA	-12	NA	NA	NA
CV of root ¹⁵ N	<i>Arrhenatherum elatius</i> (n = 10)	NA	10	NA	NA	NA

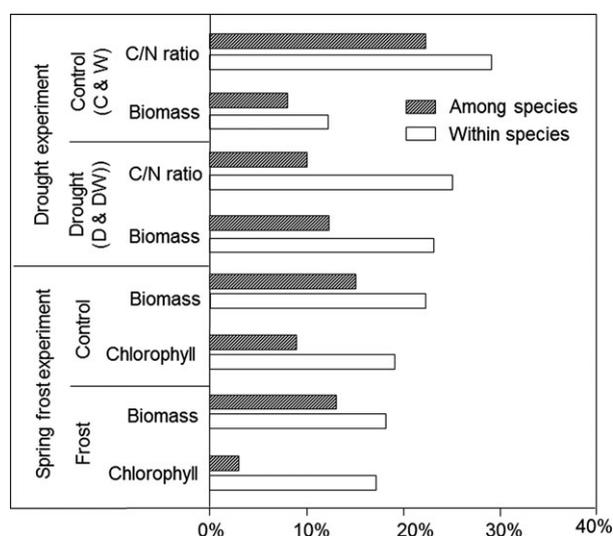


Fig. 5 Variation partitioning of drought as well as spring frost experiments, with respect to each measured parameter and treatment. Both species and ecotypes (their origin) are factors, with ecotypes nested in species (one species has several ecotypes). Within-species differences explained an additional mean of 9% of total variation, after accounting for among-species differences. No additional variation could be explained by species after accounting for the within-species factor as ecotypes are nested within species.

species translocations, such as the threat of invasions (Aitken & Whitlock, 2013).

Local adaptation

All grass ecotypes had stemmed from multiple mother plants and were grown in a common environment before being exposed to the different stresses.

Therefore, a genetic component to the observed phenotypic variation is likely. The genetic differences among ecotypes were probably due to local adaptation, as shown by the correlations between the climate parameters at the ecotype origins and their treatment responses (Appendix S3). Due to the presence of only four or five ecotypes for two of the three experiments, such correlations are difficult to detect. In the winter warming plus frost experiment, stronger correlations were present, although even here it would be more logical to correlate the ecotype responses with the frequency of frosts which occur after warm spells, and those data are not available. As tools to quantify local adaptation, we propose (i) establishment of multiple common gardens along latitudinal/altitudinal gradients, (ii) *in situ* additional climate manipulations, and (iii) manipulations in climate chambers. Detailed methods on these procedures in the context of frost adaptation can be found in Malyshev *et al.* (2014). Overall, the speed of evolution of local adaptation should always be evaluated to the same degree as species-specific adaptations for theoretical considerations in ecology, such as coexistence theories.

Implications for predicting changes in species distributions

Our result of within-species variation in drought and frost tolerance being as high as among-species variation in different functional groups emphasizes the importance of incorporating within-species variation into projections of climate change responses (Valladares *et al.*, 2014). However, the speed at which ecotypes that are ill adapted for future climate might be replaced by better-adapted ecotypes is an important, yet hardly known,

piece of information required for sound projections of species' responses to climate change. Decision-makers responsible for plant transplantations (e.g., foresters, urban landscape planners) and ecotypic seed mixing (e.g. farmers) thus need to acquire the necessary information on ecotype performance to make informed decisions.

Micro-evolutionary adaptation to drought can occur within short geographic distances in forest tree species, and such adaptations can easily spread via gene flow (Pluess & Weber, 2012). Alternatively, the assisted colonization of pre-adapted ecotypes of key species within their current range may contribute to the functional integrity of ecosystems, without the need to introduce exotic species with unknown risks (Kreyling *et al.*, 2011). The level of ecotypic variation and ability to evolve new ecotypes within a species are therefore important characteristics to consider when evaluating range shifts of species driven by environmental stressors. Local adaptation has been detected in only 71% of transplant studies (Leimu & Fischer, 2008; Hereford, 2009), which could be explained by species-specific differences in the extent of within-species variation under stress. Therefore, our results highlight the importance of identifying factors and species traits responsible for evolving new ecotypes, both of which might play a crucial role in determining the most vulnerable species under climate change.

Ecological implications of drought and frost responses

Drought, spring frost damage, and winter warm spells are likely to increase in the future (IPCC, 2012). Drought duration and spring frost magnitude in our experiments were selected based on local climate patterns and projections (see Beierkuhnlein *et al.*, 2011 and Kreyling *et al.*, 2012) and therefore represent realistic scenarios. Likewise, our winter warming plus frost simulation resembles natural winter warming events lasting 5 days or more with temperatures reaching over 5 °C, which have occurred approximately once every seven years between 1913 and 2000, even at locations much colder than our sampled sites (Bokhorst *et al.*, 2008). The latter implies that warm spells of this magnitude and duration are also likely to occur at lower latitudes as well. Field experiments have shown comparable growth reduction after extreme winter warming, as observed in our study, with week-long temperatures in winter of around 7 °C reducing summer growth by 87% in dwarf shrubs (Bokhorst *et al.*, 2009). In this respect, the testing of frost responses after winter warm spells lasting two and six days at 9 °C was realistic in the context of winter climate change.

Considerations regarding interpretation of results

We compared among- and within-species variation only in species from extensively used grasslands which are widespread across Europe, species rich (commonly >20 species per m²), characterized by low management intensity (not plowed, not sown, unfertilized or subject to low, mainly organic, fertilization), and used as meadows for hay or silage or as pastures (Beierkuhnlein *et al.*, 2011). Still, these grasslands depend on human use (if missing, succession toward forests takes place in a few decades). This specific disturbance regime might have selected for rather similar species. Phenology has been shown as a key predictive factor in explaining tree species distributions (Chuine & Beaubien, 2001; Morin *et al.*, 2007). Among- and within-species differences in phenology with respect to temperature changes were not tested here and are suggested to be compared in future studies. Nonetheless, for a defined set of growth response parameters, we have shown that for each of the four common grassland species, within-species variation was not lower than among-species variation. One promising future research direction is thus to quantify the effect of within-species variability for different plant functional groups.

Species interactions can influence the evolutionary paths of different plant traits (Tilman & Snell-Rood, 2014). Greater trait differentiation develops in species grown in more diverse communities, which promotes stronger selection pressure compared with species grown in isolation (Zuppinge-Dingley *et al.*, 2014) or under anthropogenic influence such as fertilization (Hautier *et al.*, 2014). In this respect, species mixtures may still be more influential in leading to trait diversity of a community compared with ecotype mixtures. Experiments studying evolutionary processes in different ecotype and species mixtures are therefore currently of high importance. Overall, we suggest five research questions to spur further research to explain and make use of our finding of potentially equal variation in growth within- and among-species after stress: (i) What is the speed of evolution of local adaptations and plant ecotypes? (ii) What happens, with respect to performance and response variability, if geographically isolated ecotypes are mixed by humans (Fig. 5)? (iii) How does within-species variability in stress responses vary among species and what drives this variability (species, functional groups, generation length, range size etc.)? (iv) Are certain factors more influential in the development of within-species variability (adaptation to climate, environmental opportunity, space, genetic isolation by geographic isolation)? (v) Are certain environmental thresholds (e.g., minimum temperature) harder to

cross by within-species variability in stress tolerance than among species?

In summary, our study explored the relative importance of within- vs. among-species variation in response to multiple stress factors and disturbance interactions – drought, warming, frost, and their combinations. In addition, we explored within-species variation in four grass species and among-species variation in multiple functional groups and quantified several response traits. Taken together, we present general evidence that response variation within single species across their ranges can match the response variation encompassing different plant functional groups at single sites under stress. This contrasts previous reports, conducted under nonstressful conditions, where among-species trait variation dominates. Within-species variation should therefore be included in the refinement and testing of general ecological theories and ecological applications such as species distribution modeling and biodiversity conservation.

Acknowledgements

This study was funded by the German Research Foundation (DFG KR 3309/6-1 and DFG KR 3309/7-1), by the 'Bavarian Climate Programme 2020' in the joint research center 'FORCAST'. We thank Daniel Thiel, Luis Pérez Fernández, Christian Schemm, Elke König, Stefan König and Christine Pils for setting up and maintaining the experimental facilities. We also thank the reviewers for making a major contribution in improving the manuscript.

References

- Abeli T, Jäkäläniemi A, Gentili R (2014) Living with extremes: the dark side of global climate change. *Plant Ecology*, **215**, 673–675.
- Aitken SN, Whitlock MC (2013) Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 367–388.
- Albert CH, Thuiller W, Yoccoz NG, Douzet R, Aubert S, Lavorel S (2010) A multi-trait approach reveals the structure and the relative importance of intra- vs. interspecific variability in plant traits. *Functional Ecology*, **24**, 1192–1201.
- An YA, Wan SQ, Zhou XH, Subedar AA, Wallace LL, Luo YQ (2005) Plant nitrogen concentration, use efficiency, and contents in a tallgrass prairie ecosystem under experimental warming. *Global Change Biology*, **11**, 1733–1744.
- Annicchiarico P, Iannucci A (2007) Winter survival of pea, faba bean and white lupin cultivars in contrasting Italian locations and sowing times, and implications for selection. *The Journal of Agricultural Science*, **145**, 611–622.
- Atkins KE, Travis MJM (2010) Local adaptation and the evolution of species' ranges under climate change. *Journal of Theoretical Biology*, **266**, 449–457.
- Augsburger CK (2013) Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology*, **94**, 41–50.
- Beierkuhnlein C, Thiel D, Jentsch A, Willner E, Kreyling J (2011) Ecotypes of European grass species respond specifically to warming and extreme drought. *Journal of Ecology*, **99**, 703–713.
- Bokhorst S, Bjerke JW, Bowles FW, Melillo J, Callaghan TV, Phoenix GK (2008) Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. *Global Change Biology*, **14**, 2603–2612.
- Bokhorst SF, Bjerke JW, Tømmervik H, Callaghan TV, Phoenix GK (2009) Winter warming events damage sub-Arctic vegetation: consistent evidence from an experimental manipulation and a natural event. *Journal of Ecology*, **97**, 1408–1415.
- Booth RE, Grime JP (2003) Effects of genetic impoverishment on plant community diversity. *Journal of Ecology*, **91**, 721–730.
- Breed MF, Stead MG, Ottewill KM, Gardner MG, Lowe AJ (2012) Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. *Conservation Genetics*, **14**, 1–10.
- Chevin L, Lande R (2010) When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evolution*, **64**, 1143–1150.
- Chuine I, Beaubien EG (2001) Phenology is a major determinant of tree species range. *Ecology Letters*, **4**, 500–510.
- Epstein E, Schmid WE, Rains DW (1963) Significance and technique of short-term experiments on solute absorption by plant tissue. *Plant and Cell Physiology*, **4**, 79–84.
- Faraway JJ (2005) *Linear Models with R*. Chapman & Hall/CRC, Boca Raton.
- Fitzpatrick SW, Gerberich JC, Kronenberger JA, Angeloni LM, Funk WC (2015) Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, **18**, 37–47.
- Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. *Conservation Biology*, **25**, 465–475.
- Franks SJ, Weber JJ, Aitken SN (2014) Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications*, **7**, 123–139.
- Garamszegi LZ, Moller AP (2010) Effects of sample size and intraspecific variation in phylogenetic comparative studies: a meta-analytic review. *Biological Reviews*, **85**, 797–805.
- Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J. (2008) Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology*, **17**, 167–178.
- Grime JP, Thompson K, Hunt R *et al.* (1997) Integrated screening validates primary axes of specialisation in plants. *Oikos*, **79**, 259–281.
- Hautier Y, Seabloom E, Borer ET *et al.* (2014) Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, **508**, 521–525.
- Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist*, **173**, 579–588.
- Hettwer G, Eduardo L, Jarenkow JA (2012) Niche conservatism and the differences in species richness at the transition of tropical and subtropical climates in South America. *Ecography*, **35**, 933–943.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.
- Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution*, **18**, 147–155.
- Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.
- IPCC (2012) Summary for policymakers. In: *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change* (eds Field CB *et al.*), pp. 1–19. Cambridge Univ. Press, Cambridge, UK.
- IPCC (2013) Climate change 2013: the physical science basis. In: *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM), pp. 1–1535. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jentsch A, Beierkuhnlein C (2010) Simulating the future – responses of ecosystems, key species and European provenances to expected climatic trends and events. In special issue: continents under climate change. *Nova Acta Leopoldina*, **112**, 89–98.
- Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate change experiments: events, not trends. *Frontiers in Ecology and the Environment*, **5**, 365–374.
- Jentsch A, Kreyling J, Elmer M *et al.* (2011) Climate extremes initiate ecosystem-regulating functions while maintaining productivity. *Journal of Ecology*, **99**, 689–702.
- Joseph G, Henry HA (2008) Soil nitrogen leaching losses in response to freeze-thaw cycles and pulsed warming in a temperate old field. *Soil Biology and Biochemistry*, **40**, 1947–1953.
- Jung V, Violle C, Mondy C, Hoffmann L, Muller S (2010) Intraspecific variability and trait-based community assembly. *Journal of Ecology*, **98**, 1134–1140.
- Kaliberer SR, Wisniewski M, Arora R (2006) Deacclimation and reacclimation of cold-hardy plants: current understanding and emerging concepts. *Plant Science*, **171**, 3–16.
- Kichenin E, Wardle DA, Peltzer DA, Morse CW, Freschet GT (2013) Contrasting effects of plant inter- and intraspecific variation on community-level trait measures along an environmental gradient. *Functional Ecology*, **27**, 1254–1261.
- Kodra E, Steinhilber K, Ganguly AR (2011) Persisting cold extremes under 21st-century warming scenarios. *Geophysical Research Letters*, **38**, L08705.

- Kreyling J, Bittner T, Jaeschke A, Jentsch A, Steinbauer MJ, Thiel D, Beierkuhnlein C (2011) Assisted colonization: a question of focal units and recipient localities. *Restoration Ecology*, **19**, 433–440.
- Kreyling J, Thiel D, Simmnacher K, Willner E, Jentsch A, Beierkuhnlein C (2012) Geographic origin and past climatic experience influence the response to late spring frost in four common grass species in central Europe. *Ecography*, **35**, 268–275.
- Legendre P (2008) Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *Journal of Plant Ecology*, **1**, 3–8.
- Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PLoS ONE*, **3**, e4010.
- Malyshev AV, Henry HAL, Kreyling J (2014) Common garden experiments to characterize cold acclimation responses in plants from different climatic regions. In: *Plant Cold Acclimation*, (eds DK Hincha, E Zuther), pp. 65–78. Springer, New York.
- Marchand FL, Nijs I, Heuer M *et al.* (2004) Climate warming postpones senescence in High Arctic tundra. *Arctic Antarctic and Alpine Research*, **36**, 390–394.
- Meusel H, Bräutigam S (1992) Vergleichende Chorologie der zentraleuropäischen Flora Band 3: Karten, Literatur, Register. Fischer, Jena, 333 pp.
- Michalski SG, Durka W, Jentsch A *et al.* (2010) Evidence for genetic differentiation and divergent selection in an autotetraploid forage grass (*Arrhenatherum elatius*). *Theoretical and Applied Genetics*, **120**, 1151–1162.
- Morin X, Augspurger C, Chuine I (2007) Process-based modeling of species' distributions: what limits temperate tree species' range boundaries? *Ecology*, **88**, 2280–2291.
- Murren CJ, Auld JR, Callahan H *et al.* (2015) Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Hereditas*, **115**, 293–301.
- Naem S, Li SB (1997) Biodiversity enhances ecosystem reliability. *Nature*, **390**, 507–509.
- Ogura T, Busch W (2015) From phenotypes to causal sequences: using genome wide association studies to dissect the sequence basis for variation of plant development. *Current opinion in Plant Biology*, **23**, 98–108.
- Oney B, Reineking B, O'Neill G, Kreyling J (2013) Intraspecific variation buffers projected climate change impacts on *Pinus contorta*. *Ecology and Evolution*, **3**, 437–449.
- Pluess AR, Weber P (2012) Drought-Adaptation Potential in *Fagus sylvatica*: Linking Moisture Availability with Genetic Diversity and Dendrochronology. *PLoS ONE*, **7**, e33636.
- Poirier M, Durand J, Volaire F (2012) Persistence and production of perennial grasses under water deficits and extreme temperatures: importance of intraspecific vs. interspecific variability. *Global Change Biology*, **18**, 3632–3646.
- Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous-equations for assaying chlorophyll-a and chlorophyll-b extracted with 4 different solvents – verification of the concentration of chlorophyll standards by atomic-absorption spectroscopy. *Biochimica et Biophysica Acta*, **975**, 384–394.
- R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/>. (accessed 2 February 2014)
- Reusch TBH, Ehlers A, Ha A (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 2826–2831.
- Sakai A, Weiser CJ (1973) Freezing resistance of trees in North America with reference to tree regions. *Ecology*, **54**, 118–126.
- Sardans J, Peñuelas J, Estiarte M, Prieto P (2008) Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. *Global Change Biology*, **14**, 2304–2316.
- Schär C, Vidale PL, Luthi D, Frei C, Haberli C, Liniger MA, Appenzeller C (2004) The role of increasing temperature variability in European summer heatwaves. *Nature*, **427**, 332–336.
- Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. *Nature*, **465**, 609–612.
- Smith MD (2011) An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology*, **99**, 656–663.
- Thomas CD, Cameron A, Green RE *et al.* (2004) Extinction risk from climate change. *Nature*, **427**, 145–148.
- Thuiller W, Lavorel S, Araujo MB, Sykes MT, Prentice IC (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 8245–8250.
- Tilman D, Snell-Rood EC (2014) Ecology: diversity breeds complementarity. *Nature*, **515**, 44–45.
- Turrill WB (1946) The ecotype concept. *New Phytologist*, **4**, 34–43.
- Valladares F, Sanchez-Gomez D, Zavala MA (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*, **94**, 1103–1116.
- Valladares F, Matesanz S, Guilhaumon F *et al.* (2014) The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, **17**, 1351–1364.
- Walker MD, Ingersoll RC, Webber PJ (1995) Effects of interannual climate variation on phenology and growth of 2 alpine forbs. *Ecology*, **76**, 1067–1083.
- Walker B, Kinzig A, Langridge J (1999) Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. *Ecosystems*, **2**, 95–113.
- Whitlock R, Grime JP, Booth RE, Burke T (2007) The role of genotypic diversity in determining grassland community structure under constant environmental conditions. *Journal of Ecology*, **95**, 895–907.
- Whitlock R, Stewart GB, Goodman SJ, Piortney SB, Butlin RK, Pullin AS, Burke T (2013) A systematic review of phenotypic responses to between-population outbreeding. *Environmental Evidence*, **2**, 13.
- Whitman DW, Agrawal AA (2009) What is phenotypic plasticity and why is it important? In: *Phenotypic Plasticity of Insects: Mechanisms and Consequences* (eds Whitman DW, Ananthakrishnan TN), pp. 1–63. Scientific Publishers, Enfield.
- Wilder, S. M.
- Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 1463–1468.
- Zuppinge-Dingley D, Schmid B, Petermann JS, Yadav V, Deyn D, Gerlinde B, Flynn DFB (2014) Selection for niche differentiation in plant communities increases biodiversity effects. *Nature*, **515**, 108–111.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Key recent studies comparing among vs. within-species variation in the same paper, showing the dominant variation source in each study.

Appendix S2. Species and ecotype-specific treatment effects on each measured parameter and their interactions for drought and warming experiment (a) for spring frost experiment (b) and for winter warming plus frost experiment (c).

Appendix S3. Linear correlations between climate of origin and the mean relative responses of each ecotype for every species.

Figure S1. Sequence of the three experiments and their respective constituents, making up among- and within-species variation.

Figure S2. Natural distribution ranges of all species used in the experiments.

Figure S3. Drought and warming experiment.