Beyond productivity: Effects of extreme weather events on ecosystem processes and biotic interactions

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1. Short summary of the thesis/ Kurze Zusammenfassung der Doktorarbeit

Under global climate change, extreme weather events, such as heat waves, drought or heavy rain spells, are projected to increase in magnitude and frequency. As these may affect vegetation and ecosystems more than gradual shifts in mean climatic parameters, investigating the consequences of extreme weather events recently became an important issue in climate change research. The main focus of most experiments investigating effects of extreme weather events on vegetation is on primary productivity. In our experiment in artificially planted communities, even an extreme drought of 1000-year recurrence did not have effects on above- or below-ground biomass production from 2005-2010 (manuscript 1).

Thus, the main objectives of this thesis were (1) to investigate if extreme weather events have an effect on ecosystem functions[1] beyond productivity, (2) to test if such a high resistance or resilience[2] in response to drought regarding productivity also exists in more naturally grown plant communities and (3) to further elucidate possible mechanisms of the surprisingly large stability of the plant communities.

To investigate these objectives, several experimental studies were conducted in artificially planted, as well as in naturally grown grassland communities and consequences of extreme weather events for ecosystem processes, such as decomposition and herbivory were investigated. In a pot experiment, it was studied, if grass plants react improved towards repeated drought when compared to a first drought and thus reveal a kind of drought memory. Such a memory might be one possible, but up until now widely neglected mechanism of resilience.

Even though biomass production remained stable in our experiment in artificially planted communities (manuscript 1), biomass quality was severely affected by extreme drought, thereby strongly affecting the development of a herbivore caterpillar feeding on drought-exposed leaves (manuscript 2). Further, plant compounds of the host plant depended on the composition of the plant community it was grown in. This in turn resulted in strong effects on the larval mortality of herbivores feeding on such plants.

In contrast to the study in artificially planted communities (manuscript 1), aboveground net primary productivity (ANPP) was reduced in naturally composed grassland in response to extreme rainfall variability, including an extreme drought followed by heavy

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[1] Ecosystem functions: Processes that involve more than one ecosystem or trophic level and are important for the maintenance of the whole ecosystem (e.g. decomposition, which is important for nutrient turnover, or providing food of good quality to sustain food webs)

[2] Resilience is understood here as the time required to return to a steady-state following disturbance (Holling (1973); Gunderson (2000))

rainfall (manuscript 6). Forage quality was altered by drought. Furthermore, mowing frequency strongly altered forage quality and biomass production, but did not interact with rainfall variability and thus did neither buffer, nor amplify effects of extreme rainfall variability. Despite effects of rainfall variability on ANPP, grassland showed high resilience after drought followed by heavy rain, as effects were large shortly after the extreme event, but did not persist until a second harvest later in the year.

In natural grassland, rainfall variability and drought also affected ecosystem processes, here litter decomposition, beyond productivity (manuscript 7). Drought followed by heavy rain pulses decreased decomposition rates. Decomposition in more frequently mown meadows was more vulnerable towards drought exposure. Winter warming and additional winter rain had no long-term effect on decomposition. To conclude, projected increases in drought frequency under climate change may inhibit decomposition and alter nutrient and carbon cycling along with soil quality in temperate grassland, whereas a reduction of snow cover leading to more variable soil surface temperatures may counteract increased decomposition under winter warming.

In this thesis, an ecological stress memory as one possible mechanism of resilience is defined as any response of a single plant after a stress experience that improves the reaction of the plant towards future stress experience and which is assessed on a whole plant level (manuscript 3). This thesis further provides evidence of a drought memory in grass plants (manuscript 4): Plants repeatedly subjected to drought showed improved photo-protection and a higher rate of living biomass when compared to plants faced with their first drought. Similarly, tree seedlings exposed to drought in summer revealed higher frost resistance during winter, providing evidence of a long-lasting “cross-stress-memory” (manuscript 5).

To sum up, the thesis shows that extreme weather events, even though neither severely affecting biomass production in artificially composed, nor in naturally growing communities in the long-term, exert strong influence on physiological or biogeochemical parameters, such as plant compounds or soil biotic activity. These changes in turn modify ecosystem functions beyond productivity, for example herbivory or decomposition, possibly altering biotic interations and nutrient cycling. Furthermore, the findings imply that plants exhibit a stress memory after stress exposure, which may be one mechanisms leading to a high stability and resilience upon frequent stress.

Kurze Zusammenfassung der Doktorarbeit

Im Zuge des globalen Klimawandels werden extreme Wetterereignisse, wie Hitzewellen, Dürren oder Starkregenereignisse sehr wahrscheinlich häufiger und auch intensiver werden. Da diese Vegetation und Ökosysteme stärker beeinflussen können als graduelle Änderungen in klimatischen Durchschnittsparametern, ist die Untersuchung der Konsequenzen extremer Wetterereignisse in letzter Zeit verstärkt in den Fokus der Klimawandelforschung getreten. Das Hauptaugenmerk der meisten Experimente, die Folgen extremer Wetterereignisse für die Vegetation untersuchen, liegt auf der Primärproduktivität. Innerhalb unseres Experiments in künstlich zusammengesetzten Gemeinschaften wurde die ober- und unterirdische Biomasseproduktion durch eine extreme Dürre nicht beeinflusst (Manuskript 1).

Daher sind die Ziele dieser Arbeit, zu untersuchen, (1) ob extreme Wetterereignisse einen Effekt auf Ökosystemfunktionen[1], außer der reinen quantitativen Produktion von Biomasse haben, (2) ob die Ergebnisse der hohen Stabilität in den künstlich zusammengesetzten Artengemeinschaften auch für die natürlich gewachsenen Gründlandbeständen gelten und (3) mögliche Mechanismen der erstaunlichen Stabilität der Pflanzengemeinschaften näher zu beleuchten.

Dafür wurden mehrere Experimente in künstlichen und natürlichen Pflanzengemeinschaften durchgeführt, in denen Folgen extremer Wetterereignisse für Ökosystemprozesse, wie z. B. Streuabbau oder Herbivorie, untersucht wurden. In einem Topfexperiment wurde außerdem untersucht, ob Graspflanzen besser mit einer wiederholten Dürre im Vergleich zu einer ersten Dürre umgehen können, und damit eine Art Dürregedächtnis aufweisen. Ein solches Gedächtnis könnte ein möglicher, aber bisher wenig erforschter Mechanismus von Resilienz[2] sein.

Obwohl die Biomasseproduktion künstlich zusammengesetzter Gemeinschaften stabil blieb (Manuskript 1), änderte sich die Biomassequalität stark durch extreme Dürre. Dadurch veränderte sich die Entwicklung einer phytophagen Raupe, wenn sie Blätter fraß, die einer Dürre ausgesetzt worden waren (Manuskript 2). Außerdem beeinflusste die Artenzusammensetzung der Gemeinschaft, in der die Futterpflanze wuchs, die Pflanzeninhaltsstoffe, was die Sterberate der Larven veränderte.

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[1] Prozesse, die mehr als eine Ökosystemebene betreffen und die für die Aufrechterhaltung des gesamten Systems notwendig sind

[2] Benötigte Zeit, um nach einer Störung wieder einen stabilen Zustand zu erreichen (Holling (1973); Gunderson (2000))

Im Gegensatz zur Studie in künstlich zusammengesetzten Gemeinschaften (Manuskript 1) wurde die oberirdische Nettoprimärproduktion (NPP) durch den Einfluss von extremer Niederschlagsvariabilität, also extremer Dürre gefolgt von starkem Regen, reduziert (Manuskript 6). Auch die Futterqualität wurde durch die Dürre modifiziert. Des Weiteren beeinflusste die Mahdfrequenz Futterqualität und Biomasseproduktion. Allerdings konnte die Mahdfrequenz die Effekte der extremen Niederschlagsvariabilität weder abpuffern, noch verstärken; es gab keine Interaktion zwischen den beiden Faktoren. Trotz der Effekte der Niederschlagsvariabilität auf die NPP zeigte sich wieder eine hohe Resilienz von Grünland nach Dürre und Starkregen, da die negativen Effekte direkt nach dem extremen Wetterereignis sehr stark waren, aber nicht bis zur zweiten Ernte Ende des Jahres anhielten.

Auch im natürlichen Grünland wurden Ökosystemprozesse, hier Streuabbau, neben der Produktivität beeinflusst: Dürre, gefolgt von Starkregen, verringerte Streuabbauraten. Streuabbau in öfter gemähten Wiesen wurde durch die Dürre stärker beeinträchtigt. Wintererwärmung und zusätzlich applizierter Winterniederschlag hatten keine langfristigen Effekte auf den Abbau. Zusammenfassend lässt sich sagen, dass die vorhergesagte Zunahme von Dürren den Streuabbau behindern und dadurch in Nährstoff- und Kohlenstoffkreislauf eingreifen könnte. Das Tauen der Schneedecke bei Wintererwärmung führte zu einer erhöhten Variabilität der Bodenoberflächentemperatur und könnte damit erhöhten Abbauraten durch Wintererwärmung entgegen wirken.

Die vorliegende Arbeit definiert ökologisches Stressgedächtnis als die Stressantwort einer Einzelpflanze, die die Reaktion dieser gegenüber wiederholtem Stress verbessert. Ein solches Stressgedächtnis könnte ein möglicher Mechanismus von Resilienz sein (Manuskript 3). Die Arbeit zeigt erste Hinweise auf ein Dürregedächtnis bei Grasspflanzen. Pflanzen, die wiederholter Dürre ausgesetzt waren wiesen einen besseren Schutz vor oxidativem Stress und dadurch mehr lebende Biomasse auf als Pflanzen, die das erste Mal einer Dürre ausgesetzt wurden. Auch waren Baumkeimlinge, die im Sommer eine Dürre erfuhren, im Winter frostresistenter, was auf ein „Cross-Stressgedächtnis“ hinweist (Manuskript 5).

So zeigt diese Arbeit, dass extreme Wetterereignisse, selbst wenn sie die Biomasseproduktion nicht stark oder langfristig beeinflussen, physiologische oder biogeochemische Parameter, wie z. B. Pflanzeninhaltsstoffe oder die Aktivität der Bodenfauna, verändern. Diese Änderungen modifizieren wiederum Ökosystemfunktionen, wie Herbivorie oder Streuabbau, wodurch möglicherweise langfristig in biotische Interaktionen oder Stoffkreisläufe eingegriffen wird. Weiterhin legt diese Arbeit nahe, dass

Pflanzen, nachdem sie Stress ausgesetzt waren, ein Stressgedächtnis entwickeln können, das zu erhöhter Stabilität und Resilienz unter häufigen Stressereignissen führt.

2. Background of the thesis

2.1. Climate change and extreme weather events

Instrumental temperature records show that a warming of the climate system over the last century is unequivocal (Hulme, 2005; Blenkinsop and Fowler, 2007; Trenberth et al., 2007). The global mean surface temperatures have risen by 0.74 ° C (±0.18 ° C) on average from 1905-2006 (Hulme, 2005; Blenkinsop and Fowler, 2007; Trenberth et al., 2007). Warming was most pronounced over land regions, especially over the northern hemisphere during winter and spring (Trenberth et al., 2007). In Germany temperatures have risen by 1° C from 1901-2000, with a more pronounced warming during winter (Schönwiese et al., 2005; Zebisch et al., 2012). Other temperature indices, such as the global sea level rise of around 17 cm in the last century, the reduction of snow cover in the northern hemisphere or the widespread glacier retreat are consistent with the record showing rising temperatures (Trenberth et al., 2007). Furthermore, global warming is accelerating quickly: the warming rate of 0.13° C per decade from 1955-2005 is almost double the warming rate of 0.07 ° C per decade for 1906-2005 (Beierkuhnlein and Foken, 2008; Trenberth et al., 2007). It is now widely acknowledged, that anthropogenic green house gas emissions account for the largest part of observed warming since preindustrial times and that the observed warming can not be explained by internal forcing or natural external radiative forcing only (Hegerl et al., 2007; Trenberth et al., 2007).

Future projections indicate a further warming of between 1.1° C and 6.4 ° C until 2100, depending on the emission scenario used in the model. Even if CO_2 emissions were held constant on the level of the year 2000 (which is already not fulfilled), temperatures continued to rise for at least the first third of the 21st century (Meehl et al., 2007).

Along with rising temperatures, other components of the climate system, for example precipitation, are observed and projected to change.

Modifications in the magnitude, as well as in the frequency and duration of extreme weather events are of increasing concern: Such changes may occur both through changes in the mean or in the variability of the distribution of a climate variable, causing disproportionally large changes in the frequency or intensity of weather extremes, compared with the changes in the mean (Meehl et al., 2000b; Nicholls and Alexander, 2007) (Fig. 1). Extreme weather events are more and more responsible for a large part of climate related damage to society and ecosystems (Field et al., 2012).

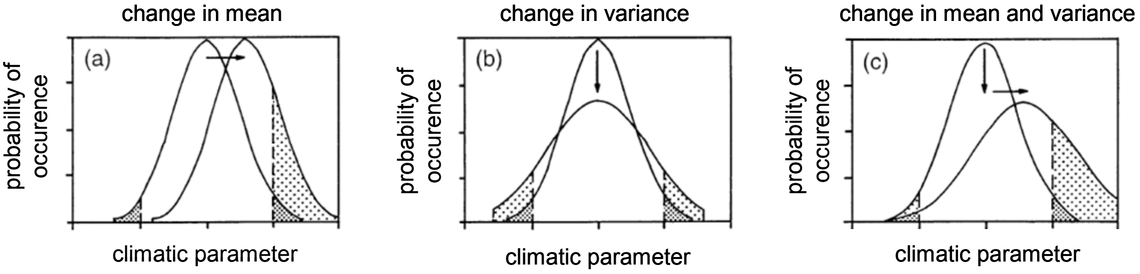

Fig.1 Schematic diagram depicting how changes in mean and variance can affect extreme weather events. Small changes in the mean of the distribution of a climate variable, indicated by the arrow, can lead to large changes in the frequency of extreme weather events (dashed areas) (a). When variance of a climatic variable enlarges, the frequency of extreme events at both ends of the distribution enlarges (b). Simultaneous changes in mean and variance of the frequency distribution results in the largest shifts in the frequency of extreme weather events (c) (modified from Meehl et al., 2000).

Changes in extremes are not as easy to assess as changes in the mean of a climate variable. Highly resolved long-term data sets are necessary to carry out extreme values statistics, and such data sets are lacking in many parts of the world (Easterling et al., 2000; Jentsch et al., 2007; Trenberth et al., 2007). As extreme weather events are infrequent per definition, enough instances in the climate record to estimate return intervals, frequency and intensity of such an event are often lacking (Tebaldi et al., 2006). Furthermore, extreme weather events are spatially quite variable, thus requiring high-resolution RCMs (regional climate models) for projections. The lack of a common definition of extreme weather event, partly due to its spatial and historical context-dependence, or of a common statistical approach to quantify weather extremes further complicates the issue (Smith, 2011a).

Nevertheless, substantial progress in analysing and predicting extreme weather events has been made in the last 20 years. In the first supplemental report (Houghton et al., 1992) and in the second assessment report (Houghton et al., 1995) of the IPCC (Intergovernmental Panel on Climate Change, founded in 1988), data were inadequate to reliably assess changes in weather extremes. Since then, data has been digitized and new software and indices for defining weather extremes have been developed. Based on this, the third (Houghton et al., 2001, TAR) and fourth (Meehl et al., 2007; Trenberth et al., 2007) assessment reports of the IPCC could indicate several observed changes in extreme weather events and also give projections about possible intensification or a higher frequency of extreme weather events in the future (Nicholls and Alexander, 2007). In 2012, the IPCC published a special report about the increasing risks of extreme events (Field et al., 2012).

In the following, the various observed and projected changes in weather extremes are reviewed, with a special focus on changes in Europe and Germany.

2.1.1. Temperature extremes

Under rising mean temperatures the occurrence probability of extremely warm temperatures increases, while the occurrence probability of extremely cold temperatures decreases (Meehl et al., 2000a; Fig.1). Conclusions about changes in temperature extremes were among the earliest results related to changes of extreme weather events, as data records monitoring temperature are globally well developed and spatially quite coherent. A decrease in frost days and an increase in the number of extremely hot days had been observed world-wide in the 20th century (Easterling et al., 2000; Salinger, 2005; Nicholls and Alexander, 2007; Trenberth et al., 2007). The magnitude of changes in extremes varies spatially, along with unevenly distributed changes in mean temperature. Across Europe, an increasing frequency in the number of heat waves has been observed in the 20th century and especially the Mediterranean was faced with more extremely hot days (Klein Tank and Können, 2003; Schaer and Jendritzky, 2004; Alexander et al., 2006; Beniston et al., 2007; Trenberth et al., 2007; Bartholy et al., 2008; Kioutsioukis et al., 2010). At the same time the number of frost days decreased and the start of the growing season advanced (Alexander et al., 2006; Beniston et al., 2007; Bartholy et al., 2008). These changes will exacerbate in the 21st century world-wide and might even be larger than widely expected, as not only mean temperature, but also the variability in temperature might increase (Schaer and Jendritzky, 2004; Beniston et al., 2007; Meehl et al., 2007; Jacob, 2009; Field et al., 2012).

Although the number of frost days is further projected to decrease, an increase in the minimum temperature reached during winter is regarded unlikely (Kodra et al., 2011) and the fewer frost days are predicted to be more scattered over time (Jylhä et al., 2008).

2.1.2. Precipitation extremes

In general, observations and model predictions for precipitation changes are spatially and temporally more variable and show a larger inter-model variability than those for temperature trends and extremes (Blenkinsop and Fowler, 2007).Theory predicts that global warming will be accompanied by an intensification of the hydrological cycle: Along with rising temperatures, surface evaporation as well as the water holding capacity of the atmosphere rise, the latter by almost 7% per degree K, according to the Clausius-Clapeyron relation (Allen and Ingram, 2002; Trenberth et al., 2003; Christensen and Christensen, 2004; Huntington, 2006; Allan and Soden, 2008; O'Gorman and Schneider, 2009). Data records since 1973 have shown that atmospheric moisture amounts have been rising since then, which resulted in a 10% increase of precipitable water in all regions where reliable data were available (Trenberth et al., 2003; Huntington, 2006). Warming and increased moisture holding capacity also lead to increased lateral convergence of low level moisture and this in turn causes an intensification of rainfall variability, leading to fewer, but more intense rainfall events (Allen and Ingram, 2002; Trenberth et al., 2003; Christensen and Christensen, 2004; Groisman et al., 2005; O'Gorman and Schneider, 2009; Min et al., 2011).

An increase in the frequency of heavy rainfall events has been observed in many regions, even in areas with declining mean annual precipitation (Karl and Knight, 1998; Easterling et al., 2000; Trenberth et al., 2003; Groisman et al., 2005; Tebaldi et al., 2006; Marengo et al., 2010). Already in the TAR (Houghton et al., 2001), a significant increase in the frequency of heavy rainfall events by 2-4% over mid and high latitudes has been stated. Within Europe, seasonal and regional differences exist for trends in heavy rainfall events. During winter, heavy rainfall has become more frequent in Northern Europe and less frequent in southern Europe, according to changes in mean precipitation (Klein Tank and Können, 2003; Haylock and Goodess, 2004; Groisman et al., 2005; Beniston et al., 2007). During summer, more extremes occurred again in Northern Europe and for Central- and Eastern Europe, although for the latter, total precipitation declined during summer in many parts (Raisanen and Joelsson, 2001; Klein Tank and Können, 2003; Christensen and Christensen, 2004; Beniston et al., 2007).

Over the 21st century, the frequency of heavy rainfall events is likely to increase further in many regions (Field et al., 2012). European models predict an increase in magnitude and frequency of extreme precipitation events in northern, central and eastern Europe (Raisanen and Joelsson, 2001; Beniston et al., 2007; Bartholy et al., 2008; Boberg et al., 2010), but also for some parts of southern Europe (Coppola and Giorgi, 2010; Kioutsioukis et al., 2010) and the UK (Fowler and Ekstroem, 2009). Germany is also projected to further experience more intense heavy rainfall events, especially during winter (Jacob, 2009).

As variability of rainfall is projected to increase, leading to more intense, but less frequent events and as warming accelerates surface drying, the risk for droughts rises under global warming (Blenkinsop and Fowler, 2007; Allan and Soden, 2008). Assessment and quantification of droughts is complicated by several issues (Field et al., 2012): “Drought” can be defined in numerous ways and each drought type can be assessed using various drought indices (e.g. the prominent Palmer drought severity index PDSI)(Keyantash and Dracup, 2002; Dai et al., 2004). Historical datasets to directly quantify and determine drought, like soil moisture data, are relatively sparse (Robock et al., 2000; Dai et al., 2004).

Nevertheless, numerous studies and modelling approaches in recent years investigated whether drought frequency and severity increased due to global climate change and how droughts are projected to change in the future. Since the 1970s, areas affected by drought have markedly amplified by up to 50%, especially in the tropics and subtropics (Dai et al., 2004; Huntington, 2006; Wang et al., 2010; Trenberth et al., 2007), and droughts are projected to intensify further in many parts of the world, including central North America, Central America and Mexico, northeast Brazil, and southern Africa (Allan and Soden, 2008; Li et al., 2009; Wang et al., 2010; Field et al., 2012). Many regions in Europe have been faced with severe summer droughts in the last decades, especially the Mediterranean and parts of Central Europe (Lopez-Moreno et al., 2010; Dai et al., 2004; Beniston et al., 2007; Briffa et al., 2009), and an exacerbation of this situation is predicted as mean summer precipitation is projected to decrease in these areas by up to 30 % (Beniston et al., 2007; Blenkinsop and Fowler, 2007; Meehl et al., 2007; Jacob, 2009; Coppola and Giorgi, 2010; Iglesias et al., 2010; Moriondo et al., 2010; Field et al., 2012).

This will likely affect Germany, where summer precipitation already decreased over the last decades and is projected to decrease further, especially in Southern, South-Western and North-Eastern Germany (Schönwiese et al., 2005; Jacob, 2009).

2.2. Plant and ecosystem response to extreme weather events

The abruptness of extreme events gives only little time for acclimation and their novel magnitude might push single plants, plant communities or whole ecosystems beyond their thresholds of survival and equilibrium (Easterling et al., 2000; Jentsch et al., 2007; Smith, 2011b). Thus, extreme weather events may exert stronger effects on plants and plant communities than gradual shifts in means (e.g. warming or rising CO_2-levels) and their ecological consequences are expected to be out of proportion to their relatively short duration (Jentsch et al., 2007). In the following, the response of single plants as well as of plant communities towards extreme weather events will be shortly summarized, including observational and experimental evidence.

2.2.1. Morphological and physiological response of single plants to various climatic stress[3] types

Plant response to heat

Extreme heat (for mesophil plants this often means temperature above 35 ° C (Schulze et al., 2005)) causes metabolic imbalances, due to the temperature dependence of biochemical reactions, as well as protein denaturation. Plants growing in heat-prone environments often avoid heat by morphological adaptations, such as pubescent or splitted leaves. Short-term morphological avoidance mechanisms include changing the leaf orientation or cooling via transpiration, which, however, may additionally cause water stress. An acclimation mechanism to increase heat tolerance is the heat-shock reaction of cells. It begins with a down-regulation of housekeeping-gene-expression and an up-regulation of heat-shock proteins (Schulze et al., 2005; Lambers et al., 2008). These prevent damage of the photosynthetic apparatus, repair denatured proteins or break down irreversible damaged proteins (Parcellier et al., 2003; Schulze et al., 2005).

Plant response to frost

Despite the general decrease of frost days under global warming, the projected increase in the variability of air temperature along with a reduction in snow cover, acting as an insulation for many plants (Marchand, 1996), could increase the impact of frost in many regions of the northern hemisphere (Groffman et al., 2001; Kreyling, 2010). Along with an earlier onset of the growing season under global warming, the risk of late frost damage might also increase (Rigby and Porporato, 2008; Woldendorp et al., 2008). Exposure to cold temperatures causes changes in membrane fluidity, damage to biomembranes, metabolic imbalances and oxidative stress due to formation of reactive oxygen species (ROS)[4] (Schulze et al., 2005; Lambers et al., 2008). Frost stress leads to the additional problem of cell dehydration caused by apoplastic ice formation and to cell death by symplastic ice crystal formation (Janska et al., 2010; Thomashow, 1999; Schulze et al., 2005). Many plants avoid frost stress by dormancy or by completing their life cycle within the frost-free period. Plants adapted to frost show frost hardening that enables them to survive frost without cell damage and which is triggered by low temperatures and the photoperiod, (Janska et al., 2010;

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[3] Stress is understood here as deviation from the optimum environmental conditions of plants

[4] ROS accumulate under various stressors, when the light reaction of the photosynthesis produces reduction equivalents (NADPH) via the electron transport chain that cannot be used in the calvin cycle, e.g. caused by a lack of CO_2 due to stomata closure or by low temperatures and thus slow biochemical reactions. The resulting over-reduction or over-energetization causes reduction of O_2 to the very reactive superoxide. This can convert rapidly into other ROS, that lead to cell and membrane damages.

Thomashow, 1999). Frost hardening involves an enhancement of membrane fluidity, e.g. by increasing desaturation of fatty acids, the upregulation of cold-related proteins (COR), which often serve to stabilize membranes, an upregulation of substances to detoxify ROS (e.g. superoxiddismutase, xanthophyll) and mechanisms to avoid dehydration (accumulation of cryoprotectives, such as compatible solutes or dehydrins, see next section) (Janska et al., 2010; Schulze et al., 2005). While hardening of perennial plants in autumn takes several weeks, dehardening may occur within hours to days (Strimbeck et al., 1995; Rapacz et al., 2000; Sakai and Larcher, 1987), leaving the plants vulnerable to short-term late frost events in the early growing season or after winter warming events.

Plant response to drought

Drought is one of the major limitations for plant growth world wide (Chaves et al., 2002). Plants in drought-prone environments show adaptations to avoid drought stress by dormancy or morphological modifications such as an enlargement of the root system (Lambers et al., 2008; Newman et al., 2006). Many mesic plants are able to acclimate to drought stress to a certain extent, thereby increasing their drought tolerance. The phytohormone abscisic acid (ABA) plays a key role in drought perception and reaction (Wasilewska et al., 2008). A rapid ABA-mediated response to water shortage is the closure of stomata to prevent further transpiration. Morphological mechanisms of drought acclimation include the diminishment of the leaf area by leaf rolling or even leaf shedding. The depletion of CO_2 in the cells when stomata are closed can lead to a formation of ROS, especially under high light conditions when the plants ability to dissipate excess energy is exceeded. To avoid oxidative damage, enzymes and substances to detoxify or scavenge ROS are increasingly synthesized (Munne-Bosch and Alegre, 2000). Furthermore, compatible solutes, e.g. soluble carbohydrates, proline and betaines are synthesized to prevent further cell dehydration and to protect biomembranes from damage by charged ions (Bohnert, 2000; Schulze et al., 2005). Another mechanism to protect biomembranes is the synthesis of dehydrins, which are often amphiphil and serve to stabilize other proteins (Bohnert, 2000; Schulze et al., 2005).

Plant response to heavy rainfall

Single plant response to heavy rainfall has rarely been studied. Plants do not suffer from an increased water supply in the soil, as long as the soil is not waterlogged. In waterlogged soils, air in soil pores is replaced by water, limiting oxygen supply to the roots, as oxygen diffuses and dissolves slowly in water. Plants adapted to flooding-prone environments (mangroves, for instance) have evolved mechanisms to supply their roots with oxygen, for example by developing air roots. Plants not adapted to flooding can sometimes acclimate by histological modifications, like aerenchymes. Otherwise, they experience hypoxia or even anoxia. This causes fermentation instead of respiration in the root cells, which restricts growth by a fast depletion of stored carbohydrates. Lactate and ethanol accumulate and might cause cell damage by increasing acidity. After re-aeration plants might suffer oxidative damage by formation of ROS (Schulze et al., 2005; Lambers et al., 2008). Often mykorrhiza are damaged in hypoxic soils, which impairs the plants nutrient supply.

2.2.2. Impact of extreme weather events on plant communities and ecosystems

Observational studies

Besides physiological and morphological alterations in single plants, climatic variables affect species distribution and ranges, phenological life cycle events, community composition and species interactions (Hughes, 2000; Visser and Holleman, 2001; Walther et al., 2002).

Many observational studies document the effect of the gradual warming on vegetation: Polewards or upwards range shifts in response to warming have been observed for various species, e.g. an upward shift of the treeline and of alpine plants in Europe in the last decades (Hughes, 2000; Walther et al., 2002; Parmesan and Yohe, 2003; Thuiller, 2007). The rising temperatures also led to phenological shifts in many plant species, for instance to an earlier onset of bud burst or flowering (Walther et al., 2002). Warmer conditions often match the needs of invasive plants, that can possibly establish more rapidly and more widespread under new conditions. An increase of thermophilic invasive species has been documented in several ecosystems (Walther et al., 2002). Climate change may also lead to species extinctions, with species in mountain habitats or the Mediterranean especially endangered (McCarty, 2001; Thomas et al., 2004; Thuiller et al., 2005; Schröter et al., 2005).

Compared to observations of the effects of gradual warming for plant communities, populations and species distribution, observational studies investigating the consequences of extreme weather events are rare, as the occurrence of extreme climatic events is also rare (Meehl et al., 2000a; Gutschick and BassiriRad, 2003; Jentsch et al., 2007). Rapid catastrophic shifts in community composition often follow disturbances caused by extreme climatic events (e.g. storms)(Scheffer et al., 2001). Even less dramatic events may cause changes in species competitive and facilitative interactions (Bertness and Callaway, 1994; Jentsch et al., 2007). For instance, competition intensifies in plant-plant interactions under extreme drought (Tielborger and Kadmon, 2000; Ludwig et al., 2004; Maestre and Cortina, 2004). Thus, naturally occurring droughts cause long-lasting shifts in plant community composition (Allen and Breshears, 1998; Breshears et al., 2005; Mueller et al., 2005). Drought further reduces forest resilience and productivity and is projected to increase tree mortality (Thompson et al., 2009; Lloret et al., 2004; Noormets et al., 2008; Allen et al., 2010). Many tree-species in the Mediterranean are projected to decrease their distribution due to more severe droughts (Schröter et al., 2005). In mesic grassland, however, increased precipitation variability, leading to longer dry periods followed by more extreme rainfall events, promoted plant coexistence and thus stabilized diversity (Adler et al., 2006).

The extreme summer heat waves in Central- and Western Europe in 2003 and in Eastern Europe in 2010, accompanied by severe drought, caused crop failure and Europe-wide reductions in primary productivity (Ciais et al., 2005; Barriopedro et al., 2011).

Warm spells during winter have also been observed to cause damage, as they may lead to a loss of frost acclimation and thus increased damage upon recurring frost. A winter “heat wave” in 2007 in northern Scandinavia, accompanied by thawing, led to extensive damage of the dominant dwarf-shrubs (Bokhorst et al., 2009). Strimbeck et al. (1995) found that a natural thaw during midwinter caused dehardening of montane red spruce. As global warming advances the beginning of the growing season, increasing damage caused by late frost events has been observed (Gu et al., 2008).

Experimental evidence on extreme weather events and plant communities

As observational evidence on the impacts of extreme weather events is limited, several controlled field-experiments assessed effects of extreme climatic events on natural or artificially composed vegetation. The advantages of well-conducted experiments[5] are the possibility to incorporate control treatments and to minimize the influence of confounding factors. However, as such a reductionist approach implies rather artificial conditions rarely found in reality, the transfer of experimental evidence on complex, natural systems might be limited.

In the beginning of experimental climate change research (1990s), studies testing effects of extreme weather events on plant communities were scarce (Jentsch et al., 2007; manuscript 1) and the majority of the experiments implemented changes in weather trends, such as warming or increased CO_2. Until 2006, research investigating the effects of extreme events accounted for only one fifth of the experimental climate change studies published

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[5] Well-conducted experiments should include proper control treatments varying only the factor studied, should work with enough replicates to ensure statistical power and should randomly assign treatments and replicates. Further, treatment artifacts and biases caused by the experiment conductors have to be avoided (Hurlbert, 1984)

(Jentsch et al., 2007). Most experiments assessed aboveground productivity as main response parameter and investigated effects of drought (manuscript 1).

Precipitation manipulations:

Experimentally applied drought decreased grassland productivity in some studies (Morecroft et al., 2004; van Ruijven and Berendse, 2010; de Boeck et al., 2011). However, productivity was often only affected in response to drought in arid habitats (Gilgen and Buchmann, 2009; Miranda et al., 2009) or in generally dry years (Bloor et al., 2010). The VULCAN experiments assessing data at shrubland sites across Europe, also found a trend to reduced biomass production after drought only at the drier sites (Penuelas et al., 2004; Penuelas et al., 2007). In mesic grassland, drought often had no long-term effects on productivity (Naudtsa et al., 2011), which was also found for the EVENT I experiment (manuscript 1). Despite often not having large effects on productivity, drought alters belowground processes, e.g. by reducing soil respiration (EVENT I and CLIMOOR experiment: Emmett et al., 2004; Kreyling et al., 2008; Sowerby et al., 2008; Toberman et al., 2008; manuscript 1).

Several studies did not test the direct effects of drought, but the effects of increased rainfall variability (fewer, but larger events, including long dry intervals and heavy rain spells) on grassland parameters. Some studies showed a larger effect of mean annual precipitation on productivity (Barrett et al., 2002; Chou et al., 2008), while others found rainfall variability to be a more important driver for ANPP (Knapp et al., 2002; Fay et al., 2003). In the Rain Manipulation Plots (RaMPs) experiment at Konza Prairie Biological Station in Kansas, USA, a reduction in soil respiration, plant CO_2 uptake (Harper et al., 2005) soil water content (Fay et al., 2003) and productivity (Fay et al., 2003; Knapp et al., 2002) and an increase in soil nitrogen availability and in plant diversity (Knapp et al., 2002) was found in temperate continental grassland under increased rainfall variability (larger but fewer rainfall events with a constant overall rainfall amount (Heisler and Weltzin, 2006). Heisler-White et al. (2008, 2009) found a decrease in productivity at the temperate part of a transect and an increase in the semi-arid end under fewer, but larger rainfall events. In a Californian grassland, changes in precipitation patterns caused changes in trophic interactions, e.g. a reduction in consumer abundance on a longer time scale that overrode direct, autecological short-term effects (Suttle et al., 2007).

The drought studies not applying compensating rain pulses show that arid systems or mesic systems in dry years are more vulnerable towards drought. Thus, a sufficient overall rainfall amount seems to be important for grassland recovery, which was also found in our study within the EVENT II experiment (manuscript 6). The impact of drought in arid ecosystems seems to depend largely on overall rainfall amount or the occurrence of several larger rain pulses.

Experiments testing effects of heavy rainfall events on vegetation are rare. In the EVENT I experiment heavy rainfall events had only minor effects on productivity (Kreyling et al., 2008).

Temperature manipulations:

Experiments applying not only gradual warming, but extreme heat pulses, are scarce. Arnone et al. (2011) found only short-termed effects of an experimental heat wave on the productivity of the dominant grass species in tallgrass-prairie of Oklahoma, but no changes in most of the studied species. In cold biomes plants performed better during a warming pulse, but worse afterwards, possibly due to a loss of cold resistance and subsequent higher stress levels under the recurring cold (Marchand et al., 2005; Marchand et al., 2006; Bokhorst et al., 2009), whereas fresh litter decomposition was unaffected by warming pulses (Bokhorst et al., 2010). In the EVENT I experiment, repeated soil freeze-thaw cycles caused an increase in productivity of temperate grassland (Kreyling et al., 2010). However, lagged stress effects in heath communities diminished biomass two vegetation periods after applying warming pulses (Kreyling et al., 2010).

Combined manipulations of multiple climatic variables:

Few experiments apply multiple, combined climatic stressors on vegetation:
The CLIMAITE project (Mikkelsen et al., 2008) applying elevated CO_2, drought and warming as single factors and in combination on shrubland systems in Denmark found mostly smaller responses of nutrient cycling to the combined treatments than to the single treatments. Nevertheless, the future climate scenario combining all factors led to reduced N turnover (Larsen et al., 2011). Grime et al. (2008) found a large long-term resistance of infertile, established grassland in response to warming, droughts and water additions over 13 years. A mesocosm experiment including herbaceous species in Belgium also applied heat waves and drought as single and combined factors (de Boeck et al., 2011; van Peer et al., 2004). They found that negative effects of drought on CO_2 exchange, growth, survival and biomass production were exacerbated by heat waves, whereas heat waves alone had no effect, due to transpirative cooling.

The summarized results demonstrate that intensifying droughts might reduce productivity and also agricultural yield, especially under already dry conditions, with smaller to no effects in mesic grassland. Furthermore, some studies show that extreme events alter net carbon balance and soil processes, thereby altering nutritional pathways and soil quality. There is an urgent need to further combine multiple climatic stressors, as effects of such multifactor experiments might point in totally different directions as expected out of the response towards single factors (Mikkelsen et al., 2008). Studies investigating parameters other than productivity and soil respiration are needed to elucidate effects on biotic interactions and ecosystem processes on multiple levels.

3. On this thesis

3.1. Objectives of this thesis

The prevailing response parameter of most experiments investigating effects of extreme weather events on vegetation is primary productivity. In the EVENT I experiment, in which statistically extreme weather events were applied on artificially planted communities of varying species- and functional diversity, the extreme weather events did surprisingly not cause large and detrimental changes in grassland productivity (Kreyling et al., 2010). The applied treatments could consequently not be called “extreme climatic events” sensu Smith (Smith, 2011a), as, although being extreme in their magnitude and length relative to the reference period, they did not cause an extreme response of plant communities, such as widespread species mortality or community breakdown.

However, although not severely affecting productivity, the weather treatments caused more subtle changes on a physiological and biogeochemical level that are summarized in manuscript 1. Slight changes, for example in plant metabolic compounds can affect multiple ecosystem processes and levels, for instance by decreasing palatability for herbivores or by changing decomposition rates, which in turn alters trophic interactions and nutrient cycling. Thus, one objective of this thesis was to elucidate how extreme weather events affect ecosystem functions beyond productivity, such as plant-herbivore interactions or decomposition.

Especially mesic grassland communities are often very stable when faced with extreme drought (see section 2.2.2.), which was also shown in the EVENT I experiment. Yet, the underlying mechanisms of such a high stability are not well understood. Another objective of this thesis is to further elucidate possible mechanisms of the surprisingly large resistance or resilience of plants and plant communities when faced with extreme weather events. Here, the focus is on a possible stress memory, as up until now it is unclear, how plants and plant communities react when stress is applied repeatedly over a relatively short time span. On the one hand, this might lead to a step-wise reduction in the ability to recover, until a total breakdown of the system (Scheffer et al., 2001). On the other hand, stress acclimation may lead to a persisting increase in stress resistance, a mechanisms that could be regarded as kind of stress memory. The consideration of not only an increased event magnitude, but also of an increased frequency of events is urgently needed in studying climatic extremes (Smith, 2011b).

The EVENT I experiment is highly controlled in terms of species composition, as the planted community compositions were kept constant over the years by periodically weeding. To investigate if the findings of high stability in the artificially composed plant communities can be conferred to more natural systems, the EVENT II experiment was established on a semi-natural meadow in 2008. Here, not only rainfall was manipulated, but also different land-use scenarios were implemented. This experiment was also designed to answer the question whether the effects of drought or heavy rain are caused by an overall alteration in mean annual rainfall amount, or by increased rainfall variability (larger, but fewer rainfall events) under constant annual rainfall amount. For this reason, in EVENT II rainfall amount was kept constant from 2009 onwards and only the size of and the intervals between the rainfall events were varied.

To sum up, the main objectives of this thesis were (1) to investigate if extreme weather events have an effect on ecosystem functions beyond productivity, (2) to test if the high stability or resilience in response to drought regarding productivity also exists in more naturally grown plant communities and (3) to further elucidate possible mechanisms of the surprisingly large resistance or resilience of the plant communities.

3.2. Outline of manuscripts

The first manuscript summarizes 5 years of drought research in the artificially planted grassland communities of EVENT I. Extreme drought had no effect on aboveground- or belowground productivity. Nevertheless, several other physiological and biogeochemical parameters were affected. If physiological changes on a leaf level influence other ecosystem levels and processes in the long-term had thus to be investigated.

The second manuscript therefore deals with changes in leaf compounds caused by extreme drought and resulting effects on herbivores feeding on such leaves. A second focus of this study was to elucidate effects of plant community composition on leaf compounds and, as a consequence, herbivore development. The study showed that changes in grass compounds caused by severe drought affected herbivores feeding on such grass: Caterpillars fed with drought-subjected leaves showed significantly higher survival, a longer duration of larval development and higher pupal weight. Further, plant compounds of our target grass depended on the composition of the plant community it was grown in, which in turn affected herbivore development: Larvae feeding on species-richest communities without legumes showed the highest mortality, which was closely linked to low protein content in these leaves. This study provides evidence that even quite subtle changes in plants caused by drought or community composition are able to influence biotic interactions and may even lead to desynchronisation of trophic and phenological adjustments under climate change. Furthermore, as climate change is likely to affect plant community composition, this will further affect leaf quality and thus plant-herbivore interactions.

The second objective of this thesis was to elucidate possible mechanisms of the high stability of grassland productivity under climatic extremes. In the first three years of the EVENT I experiment, a drought of 100-year recurrence was applied (leading to 32 days of consecutive drought), and in the next years, a drought of 1000 year recurrence was applied. In every year, the same plots were subjected to drought. One possible mechanisms of resilience might be that the communities built up an ecological memory that helped them to cope with drought in the following years. As ecological memory on a community level is difficult to assess, we focused on an ecological stress memory on a single plant level. Surprisingly few studies investigated if whole plants are able to remember stress and to react improved towards a recurrent stress event. This issue is especially important as frequency of extreme weather events is projected to increase under climate change (Smith, 2011b). Further, a common definition of stress memory for ecologists is missing. The third manuscript thus first defines the concept of an ecological stress memory on a whole plant level, reviews the few existing studies indicating stress memory after climatic stress (drought, frost, heat) and discusses possible mechanisms of an ecological stress memory, including epigenetic ones.

A drought memory in grass plants was investigated within a pot-experiment in which one group of plants was subjected to a single drought and the other to recurrent drought (manuscript 4). This study provided evidence that grass plants are able to remember drought even after a harvest and resprouting and to show a higher percentage of living biomass, due to improved photoprotection, when compared to plants subjected to their first drought. Similarly, the experiment pertaining to manuscript 5 tested frost hardiness of *Pinus nigra* juveniles and showed that plants exposed to drought during summer revealed higher frost hardiness in winter (manuscript 5). As both, frost and drought stress, involve dehydration stress, it might well be that an ecological cross-stress memory was involved here. Plant frost hardiness in this study was related to a higher concentration of carbohydrates. Content of carbohydrates is also often increased under drought (e.g. manuscript 2). Thus, the cross-stress memory indicated in manuscript 5 might be related to the faster synthesis of soluble carbohydrates.

To test if the findings of the artificially composed plant communities also hold under more realistic conditions, an extreme drought was also applied on naturally grown grassland communities in the EVENT II experiment. Here, effects of increased rainfall variability (changes in timing and distribution of rainfall, but not in overall rainfall sum) on the productivity and some aspects of forage quality of established grassland were investigated. In contrast to the findings in artificially planted communities (manuscript 1), ANPP and forage quality were reduced in naturally composed grassland in response to extreme drought followed by heavy rainfall events (manuscript 6). Mowing frequency strongly altered forage quality and biomass production, but did did neither buffer, nor amplify effects of extreme rainfall variability on productiviy, as it did not interact with rainfall variability manipulations. Despite effects of rainfall variability on ANPP, grassland showed high resilience after extreme spring drought followed by heavy irrigation, as effects were large shortly after the extreme event, but did not persist until a second harvest later in the year, when no differences between the rainfall variability manipulations appeared. In the preceding year, when the extreme spring drought was not followed by irrigations and thus also received the smallest overall amount of water, negative effects on productivity were larger and remained until the second harvest in late summer. Then, formerly drought exposed communities still showed reduced biomass production. This highlights the important role of a sufficient overall amount of rainfall for recovery processes in temperate grassland and is in accordance with the drought studies mentioned in section 2.2.2., showing severely adverse effects of drought primarily in dry years or in arid biomes. As this thesis investigates effects of extreme weather events on ecosystems beyond productivity, manuscript 7 reports findings of a long-term decomposition experiment conducted within EVENT II. Extreme drought reduced litter decomposition when litter bags were exposed to drought for six weeks within an 11 month period. Surprisingly, low rainfall variability with regular irrigation decreased decomposition. Additional winter rain accelerated decomposition, whereas winter warming had no effect on decomposition, but reduced snow cover and increased variability of surface temperatures. More frequent mowing strongly stimulated decomposition, which could be attributed to changes in litter quality. However, the stimulating effect of frequent mowing was absent under extreme rainfall variability including drought. Projected increases in drought frequency under climate change may inhibit decomposition and alter nutrient and carbon cycling along with soil quality. Especially decomposition in intensively managed grassland appears vulnerable towards drought.

3.3. Emerging research questions

3.3.1. Resilience and stress memory

Often, and also in our study (manuscript 1) grassland shows a surprisingly large resistance or resilience towards drought. Mechanisms of resilience remain to be elucidated.

One likely mechanism is a stress memory of plants that renders them less vulnerable to repeated stress events (manuscripts 3, 4, 5). However, if such a mechanisms exists also under natural conditions and also on larger scales, e.g. on a community level, is yet to be investigated, especially as findings of manuscript 6 imply that grassland resilience under more natural conditions might be diminished under generally dry conditions. Possible mechanisms of a stress memory are largely unknown. Joint research of ecologists and molecular biologist is needed to elucidate possible epigenetic mechanisms. First studies already showed the heritability of acquired stress tolerance (see manuscript 3). Besides ecological stress memory, other underlying physiological and biogeochemical processes that serve to maintain productivity and might thus be mechanisms of community stability and recovery have to be identified and addressed in future research. Maintaining ecosystem resilience is of major importance to mitigate and prevent catastrophic consequences of global climate change.

3.3.2. Extreme weather events and ecosystem processes at multiple levels

Up until now, the main response parameter studied in research on extreme climatic events is primary production (manuscript 1). However, even if primary production remains stable, other physiological and biogeochemical parameters are changed under extreme weather events (manuscripts 1, 2, 7). Such changes, e.g. food plant quality might seriously interfere in ecosystem synchronisation and ecosystem functioning. Further work to study long-term effects of extreme weather events on, e.g. biotic interactions or biodiversity is needed to estimate consequences of weather extremes and to enable policy makers to prevent destabilization of established food-webs and to seize measures for adaptation. How herbivores might react to changes in their host plant in more natural conditions than the ones described in manuscript 2 and whether specialists might react differently compared to generalist herbivores also needs further research. We showed that winter warming did not increase decomposition, due to loss of snow insulation and increased surface temperature variability. How decomposition might be affected by summer warming, also in combination with drought conditions, needs further study. Long-term changes in soil biotic activity under more frequent mowing needs to be addressed, to find explanations for the higher vulnerability of decomposition towards drought in more frequently mown communities. Preliminary results of the EVENT experiments also indicate strong effects of heavy rainfall on biotic interactions, such as mycorrhiza or decomposer fauna. As heavy rain events are expected to increase in the future, but are rarely studies yet, more investigations are needed to look at effects of heavy rain on ecosystem functions.

3.3.3. Climate change and land use

Although mowing strongly influences primary productivity in grassland (manuscript 6) it did neither buffer, nor amplify effects of extreme weather events on productivity in our experiment. However, increased mowing frequency generally increased N concentration of leaves and made them more susceptible to altered rainfall variability. The decreased C/N ratio in more frequently mown plants also led to higher decomposition rates of such litter. However, this stimulating effect was strongly reduced under drought, which indicates a higher vulnerability of decomposition towards extreme rainfall variability in more frequently mown communities. Further research is needed to investigate combined effects of mowing and rainfall variability on the nutritional value of hay meadows more in detail, including other parameters, such as fibre content. Management strategies other than mowing frequency that might be able to buffer adverse effects of increased rainfall variability on productivity and forage quality of grassland have to be identified.

List of manuscripts and declaration of own contribution

Concept: Idea for the study and development of experimental design or development of an outline for reviews
Data acquisition: being responsible for organization and execution of data acquisition and doing the measurements together with the help of students and interns
Data analysis: statistical analysis of data and their illustration in tables and figures
Writing: writing the manuscripts, including literature research
Editing: Proof-reading and grammar editing, including comments and inputs from co-authors and their integration in the manuscript and preparation for resubmissions after the manuscript was reviewed by the journals referees

Manuscript 1:

Climate extremes initiate ecosystem-regulating functions while maintaining productivity

Anke Jentsch, Juergen Kreyling, Michael Elmer, Ellen Gellesch, Bruno Glaser, Kerstin Grant, Roman Hein, Marco Lara, Heydar Mirzae, Stefanie E. Nadler, Laura Nagy, Denis Otieno, Karin Pritsch, Uwe Rascher, Martin Schädler, Michael Schloter, Brajesh K. Singh, Jutta Stadler, **Julia Walter**, Camilla Wellstein, Jens Wöllecke and Carl Beierkuhnlein
Journal of Ecology, 2011, **99**: 689–702.
Concept: 0 %
Data acquisition: 5 %
Data analysis: 5 %
Writing: 5 %
Editing: 5 %

Manuscript 2:

How do extreme drought and plant community composition affect host plant metabolites and herbivore performance?

Julia Walter, Roman Hein, Harald Auge, Carl Beierkuhnlein, Sonja Löffler, Kerstin Reifenrath, Martin Schädler, Michael Weber, Anke Jentsch

Arthropod-Plant Interactions, 2012, **6**: 15-25.

Concept: 80 %
Data acquisition: 70 %
Data analysis: 100 %
Writing: 90 %
Editing: 50 %
Corresponding author

Manuscript 3:

Ecological stress memory and cross stress tolerance in plants in the face of climate extremes

Julia Walter, Anke Jentsch, Carl Beierkuhnlein, Juergen Kreyling

Environmental and Experimental Botany, 2012, in press.

http://dx.doi.org/10.1016/j.envexpbot.2012.02.009

Concept: 60 % (invited review)
Preparation of figures: 90 %
Writing: 70 %
Editing: 70 %
Corresponding author

Manuscript 4:

Do plants remember drought? Hints towards a drought-memory in grasses

Julia Walter, Laura Nagy, Roman Hein, Uwe Rascher, Carl Beierkuhnlein, Evelin Willner, Anke Jentsch

Environmental and Experimental Botany, 2011, **71**: 34–40.

Concept: 100 %

Data acquisition: 90 %

Data analysis: 100 %

Writing: 90 %

Editing: 50 %

Corresponding author

Manuscript 5:

Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought

Environmental and Experimental Botany, 2012, **78**: 99-108.

Concept: 0 %

Data acquisition: 5 %

Data analysis: 0 %

Writing: 5 %

Editing: 15 %

Manuscript 6:

Increased rainfall variability reduces biomass and forage quality of temperate grassland largely independent of mowing frequency

Julia Walter, Kerstin Grant, Carl Beierkuhnlein, Jürgen Kreyling, Michael Weber, Anke Jentsch

Agriculture, Ecosystems and Environment, 2012, **148**: 1-10.

Concept: 30 %

Data acquisition: 30 %

Data analysis: 90 %

Writing: 90 %

Editing: 30 %

Corresponding author

Manuscript 7:

Combined effects of multifactor climate change and land-use on decomposition in temperate grassland

Julia Walter, Roman Hein, Carl Beierkuhnlein, Verena Hammerl, Anke Jentsch, Martin Schädler, Jan Schuerings, Juergen Kreyling

Submitted to *Soil Biology and Biochemistry* on July 27th

Concept: 90 %
Data acquisition: 60 %
Data analysis: 100 %
Writing: 90 %
Editing: 30 %
Corresponding author

Presentations of my work at conferences

conference	date	location	own contribution	topic
BayCEER Kolloquium, 2009	April 2009	Bayreuth	15´presentation	The ecophysiology of climate change-Effects of extreme drought on leaf fluorescence and protein content in different plant communities
39th annual Meeting of the German Society for Ecology	September 2009	Bayreuth	15´presentation	Potential role of community composition in modifying plant physiological response to extreme drought on the species level
Vavilov Seminar, IPK Gatersleben	July 2010	Gatersleben	30´presentation	Do plants remember drought? Some hints towards a “drought memory” in grasses
95th annual Meeting of the Ecological Society of America	August 2010	Pittsburgh	15´presentation	Do plants remember drought? Some hints towards a “drought memory” in grasses
40th annual Meeting of the German Society for Ecology	September 2010	Gießen	15´presentation	How do extreme weather events and plant community composition affect host plant metabolites and herbivore performance?
Conference of the Helmholtz Centre for Environmental Research-UFZ	October 2010	Leipzig	poster	How precipitation variability and mowing frequency affect quantity and quality of grassland biomass
Finale Wissenschaft Verstehen	November 2010	Leipzig	15´presentation	Friss oder stirb-Wie das veränderte Klima sich selbst auf Pflanzenfresser im Klimaschrank auswirkt

Curriculum and credit points for the postgraduate school HIGRADE and award

course name	duration	own contribution	credit points
Introduction to water resources and aquatic ecosystem management	3 days	active participation and homework	1
Introduction into biodiversity sciences	2 days	active participation	1
Advanced course terrestrial ecosystem functions and biodiversity	3 days	active participation	2
Advanced course proteomics	5 days	lab work and analysis	2
Seminar on land-use conflicts and conservation of natural resources	1 day	presentation and active participation	1
Application course “Land-use conflicts and conservation of natural resources in the Banaue region of Nothern-Luzon/ Philippines	12 days	conduction of field experiment and writing of final report	3
Soft Skills: Presentations in Englisch	2 days	active participation including short presentations	1
Soft Skills: Scientific Writing	3 days	active participiation including writing of short sections	1
Soft Skills: Grant Aquisition	1 day	participation	0.25
Four talks at international conferences		preparation and presentation of talks	2
Four presentations in the UFZ □reparatio seminar and one poster presentation at the UFZ Topic I conference		preparation and presentation of talks	1.5
Organisation of UFZ doc days 2009	several days	planning of location, activities, talks, schedule	0.75
Statistics: Data Analysis and Modelling using R	6 days	active participation	1.25
Publication of articles in ISI-listed journals		preparation of manuscripts, first and corresponding author	2
Participation at the competition “Wissenschaft Verstehen” and **AWARD for the 3rd place**	Finals were held one day	□reparation of article and 15 minute presentation	1
			20.75

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Manuscript 1: Climate extremes initiate ecosystem regulating functions while maintaining productivity

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Running title: Drought effect on multiple ecosystem services

Summary

1. Studying the effects of extreme climatic or weather events such as drought and heat waves on biodiversity and ecosystem functions is one of the most important facets of climate change research. In particular, primary production is amounting to the common currency in field experiments worldwide. Rarely, however, are multiple ecosystem functions measured in a single study in order to address general patterns across different categories of responses and to analyse effects of climate extremes on various ecosystem functions.

2. We set up a long-term field experiment, where we applied recurrent severe drought events annually for five consecutive years to constructed grassland communities in central Europe.

The 32 response parameters studied were closely related to ecosystem functions such as primary production, nutrient cycling, carbon fixation, water regulation and community stability.

3. Surprisingly, in the face of severe drought, above- and below-ground primary production of plants remained stable across all years of the drought manipulation.

4. Yet, severe drought significantly reduced below-ground performance of microbes in soil indicated by reduced soil respiration, microbial biomass and cellulose decomposition rates as well as mycorrhization rates. Furthermore, drought reduced leaf water potential, leaf gas exchange and leaf protein content, while increasing maximum uptake capacity, leaf carbon isotope signature and leaf carbohydrate content. With regard to community stability, drought induced complementary plant–plant interactions and shifts in flower phenology, and decreased invasibility of plant communities and primary consumer abundance.

5. *Synthesis*. Our results provide the first field-based experimental evidence that climate extremes initiate plant physiological processes, which may serve to regulate ecosystem productivity. A potential reason for different dynamics in various ecosystem services facing extreme climatic events may lie in the temporal hierarchy of patterns of fast versus slow response Such data on multiple response parameters within climate change experiments foster the understanding of mechanisms of resilience, of synergisms or decoupling of biogeochemical processes, and of fundamental response dynamics to drought at the ecosystem level including potential tipping points and thresholds of regime shift. Future work is needed to elucidate the role of biodiversity and of biotic interactions in modulating ecosystem response to extreme climatic events.

Keywords: below-ground, competition, decomposition, invasion, leaf chemistry, microbial, phenology, plant–climate interactions, precipitation change, productivity

Introduction

Currently, knowledge about ecological responses to climate change is based largely on effects of climatic trends such as gradual warming, precipitation change and CO_2 enrichment. However, the magnitude and frequency of extreme climatic or weather events such as severe drought, heat waves, heavy rain and late frost events are expected to increase in the near future (IPCC 2007; O'Gorman & Schneider 2009). Thus, predictions of effects of climate extremes on species, communities and ecosystems have become critical to science and society. Yet, consequences of future extreme climate events for ecosystem functions and services are largely unknown and have only recently been addressed by ecological research (Gutschick & BassiriRad 2003; Schröter *et al.* 2005; Jentsch 2006; Suttle, Thomsen & Power 2007; Jentsch, Kreyling & Beierkuhnlein 2007; Knapp *et al.* 2008; Fisher et al. 2009; Jentsch & Beierkuhnlein 2010).

There is growing concern that climatic extremes such as severe drought could negatively affect ecosystem functioning and stability. A review of the literature revealed that the focus over the last decade has been primarily on primary productivity (Figure S1a-d and Table S1 in Supporting Information), one of the major common currencies in global ecology. The findings from existing climate change studies on drought effects are highly controversial. While some field experiments showed that natural and simulated drought led to decreases of primary productivity (Olesen & Bindi 2002; Morecroft *et al.* 2004; Penuelas *et al.* 2004; Ciais *et al.* 2005), others did not find any significant effects of locally severe drought manipulations (Fay *et al.* 2000; Kreyling *et al.* 2008c). Generally, evidence suggests that an elongation of inter-rainfall intervals as well as changes in seasonal timing are more likely to cause a reduction of above-ground net primary productivity (ANPP) than reduced total rainfall quantity per se (Fay *et al.* 2000; Swemmer, Knapp & Snyman 2007).

However, further aspects confound the debate on ecosystem functioning in the light of climate change. First, the role of biodiversity in ensuring the performance of ecosystem functioning (Balvanera 2006; Worm *et al.* 2006; Hector & Bagchi 2007; Suttle, Thomsen & Power 2007) and in enhancing resistance or resilience to drought has been proven to be fundamental (Pfisterer & Schmid 2002; Kahmen, Perner & Buchmann 2005; De Boeck *et al.* 2008; van Ruijven & Brendse 2010). Second, multiple ecosystem functions in the face of climate extremes have rarely been addressed simultaneously in experiments (Jentsch, Kreyling & Beierkuhnlein 2007; Jentsch & Beierkuhnlein 2008; 2010). Prevailing response parameters in climate change experiments are above-ground production, soil C:N ratio and soil respiration (Figure S1d). However, the interrelationships between above-ground primary production and below-ground nutrient cycling, carbon fixation or water regulation are rarely addressed.

Here, we analyse the effects of recurrent severe drought (local 100-year or1000-year extreme events) on multiple ecosystem properties of a planted grassland in Central Europe in a long-term field experiment (EVENT-I) located in Bayreuth, Germany. Semi-natural European grasslands are widespread, of economic value, provide many ecological services and are important for nature conservation. They have been managed either as hay meadows or pastures in Europe for thousands of years.

Our goal was to assess whether there are general patterns across these different categories of important ecosystem functions including primary productivity, water regulation, carbon fixation, nutrient cycling and compositional stability to climate extremes.
We expected the grassland ecosystem to react sensitively to extreme recurrent drought events, and specifically hypothesized that (i) above-ground productivity would be decreased and that (ii) other ecosystem functions, such as water regulation, carbon fixation, nutrient cycling and compositional stability, would be negatively impacted.

Materials and methods

Experimental Design

The EVENT-I experiment (Jentsch, Kreyling & Beierkuhnlein 2007) is 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.) with a mean annual temperature of 8.2 ˚C and a mean annual precipitation of 724 mm (1971 - 2000). Precipitation is distributed bi-modally with a major peak in June/July and a second peak in December/January (data: German Weather Service). The experiment was carried out with two fully crossed factors: (1) extreme climatic event (severe drought, ambient control), (2) community diversity (two species of one functional group, four species of two functional groups, and four species of three functional groups, monocultures of particular species), representing key species combinations of grassland. The total setup consisted of 5 replicates of each factorial combination, 60 plots in total of 2 × 2 m in size. The factors were applied in a split-plot design with the vegetation types and diversity levels blocked and randomly assigned within each drought manipulation (Jentsch, Kreyling & Beierkuhnlein 2007). The originally installed species composition was maintained by periodical weeding. The texture of the previously homogenized and constantly drained soil consisted of loamy sand (82 % sand, 13 % silt, 5 % clay) with pH = 4.5 in the upper and pH = 6.2 in the lower soil layer (measured in 1M KCl). Data acquisition was carried out in the central square metre of each plot only, in order to circumvent edge effects.

Climatic extremes

The climate manipulations consisted of extreme drought and ambient conditions for control. Extremeness of events was determined by statistical extremity with respect to a historical reference period (extreme value theory) independent of its effects on organisms (Jentsch 2006). In particular, intensity of the treatments was based on the local 100-year extreme event in 2005, 2006 and 2007, and on the local 1000-year extreme event for 2008 and 2009. Vegetation periods (March to September) of 1961-2000 were used as the reference period (data: German Weather Service). Gumbel I distributions were fitted to the annual extremes, and 100-year and 1000-year recurrence events were calculated.

Drought was defined as the number of consecutive days with less than 1 mm daily precipitation. Accordingly, a drought period of 32 days (2005 - 2007) and of 42 days (2008 and 2009) was applied in the experiment during the peak growing season in June. Maximum values in the historical data set were 33 days without rain during June and July 1976. Drought was induced with the support of rain-out shelters that permitted nearly 90 % penetration of photosynthetically active radiation.

Unwanted greenhouse effects were avoided by starting the roof from a height of 80 cm, allowing for near-surface air exchange. After the experimental drought period, the roofs were removed. A lateral surface flow was avoided by plastic sheet pilings around treated plots reaching down to a depth of 10 cm.

The ambient control plots (C) remained without manipulation throughout the entire period. A roof artefact control with five replicates of the rain-out shelters was in place in 2006. Adding the same amount of water as occurred naturally in daily resolution below intact shelters during the drought manipulation period did not result in any significant differences in response parameters, indicating no significant effect from the slightly increased temperature caused by the rain-out shelters.

Experimental plant communities

Overall, grasslands are spatially important ecosystems in Central Europe. Five widespread plant species were chosen from the regional flora, i.e. *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Holcus lanatus* L., *Geranium pratense* L.*, Lotus corniculatus* L. and *Plantago lanceolata* L. Species were selected with respect to their affiliation to defined functional groups (grasses, forbs, leguminous forbs), to life-span (perennials), to overall importance in nearby and Central European grassland systems, and to the fact that they do naturally grow on substrate similar to the one used in this experiment. One hundred plant individuals per plot in defined quantitative composition were planted in a systematic hexagonal grid with 20 cm distance between individuals in early April 2005. Grass and forb individuals used in the experiment were grown from seeds in a greenhouse in the preceding fall. Thus, all plants were in a juvenile stage during manipulation and data acquisition. All plants had been acclimated on site since February 2005, reaching growth heights of c, 15 cm. Biomass at planting amounted to 0.1 – 0.6 g dry wt. Individual^{-1}. These experimental communities represent naturally occurring species combinations. The grassland plots were established at two levels of species diversity (2 and 4 species) and three levels of functional diversity (1, 2, 3 functional groups), resulting in three species combinations or communities in total (Table 1) plus monocultures of selected species.

Table 1 Experimental plant communities in the EVENT-I experiment (Jentsch, Kreyling & Beierkuhnlein 2007) representing grassland vegetation in central Europe: three functional diversity levels varied by number of species, growth form and presence ⁄ absence of legume

Abbre-viation	Vegetation type	Diversity level	Description	Species
$G2^-$	grassland	A	two species, one functional group (grass)	*Arrhenatherum elatius*, *Holcus lanatus*
$G4^-$	grassland	B	four species, two functional groups (grass, forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Geranium pratense*
$G4^+$	grassland	C	four species, three functional groups (grass, forb, leguminous forb)	*Arrhenatherum elatius*, *Holcus lanatus*, *Plantago lanceolata*, *Lotus corniculatus*

Response parameter

The 32 parameters measured are categorized into five key ecosystem functions (Fig. 1) and are described below in order of their appearance, except for soil moisture, which is presented first. Since complete time series data are not available for all parameters, it is indicated in Table S3 whether data from five consecutive years or from particular years were sampled. All data presented in Fig. 1 are derived from years of maximum drought effects.

Soil moisture

Soil moisture was recorded by time domain reflectance (TDR) measurements (Diviner 2000, Sentek) at -10 cm in 2005 - 2007. In 2008 - 2009, soil moisture was recorded between 2 and 7 cm in one grassland plot per treatment block in 1-h intervals by FD-sensors (Echo.EC-5/k, Decagon).

Primary production

Above-ground net primary production

Above-ground biomass harvests (ANPP) of all standing plant material (dead and alive) in all communities were conducted twice a year (early in July and mid September) in 2005 - 2009, resembling local agricultural routines. All biomass was taken out of the central square metre of each grassland plot in order to circumvent edge effects. The harvested biomass was sorted to species and dried to constant weight at 75 °C and weighed (Ohaus NavigatorTM, Ohaus Corporation, accuracy ± 0.01 g).

Nitrogen fixing legumes

According to the above-mentioned routines, harvested biomass of the legume species *Lotus corniculatus* was used to determine the performance of nitrogen-fixing plants.

Plant cover

Species-specific above-ground cover was quantified using a pin-point method, by recording the presence of plant organs in general and the presence of each species separately at 100 vertically inserted steel needles. These values were then treated as the percentage of cover. The measurement was repeated three times in each vegetation period (May, July and September).

Below-ground biomass

Root length was used as proxy for below-ground productivity. Root length was acquired by the minirhizotron technique three times a year. One clear plastic tube (5 cm diameter) was installed at a 45° angle in each plot prior to planting. Tubes were installed to a depth of 45 cm. Portions of the tubes exposed at the surface were covered with adhesive aluminium foil and the ends were capped to prevent entry of water, light and heat. Images of 4 cm^2 were collected in the main rooting zone at 15 cm in each tube by a digital camera mounted on an endoscope. Images were analysed for root length using the line intersection method (Tennant 1975) within a systematic grid (10 × 10, with a grid unit of 0.2 × 0.2 cm). Five replicates per sampling date were analysed.

Shoot-to-root ratio

Shoot-to-root ratio was evaluated using the ratio between above-ground cover and below-ground root length at 5 cm soil depth (Kreyling et al. 2008b). Both parameters were *a priori* standardized to the same mean and standard deviation.

Water regulation

Leaf water potential

Predawn (Ψ_{pd}) and midday (Ψ_{md}). leaf water potential were measured on one leaf of *Holcus lanatus* per plot using a portable pressure chamber (PMS Instruments Co. Corvallis, OR, USA). During measurements, the leaves were cut while enclosed in a plastic bag to reduce further moisture loss during transfer and fixing into the chamber. Moist tissue paper was introduced into the chamber to reduce water loss during the measurements. Measurements were confined to the period between 04:00 and 05:00 h.

Leaf carbon isotope signal

At the end of drought, a set of three fully matured leaves of *Arrhenatherum elatius* from every plot was selected. In each plot, two sun-exposed leaves of five individual plants were sampled and combined. The samples were oven-dried for 48 h at 80 °C. The dry leaves were ball-milled and sub samples of 1 mg analysed for $\delta^{13}C$ with an elemental analyser attached to an isotope ratio mass spectrometer using ConFlo III interface. The carbon isotope composition ($\delta^{13}C$) of a sample was calculated as: $\delta^{13}C = [(Rsample/Rstandard) - 1] \times 1000$, expressed in units of per thousand (‰). ^{13}C:^{12}C ratios were calculated against the P.D. Belemite Standard (precision of 0.2 ‰). The results were compared with other measurements to determine changes associated with shifts in ^{13}C. Every measurement was replicated twice and the accuracy in δ-values was better than 0.1 ‰.

Carbon fixation

Efficiency of photosynthetic light conversion

Chlorophyll *a* fluorescence in the grass species *H. lanatus* was recorded using a pulse-amplitude-modulated photosynthesis yield analyser (PAM 2000 and Mini-PAM) (WALZ, Effeltrich, Germany) with a leaf clip holder. The second or third fully-expanded leaves were measured on four different tillers of one individual. Four measurements per plant were averaged for further analysis. We obtained predawn fluorescence values at the end of the first drought treatment in May/June and throughout the early recovery period after the second drought. The maximum quantum efficiency of photosystem II was calculated as Fv/Fm. Variable fluorescence (Fv) and maximum fluorescence (Fm) were measured before dawn. Variable fluorescence was calculated as Fm-F0, Fm being the maximum fluorescence of the dark-adapted leaf after applying a saturating light pulse and F0 being the steady-state fluorescence yield of the dark-adapted leaf (Maxwell & Johnson 2000). To enable a comparison between absolute fluorescence values, a fluorescence standard material was measured before dawn and calculated as F_v/F_m ($F_v = F_m - F_0$) (Maxwell & Johnson 2000). Absolute F_0 and F_m values were taken to separate the effects of photodamage, becoming apparent with an increase of F_0, from the effects of photoprotection related to enhanced non-photochemical quenching, becoming apparent with a decrease in F_m (Walter et al., 2011).

Leaf gas exchange

Carbon dioxide assimilation (A) at the leaf was monitored in *A. elatius* in all the grassland communities. (No data could be obtained from *H. lanatus* in the particular year of data mining due to its leave status.) A series of weekly measurements were carried out using a portable gas exchange system (LI-6400, LI-Cor, Lincoln, NE, USA). A set of 3 grass tufts on each plot were identified and marked for measurements. On any measurement day, 2-3 suitable leaf blades selected from each of the tufts per plot were set parallel in the cuvette, with their upper surfaces well exposed so that they were fully illuminated during measurements. Every turn of measurements lasted one to two minutes, when a steady state was attained and a set of 10 readings per measurement logged at 10-s intervals. The selected leaves were marked and similar leaves were monitored either during midday (12:00 to 14:00 h) or throughout the day (from sunrise to sunset), when diurnal course measurements were conducted. The measured leaves were then excised at the end of the measurement period and the leaf area (LA) of the section of leaf enclosed in the cuvette determined using leaf area meter CI-202 CID, Camas, WA, USA. Leaf area information was then used to standardize the leaf gas exchange data.

Soil respiration

In situ rates of soil respiration were measured using a portable CO_2 infrared gas analyser (EGM-4, PP Systems, Amesbury, USA) linked to a soil respiration chamber (SRC-1, PP System, Amesbury, USA). At the beginning of the vegetation period, permanent PVC collars (10 cm diameter, 5 cm height, light grey colour) were installed in every plot with a 1-cm edge above soil surface to realize a closed system when the soil respiration chamber was placed on the collar during measurement. The day before each measurement, all above-ground vegetation was removed from the collar using scissors. During the timeframe of 8:00

to 12:00, the soil respiration chamber was placed for 240 seconds on the collar of every plot. An internal fan realized the even distribution of air and the infrared gas analyser monitored the build-up of CO_2 within the system. The rates of soil respiration were determined from this by fitting a quadratic equation to the change in CO_2 concentration with time. For this study, we analysed the soil respiration rates at second 240 of each high-diversity grassland plot including *A. elatius, H. lanatus, P. lanceolata* and *G. pratense* on the last day of drought manipulation.

Maximum leaf and canopy uptake rates

Net ecosystem CO_2 exchange was measured with chambers on 40 × 40 cm frames established on each of the treatment plots. Daily course of net ecosystem CO_2 exchange (NEE) was measured using manually operated, closed gas exchange canopy chambers. Light-response curves depicting the net photosynthetic CO_2 uptake rate (A) of plants at any measuring time were obtained from leaf-level gas-exchange measurements by fitting an empirical rectangular hyperbola model (Gilmanov *et al.* 2005): $\mathrm{NEE} = (\alpha+Q\ /\ \alpha Q-\beta) - \gamma$, where α is the initial slope of the light-response curve and an approximation of the canopy light utilization efficiency (mol CO_2/ mol PAR), β is the maximum CO_2 uptake capacity (μmol m^{-2} s^{-1}), Q is the photosynthetically active radiation (PAR, in μmol m^{-2} s^{-1}), and γ is an approximation of the average daytime ecosystem respiration (lmol m^{-2} s^{-1}). An approximation of maximum canopy uptake capacity was extrapolated from leaf-level measurements. Canopy net ecosystem exchange rate (NEE) was estimated from leaf photosynthetic rate at saturating light intensities (it was shown that A at PAR = 2000 μmol m^{-2} s^{-1} correlates well with canopy NEE). Maximum gross primary productivity (GPP_{max}) was calculated as: $GPP_{max} = NEE_{2000} - R_{eco}$, where A_{2000} is the maximum leaf photosynthetic rate at a saturating level of light intensity andR_{eco} is the corrected respiration term (γ) obtained from the model.

Nutrient cycling

In situ decomposition rate of cellulose

Biological activity of soil fauna and microorganisms was determined indirectly from the decay of cellulose using mini-container tubes (Kreyling *et al.* 2008a). In total, 864 mini-containers were filled with 0.2 g of cellulose (poor in phosphorus, Schleicher & Schüll, Dassel, Germany) each, closed with a 2-mm mesh, and put into container tubes, consisting of 12 mini-containers each. Two tubes were buried horizontally 1 cm below soil surface in each grassland plot. After 94 days, one tube per plot was harvested, whereas the others were harvested after 186 days. After careful cleaning and drying, the decay of cellulose was determined by subtracting final ashes-free dry mass from initial dry mass (105 °C).

Mycorrhizal colonization

One complete plant individual of *P.lanceolata* was taken from each plot on the last day of drought using a soil core sampler with 5 cm diameter (Eijkelkamp; Netherlands). This particular species was chosen, because pre-analysis revealed higher effects of drought on mycorrhizaal colonization of *P. lanceolata* than on that of other species tested. Roots were cut off and fixed in formalin-alcoholic-acid (50 % Ethanol, 40 % H_2O, 7.5 % formalin, 2.5 % acidic acid), and stained with 5 % blue ink vinegar solution after boiling in 10 % KOH. Afterwards, mycorrhization ratios were determined by scanning 15 cm fine roots of each sample for arbuscules and vesicules under a microscope (400×) using the "magnified intersection method" (McGonigle *et al.* 1990).

Soil microbial nitrogen pool

Soil microbial nitrogen was extracted from fresh soil according to a modified chloroform fumigation–extraction method (Brookes *et al.* 1985). After chloroform fumigation (24 h at room temperature), dissolved organic and microbial N was extracted with 50 mL 0.5 M K_2SO_4 and quantified (DIMA TOC-100, Dimatec, Essen, Germany). Microbial biomass and relative abundance of microbial groups were measured using phospholipid fatty acid (PLFA) analysis as described (Singh *et al.* 2006).

Potential soil enzyme activities

For soil enzyme activity measurements, enzymes involved in carbon, nitrogen and phosphorus cycling were selected, thus addressing important microbial soil functions (Waldrop & Firestone 2006). The enzyme activities tested were acid phosphatase cleaving organically bound phosphate, cellobiohydrolase, β-xylosidase and β-glucosidase related to the degradation of plant cell wall components and N-acetylglucosaminidase representing chitinases that degrade chitin from fungal or arthropod origin. Soil samples for determining soil enzyme activities were collected immediately after finishing the drought manipulations (Kreyling *et al.* 2008a). Four samples per plot (depth 0 – 5 cm) were combined, mixed and kept at 4 °C until further processing within 4 weeks after sampling. Soil suspensions (0.4 g fresh soil in 40 mL H2O) were prepared from each sample. The assay is based on the enzymatic cleavage of the below-detailed methylumbelliferone (MU) coupled substrates and the subsequent detection of MU released during incubation. In brief, 50 µL per well of soil suspensions (three replicates each sample) were dispersed in microplates and 100 µL of substrate solutions were added to start the reactions. After stopping the reaction with 100 µL of 2.5 M Tris buffer and centrifugation, MU concentrations were determined on a fluorescence spectrometer at excitation/emission wavelengths of 365/450 nm, respectively. The following enzyme substrates were used with the incubation times given: MUF-phosphate, 20 min; MUF-xyloside, 1 h; MUF-cellobiohydrofurane, 1 h; MUF-N-acetyl-β-glucosaminide, 40 min; MUF-β-glucoside, 1 h. Substrate concentrations in the incubation mix were 500 µM except for MUF-cellobiohydrofurane with 400 µM. To account for quenching and to calculate the amount of MUF released, calibration curves were included with 50 µL of soil samples as in the incubation wells and MUF-solutions to give a final amount of 0 - 500 pmol per well. Negative controls for autofluorescence of substrates were also included. Enzyme activities are expressed as MUF-release per gram soil dry weight per hour.

Plant-available soil nitrate and ammonium

Plant-available nitrogen was extracted from four homogenized, sieved (< 2 mm), mixed samples of the upper soil layer (0-10 cm) of each plot sampled in July using a 1 M KCl solution after filtration (Roth, Karlsruhe Germany, Typ 15 A Blauband) (Kreyling *et al.* 2010). Nitrate and ammonium were quantified using flow injection analysis (FIA, MLE Dresden FIA-LAB).

Leaf carbon to nitrogen ratio

Leaf carbon (C), leaf nitrogen (N) and C:N ratios were measured from mixed samples of two sun-exposed leaves of five individual plants per species and plot, sampled in July (Kreyling, Beierkuhnlein & Jentsch 2010). The samples were oven-dried for 48 h at 75 °C. The dry leaves were ball-milled and subsamples of 1 mg analysed with an elemental analyser in a mass spectrometer using ConFlo III interface. Plant-available nitrogen was extracted from four homogenized, sieved (2 mm) and filtered (Roth, Germany, Typ 15A Blauband) mixed samples of the upper soil layer (0–10 cm) of each plot using a 1 M KCl solution.

Leaf protein content

Total protein content in µg per mg fresh weight was determined as a proxy for nutritive value of the legume key species *H. lanatus*, which was growing in all plots. One leaf sample per plot was taken on the last day of drought treatment, frozen in liquid nitrogen and freeze-dried to determine protein-bound amino acids. Amino acids of the protein fraction were extracted. Amino acid concentrations were measured with an ion exchange chromatograph (Biotronik, amino acid analyser LC 3000) and protein content was calculated by pooling the content of each amino acid in the protein fraction.

Leaf nitrogen isotope signal

Equally aged, south-facing leaves of *A. elatius* were collected and oven-dried at 60 °C for 48 h, and then fine-milled. Natural abundance of δ15N and total nitrogen concentration were analysed using an elemental analyser (EA 3000, EuroVector, Italy) coupled online to a ConFlo III interface (Thermo Electron, Bremen, Germany) connected to an isotope-ratio mass spectrometer (MAT 253, Thermo Electron, Bremen, Germany) . The δ15N values were calculated as: δ15N [‰] = (Rsample/Rstandard) -1)*1000, where R represents the ratio of 15N:14N isotopes. As standard, (nitrogen in) air was used.

Community responses

Invasibility

Invasibility of the experimental communities was recorded three times per year: before and after the drought manipulations in early summer, and in fall (Kreyling *et al.* 2008c). Invading plant individuals were collected from the inner square metre of each plot and subsequently separated by species. Removal took place only after the first true leaves (after the cotyledons) emerged, but most specimens were considerably older than this and clearly established in the stand. At this point in development, we expected that number of individuals give a measure of established invaders rather than chance germinations. For each plot, the number of individuals was determined. The planted target species of the experiment were removed from the subsequent analysis. Tests confirmed that germination from the soil seed bank was negligible after one year. Thus, invasibility was only based on species invading from the matrix vegetation.

Plant compositional change

The measurements of above-ground species-specific cover (s. above) were used to evaluate shifts in the species abundance distributions of the artificial plant assemblages. Compositional change of each individual plot was evaluated by comparing the species abundance distribution at each time step to the initial species abundance distribution (five weeks after planting) by the Bray–Curtis index.

Competitive effect / facilitative effect

The Relative Neighbour Effect calculates the effect of neighbours relative to the plant with the greatest performance: RNE = P_{contr}-P_{mix}/x with x = P_{contr} if $P_{contr} > P_{mix}$ and x = P_{mix} if $P_{mix} > P_{contr}$, where RNE = Relative neighbour effect ($-1 \leq RNE \leq +1$), P_{contr} = performance per plant for a plant growing alone ,P_{mix} = performance per plant for a plant growing in mixture. Negative values indicate facilitation, and positive values indicate competition (Markham & Chanway 1996).

Senescence

Tissue die-back was quantified by cover measurements of standing-dead plant organs (Kreyling *et al.* 2008d). A pin-point method was applied, recording the presence of plant organs in general and the presence for each species separately at 100 vertically inserted steel needles. These values were treated as percentage cover. The measurement was repeated four times over the course of the vegetation period.

Variability in length of flowering

For each species, weekly observations of the flowering status of four individuals per plot and species were carried out (Jentsch *et al.* 2009). Individuals were counted as 'flowering' when the anthers were visible in at least one flower. Flowering length was calculated as the difference between the dates of the 25 and 75 percentile of the flowering curve over time. Variability in length of flowering was obtained as the standard deviation between all species for each treatment (drought and control) separately. Statistical significance of difference in variability was evaluated by the Levene test.

Variability in flower phenology

Flower phenology was obtained from the same data as length of flowering (see above). As a surrogate, the mid-flowering date was calculated for each species and plot, i.e. the date of the 50 percentile of the flowering curve over time. Variability in flower phenology was expressed as the standard deviation between all species for each treatment (drought and control) separately. Statistical significance of difference in variability was evaluated by the Levene test.

Resistance to herbivory (phenol content)

For analysis of total soluble carbohydrates and total phenolics, three mixed samples of at least two plants per plot were taken at the end of the drought period, immediately frozen in liquid nitrogen and lyophilized (n=15). Thirty miligrams were extracted in 50 % methanol. Total soluble carbohydrates were analysed using the anthrone method with glucose as a standard. Extinction was measured at 620 nm. Total phenols were analysed using FolinCiocalteu's reagent and catechin as a standard and measuring extinction at 750 nm.

Primary consumer abundance

Richness was sampled in June in one circular area (40 cm diameter) in each grassland plot using a D-Vac suction sampler (ecotech GmbH, Bonn, Germany). For each plot, the sampling bag was removed and all sampled material was stored in ethanol. Arthropod samples were quantified as the total number of individuals and identified at least to order level. However, some taxa were identified to the family level (families within the *Coleoptera, Hemiptera*, most *Hymenoptera*) and in one case to genus level (*Psylliodes [Chrysomelidae*]. The use of higher taxonomic levels has been shown to produce a good approximation of total species richness (Biaggini *et al.* 2007).

Statistical Analyses

Linear Models combined with analysis of variance (ANOVA) were applied to test for significant differences between groups at single points of time, while taking the split-plot design into account. Homogeneous groups of factor combinations (drought manipulation, vegetation type, diversity level) were identified by Tukey HSD *post hoc* comparisons. Level of significance was set to p<0.05. Statistical significance of difference in variability of length in flowering was evaluated by the Levene test.

For time series, Linear Mixed-Effects Models were employed to test for effects of drought manipulation and diversity and their respective interactions while taking the split-plot design and the repeated measures into account (time used as random factor). When no significant interaction was found, the model was simplified by using only the drought manipulations as fixed effects and time as random effect. Significance of differences ($p < 0.05$) was evaluated by Markov Chain Monte Carlo sampling of 1000 permutations. Linear Mixed-Effects Models were conducted with the function ‘lmer’ (Bates & Sarkar 2007).

Prior to statistical analysis, data was log- or square-root-transformed, if conditions of normality were not met, or to improve homogeneity of variances. Both characteristics were tested by examining the residuals versus fitted plots and the normal qq-plots of the linear models. All statistical analyses were performed using R.

Results

The effects of drought on all measured ecosystem properties are summarized in Fig. 1 using response ratios to standardize the effect size of the severe drought treatment.

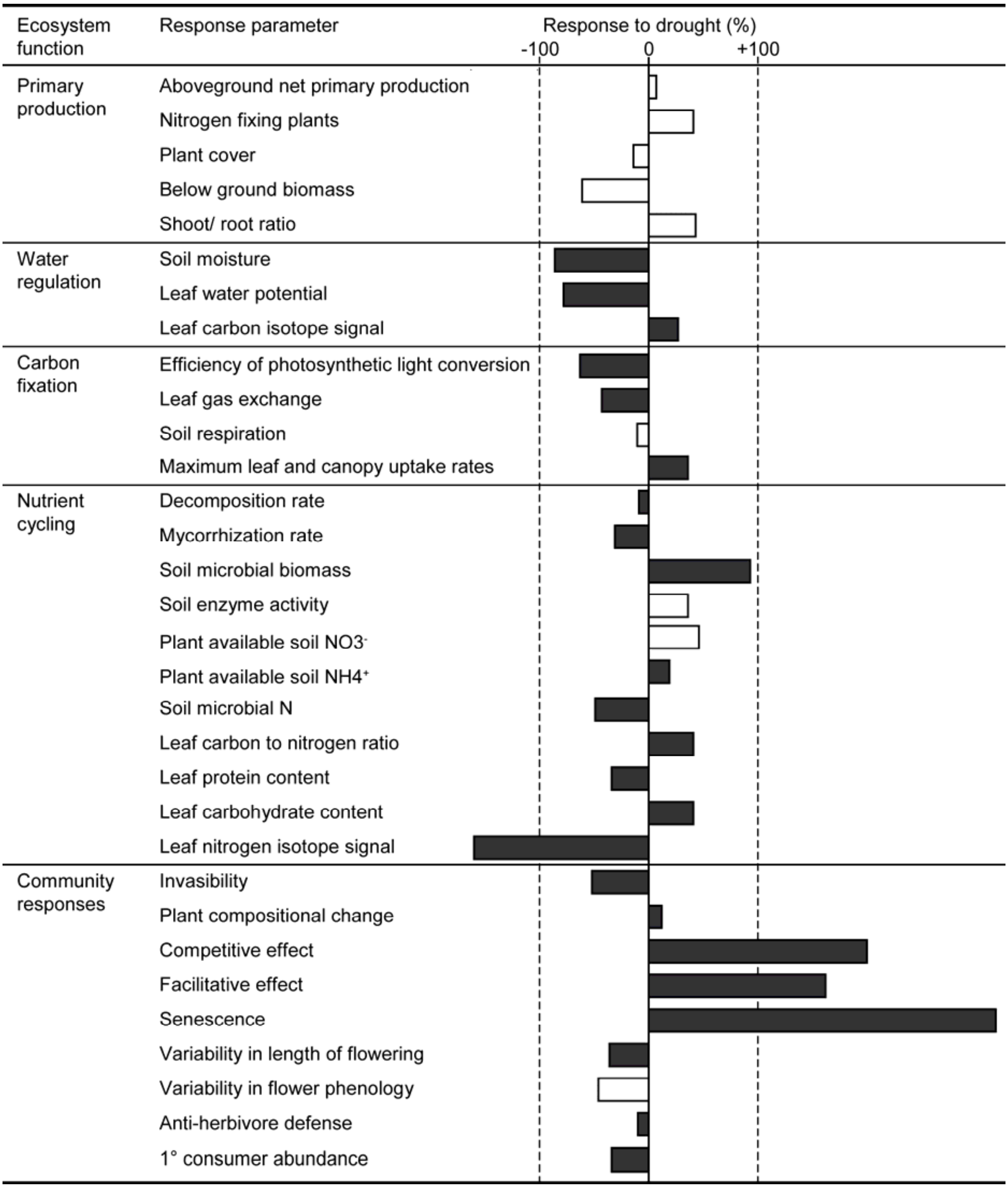

Figure 1: Effects of recurrent severe drought events on 32 response parameters organized into ecosystem functions. All data were collected at the EVENT I experimental site (Jentsch, Kreyling & Beierkuhnlein 2007) in Central Europe during the years 2005 - 2009. A parameter is marked as significant (filled black bar), if data of at least one year showed significant differences between drought and ambient conditions (ANOVA). Data shown represent maximum effects from years with highest drought effects, averaged over all three experimental grassland communities. For references of published details please refer to Materials and methods section

Water regulation

Severe drought significantly reduced soil moisture during the manipulation periods in all years (Figs 1, 2).

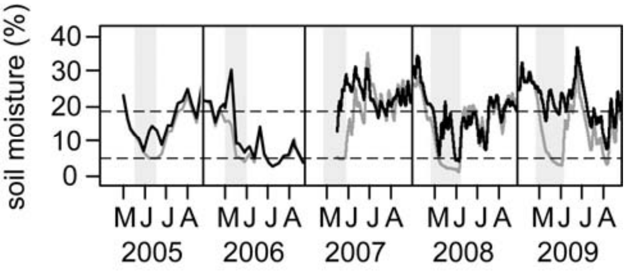

Figure 2 Soil moisture in the EVENT experiment at -2 to -7 cm during manipulation (light grey boxes) and recovery after extreme drought for control (black line) and drought (grey line). MJJA = May, June, July, August. Plant available water is shown between the dashed lines: permanent wilting point (pF = 4.2) and field capacity (pF = 1.8). See Materials and methods for technical details.

A high variability both within years and between years is evident due to inter-annual variability of precipitation (Table 2). Even though absolute minima in soil moisture were similar for drought and control in most years, soil moisture of the drought plots remained considerably longer below the approximate permanent wilting point (pF = 4.2) for the soil substrate. The manipulation effect vanished within days for all years except 2009, where a lag phase of about two months until August occurred.

Table 2 Temperature and precipitation sums (added daily amount) for each year until the start of the drought manipulation and the respective alteration from the long-term mean (1971-2000, data: German Weather Service station Bayreuth)

year	temperature sum (1 January to start of manipulation)	relative change of temperature sum compared to long-term mean (%)	precipitation sum (1 January to start of manipulation)	relative change of precipitation sum compared to long-term mean (%)
2005	824.7	-3	259.7	-9
2006	394.7	-38	208.3	+10
2007	978.7	+77	258.6	+9
2008	757.6	+40	282.2	+19
2009	574.9	+4	246.4	+4

Further, drought decreased leaf water potential, while increasing leaf carbon isotope signal in some species (Figure 1).

Primary production

At the level of the grassland community or ecosystem, respectively, local, annually recurrent 100-year and 1000-year extreme drought events had no significant effect on various processes that contribute to primary production in any of the five years from 2005 to 2009 (Figs 1, 3). Surprisingly, neither above-ground primary production (ANPP), nor green cover of vegetation or below-ground production recorded as root length in the main rooting horizon were affected by drought (Figs 1, 3).

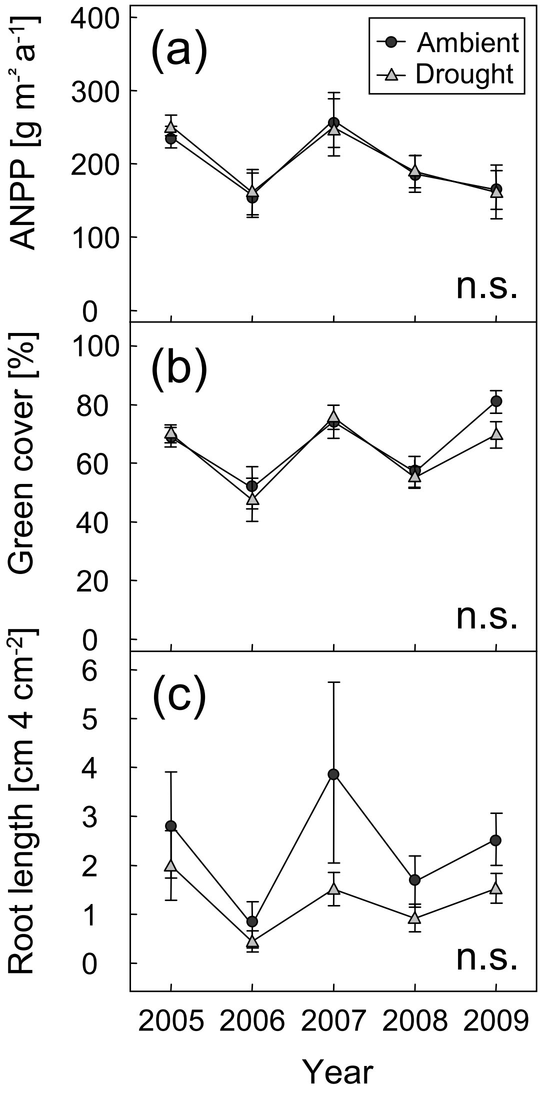

Figure 3: (a) Above-ground Net Primary Production (ANPP) , (b) cover of green biomass, and (c) root length over five growing seasons (mean ± SE over all species compositions in grassland, n = 15 per data point). An asterisk marks significant treatment effects (ANOVA, Tukey HSD *post hoc* comparison: $p < 0.05$)

Further, there was no significant drought effect on biomass production of the nitrogen-fixing plant *L. corniculatus* (Figure 1).

Carbon fixation

Drought increased the maximum uptake capacity (GPPmax) in grassland by 36 % (Figure 1). The soil respiration rate (R_{eco} calculated by the model was lower under drought than under ambient conditions. Soil respiration was slightly but not significantly decreased at the end of the drought.

Nutrient cycling

Nutrient cycling in soil was clearly affected by drought (Fig. 1). The annually recurrent drought events increased ammonium content in soil, whereas soil microbial N was decreased. Overall turnover rates were reduced, indicated by decreased decomposition rate of cellulose and potential enzymatic activities. The relative abundance of different microbial groups except for arbuscular mycorrhiza remained unchanged.

Remarkably, despite stability in biomass production, drought decreased leaf protein content and the leaf nitrogen isotope signature and increased C:N ratio and carbohydrate content in leaves, thus decreasing feed value of plant tissue.

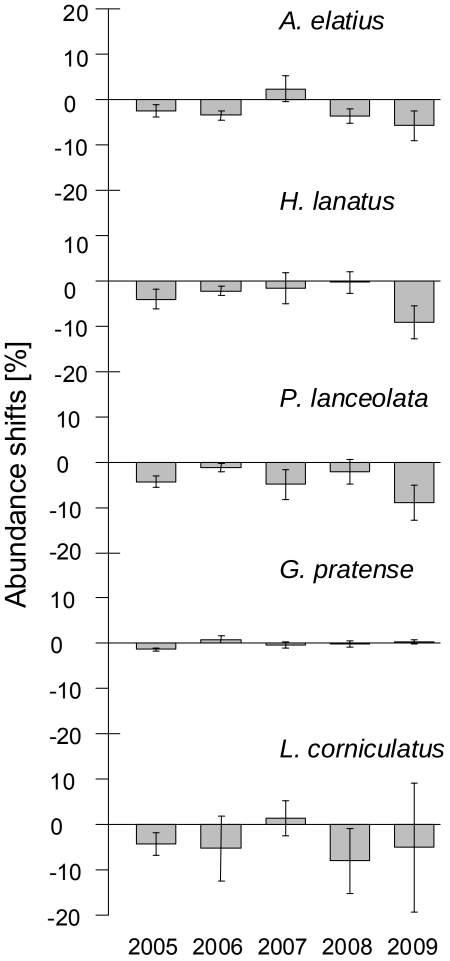

Figure 4: Abundance shift (%) of the grassland species *Arrhenatherum elatius, Holcus lanatus, Plantago lanceolata, Geranium pratense* and *Lotus corniculatus* for the years 2005 to 2009 (mean ± SE of the absolute deviation of species cover under drought from the mean of control, n = 15 for *Arrhenatherum elatius & Holcus lanatus*, n = 10 for *Plantago lanceolata*, n = 5 for *Geranium pratense & Lotus corniculatus* per data point). No significant drought effects on species cover (ANOVA for each year and Linear Mixed-Effect Model for long-term trends: $p < 0.05$)

Community responses

Some ecosystem properties associated with community stability were positively affected by drought. For example, annually recurrent drought events reduced the invasibility of plant communities and, thus, increased community stability. Remarkably, recurrent severe drought did not cause any shift in the absolute abundance of species, thus, it did not cause any compositional change within five years (Figure 4), although it induced complementary, species-specific plant–plant interactions resulting in shifts in species-specific biomass contribution to overall community production. For example, the competitive effect of neighbouring plants on *L. corniculatus* was increased by drought as well as the facilitative effect of neighbouring plants on *A. elatius*.

Still, a significant difference between drought and control was found in community composition when comparing the species abundance distribution at each time step to the initial species abundance distribution by a similarity index. Further, drought increased leaf senescence and caused shifts in flower phenology with regards to variability in length of flowering and mid-flowering day of particular species in some years (for detailed results on shifts in phenology see Jentsch *et al.* 2009). According to the decreased feed value of plant tissue, primary consumer abundance was decreased by drought.

Discussion

Our experimental approach has the ambitious goal to search for a synthesis of the wide range of drought responses collected in a single study. Our goal is to see whether general patterns about different categories of responses can be drawn within a single temperate grassland study system. In the following, we first discuss particular drought responses, and then suggest potential reasons why the responses may differ among the five major ecosystem functions.

Water regulation

Soil moisture dynamics and other soil-related parameters integrate how biological systems respond to climate change (Emmett *et al.* 2004). Soil water content was significantly reduced by drought in our experiment, but there were strong differences between years (Figure 3). Natural precipitation during the manipulation periods is of importance here, as the years 2005 to 2008 all included some natural dry spells and effect size of the drought manipulation therefore was bigger in 2009 when no such natural event occurred. Still, it is not completely clear how precipitation regimes translate into variation of the soil moisture regime (Weltzin *et al.* 2003; Potts *et al.* 2006; Dermody *et al.* 2007). There is a growing number of studies explicitly addressing time lags between precipitation manipulation and the soil moisture regime (Dermody *et al.* 2007; Sherry *et al.* 2008), soil moisture storage (Potts *et al.* 2006) or soil hydrological properties as affected by interacting climatic drivers (Bell *et al.* 2010). However, re-wetting dynamics (Xiang *et al.* 2008), soil drying (St Clair *et al.* 2008) and potential carry-over effects between recurrent heavy rainfall or drought events have not been analysed in much detail. The transformation of precipitation pulses to increased soil water contents available to plant roots and soil biota for uptake can be complex: soil depth, soil texture, parent material, organic matter content, vegetation type, presence of plant functional types, leaf area index and soil surface characteristics all affect the partitioning between interception, run-off, infiltration and subsequent hydraulic re-distribution, soil evaporation, plant water uptake and seepage (Loik *et al.* 2004; Bell *et al.* 2010).

Amount, frequency and seasonal timing of soil water available for plants, soil fauna and soil microbes will basically determine much of the ecosystem response to more extreme precipitation regimes. While in this experiment we only manipulated the amount of soil water available to plants, seasonality issues appear to be an emerging research frontier. Yet, the major remaining challenge is to assess how future precipitation regimes with more extreme precipitation events affect—due to alterations in soil moisture—biogeochemical cycles, biotic interactions and ecosystem functions.

Primary production

We found that drought has resulted in pronounced effects in the functional performance such as carbon fixation and nutrient cycling of plant communities and of individual species as well as in fluxes and pools. However, all ecosystem properties related to primary production remained stable throughout all five years of the experiment, despite recurrent severe drought events and despite different pre-experimental soil water status between years. In temperate grasslands, experimental drought events tend to reduce biomass productivity (Sternberg *et al.* 1999; Grime *et al.* 2000; Kahmen *et al.* 2005). Fay *et al.* (2003), however, showed that the magnitude of reduction in above-ground net primary productivity (ANPP) is the same if rainfall quantity is reduced by 30 % or if inter-rainfall-intervals are increased by 50 % without a change in the annual amount of precipitation. Presumably, complementary responses in species interactions contributed to buffering primary production at the community level without changing community composition in our experiment (Wang et al. 2007, Kreyling et al. 2008). For example, the competitive effect of neighbouring plants on

L. corniculatus was increased by drought as well as the facilitative effect of neighbouring plants on *A. elatius*. This is in accordance with a long-term study of 207 grassland plots, which demonstrated that biodiversity stabilizes community and ecosystem processes, but not population processes (Tilman 1996). Here, primary production was one of the key parameters studied. The persistence of this general ecosystem function was stronger than expected. Concerning below-ground production, several studies (Newman *et al.* 2006; Trillo & Fernandez 2005) report increased root biomass in response to chronically decreased water supply, while a complete water withdrawal over defined periods of time result in stable or decreased below-ground biomass (Kreyling *et al.* 2008a).

Carbon fixation

Results from ecosystem CO_2 measurements showed a 36% increase in GPP during drought in the grassland, but a reduction in the assimilatory capacity of the leaves (Figure 1). During water stress, there was an increase in tillering, leading to increased photosynthetic area of particular species, yet not an increase in absolute cover or green cover of the community. Thus, even though CO_2 assimilation was reduced at leaf level as a result of water stress, the overall effect of the large leaf area presented by the tillers lead to an increase in the contribution of particular species to ecosystem productivity, compensating for reduced photosynthetic rates at leaf level. Declining stomatal conductance as a result of stomatal closure was responsible for the observed low leaf-level CO_2 assimilation rates during stress. Zavalloni *et al.* (2009) reported a reduction in leaf assimilation, but increased biomass production in grassland subjected to extreme drought. In contrast, Stitt & Schulze (1994) point out that changes in photosynthesis not necessarily lead to changes in growth or biomass.

Nutrient cycling

Nutrient cycling was clearly affected by drought. The annually recurrent drought events increased leaf C:N and plant available soil ammonium, whereas they decreased the decomposition rate and mycorrhization rate. Obviously, water stress has an impact on the activity and abundance of ammonium oxidizing prokaryotes, resulting in increased ammonium in the soil, which, however, can hardly be taken up by plants (Gleason *et al.* 2010). The microbial community seems generally irresponsive to drought treatment where the only significant effect was an increase in microbial biomass, however the relative abundance of different microbial groups remained unchanged except for arbuscular mycorrhizal fungi. This is in accordance with other findings showing that drought changes community structure in arbuscular mycorrhizal fungi including their carbohydrate and nitrogen storage bodies, so that they take up less nitrogen (Shi *et al.* 2002). Our results suggested that composition of microbial groups in soils is generally resistant to drought treatment. This observation is in agreement with previous reports (Andersen *et al.* 2010, Williams 2007, Williams and Xia 2009). Both leaf C:N ratio and microbial data suggest that there was an increase in C:N ratio which may explain lower soil respiration under drought conditions. This may suggest lower activity of microbial communities which is reflected by the decreased rate of decomposition. In this study, leaf and microbial C:N ratio and litter decomposition responded to drought treatment, but biological and geochemical responses of climate treatment are complex (Andersen *et al.* 2010), and future work should include multi-factorial experiments taking into account environmental factors such as soil type, soil water and land use (Singh *et al*. 2010).

Additionally, our results show that extreme climatic events further affect the abundance of herbivores associated with the plant community. For instance, we suggest that the reduction of abundances of athropods by drought events may translate to changes in the top-down control of vegetation by herbivores and slowed decomposition dynamics due to a lower activity of decomposers.

Community responses

Relative importance of each species in a community context was affected by the drought treatments as measured by the similarity of species abundances to the starting conditions for each plot. The effect size, however, was comparably small, presumably because species compositions were held constant over the course of the experiment by weeding out non-target species. Furthermore, competitive balance, based on species-specific biomass production, was altered and variability in flowering was affected. Particularly, averaged over all species, drought advanced the mid-flowering day within the season and expanded the length of the flowering period. On the other hand, no significant shifts in relative abundance of single species were observed (Figure 4). Generally, however, shifts in species composition might require substantial lag phases (Grime *et al.* 2000; Buckland *et al.* 2001), especially as non-target species were not allowed to immigrate into our plots.

Limitations of the EVENT experiment

All the results discussed above stem from one site, i.e. one particular climate, one soil type, one form of experimental manipulations and a limited set of species. Certainly, an array of factors such as the investigated ecosystem type, time scales, level of nutrient availability, water holding capacity of soils, level of biodiversity, or particular design and execution of the experimental treatments will modify the effects of drought on ecosystem properties. Therefore, similar approaches from other sites and climatic conditions are clearly needed in order to test the generality of the observed findings. In particular, experiments with strongly controlled species compositions need to be compared to natural or semi-natural communities. Another important gap of knowledge that cannot be answered by our experiment is the importance of interactions between the climatic drivers, as there is clear evidence that effects of drivers such as warming, drought, N-deposition and CO_2-increase are not additive (Shaw *et al.* 2000; Andresen *et al.* 2010).

Generally, manipulation artefacts or hidden treatments are a concern for global change field experiments. Rain-out shelters are the usual device to simulate drought even though they are known to cause artefacts in the microclimate (Fay *et al.* 2000). Our artefact control treatment showed that the slight temperature increase and the alterations in irradiance or wind speed due to our shelters caused no effect on the measured response parameters, presumably because the shelters were active only during the short manipulation periods. Other artefacts, however, might be more important, yet less investigated, such as preferential site selection by animals due to the close proximity of different climatic conditions between the treatments blocks (Moise & Henry 2010). Such spatial patterns at small distances clearly differ strongly from drought effects at landscape levels.

We set the magnitude of the drought manipulation based on statistical recurrence of dry spells in the local climate data series (1961-2000). Recurrence of extreme events itself, however, is subject to climate change, leading to an amplification of precipitation extremes with ongoing climate change (Allan & Soden 2008). For the ambient conditions in our experiment, though, the statistical recurrence of the different manipulation years fell well within those of the long-term averages for air temperature, precipitation sum or length of rain-free periods (data not shown). This may be among the reasons, why we did not observe large effects on biomass production.

Conclusion

Our experimental data demonstrate that weather extremes initiate ecosystem-regulating functions such as water and nutrient cycling, gas exchange and compositional dynamics while maintaining primary production. They indicate an important contribution of ecological complexity to the maintenance of productivity in the face of increased temporal climate variability and extraordinary weather events. However, single species reactions can not be translated directly to the community and ecosystem level. A potential reason for different drought impacts on various ecosystem properties may lie in the temporal hierarchy of fast versus slow response patterns. In our temperate grassland, we observed the following response dynamics within half a decade of recurrent drought events: very fast alteration of soil moisture status, subsequent fast change in nutrient cycling and gas exchange, slow species-specific response in primary production, inertia in community productivity.

Such data on multiple response parameters within climate change experiments foster the understanding of mechanisms of resilience, of synergisms or decoupling in biogeochemical processes, and of fundamental response dynamics to drought at the ecosystem level.

As it was the case with the open questions on the consequences of the crisis of biodiversity, we see this complexity in studying impacts of climate extremes as a new chance for a boost in ecological theory. Additionally, comprehensive studies on the complex responses will help developing coping strategies for the adapted management of these ecosystems.

Future challenges consist of analysing responses for multiple ecosystem functions and at multiple levels of organization with the goal of assessing how they interact to influence emergent ecosystem properties, such as ecosystem function and stability. The observed stability in primary production in the face of recurrent severe drought does not mean that the responses at the ecosystem level are null. On the contrary, the observed changes in ecosystem regulating functions in terms of gas exchange, nutrient cycling, water regulation and community stability suggest a prominent role of extreme weather events in ecosystem response to climate change. However, modelling the behaviour of ecosystems during and after extreme climatic events at larger spatial scales and over longer periods of time requires more in-depth knowledge on possible response mechanisms at the level of plant communities. Potential epigenetic, physiological or trophic responses need to be rigorously further explored experimentally. Laboratory studies on molecular mechanisms have to be related to studies with the same species in the field. Field studies must integrate various levels of functional diversity (Beierkuhnlein *et al.* in press). Phenotypical diversity of populations has to be considered. Life cycles of plant species and cohorts can be of crucial importance. Gradients in soil types have to be integrated. Then, we can reach a better understanding of the mechanisms that are initiated in plant communities by extreme events.

Future work is needed to elucidate the role of biodiversity and of biotic interactions in modulating ecosystem response to extreme weather events. Further, we need more data on impacts of climate extremes on multiple ecosystem properties from various ecosystems and biomes, in order to foster the search for generality across different categories of response. Here, a major challenge is to assess the speed of response across various parameters, including long-term feedbacks, i.e. caused by a nitrogen-dependent feedback on productivity (Haddad *et al*. 2002).

Generally, scientists are challenged by relating the ecosystem properties measured (here: net ecosystem exchange, biomass above and below ground, carbon fixation by photosynthesis, nutrient ratios) to ecosystem functions and services, such as productivity, carbon fixation, nutrient cycling, decomposition and water regulation. Measuring ecosystem services is a fast-developing research area with many debates on how to assess the services adequately.

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Online Supporting Information

Table S1: Search items for searching the ISI Web of Science® Database for publications on weather events and climate extremes. Asterisks are place holders within the search string

Main category	Search items in ISI Web of Knowledge
Frost	*frost event* OR severe night frost* OR *spring frost* OR freeze-thaw* OR thaw-freeze* OR late frost* OR severe frost* OR *ground frost* OR extreme frost* OR extreme cold*
Heat & drought	heat wave* OR heatwave* OR severe heat* OR *temperature event* OR dry spell* OR extreme heat* OR *winter warming* OR warm* winter OR summer drought* OR spring drought* OR autumn drought* OR severe drought*
Storm	extreme storm* OR *winter storm* OR hurricane* OR typhoon* OR cyclone* OR tornado* OR storm surge* OR *windstorm* OR *wind storm* OR *tropical storm* OR ice storm*
Heavy rain	extreme flood* OR summer flood* OR extreme rain* OR torrential rain* OR extreme precipitation OR *rainfall event* OR heavy rain* OR hail* OR wet spell*
Extreme	extreme event* OR extreme weather event* OR climat* extreme* OR extreme meteorological event* OR extreme weather* OR extreme climat* event*

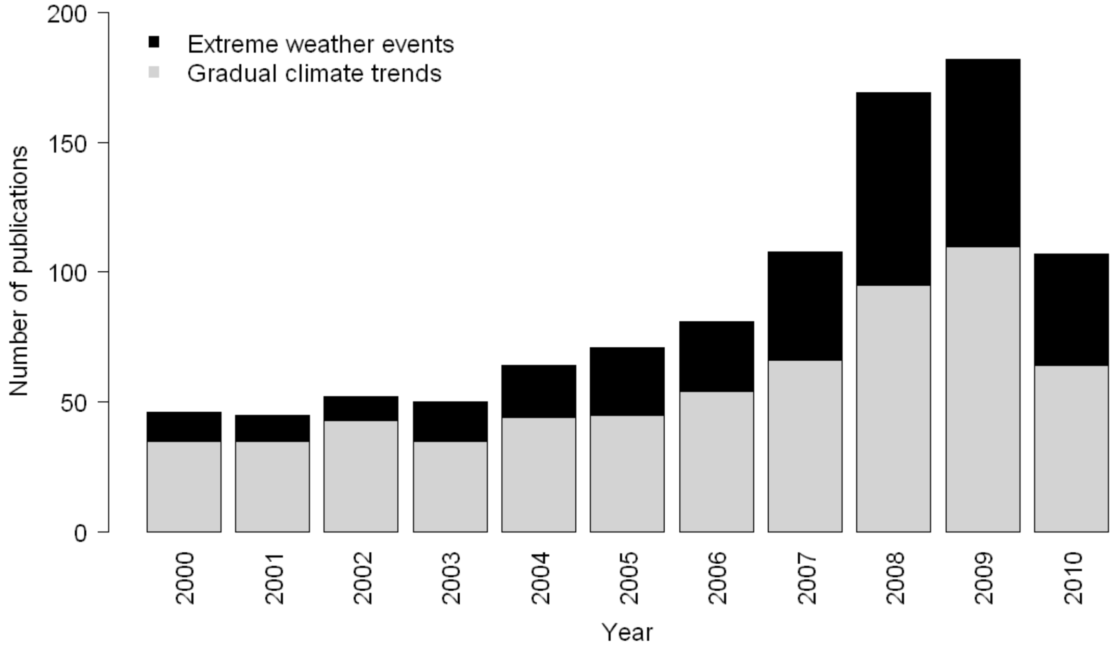
Table S2: Links for searching the ISI Web of Science® Database for publications on weather events and climate extremes

Links	Search items in ISI Web of Knowledge
Main items in "Topic"	Frost, Heat & drought, Storm, Heavy rain, Extreme
AND in "Topic"	wetland* OR floodplain* OR peat* OR bog* OR fen* OR swamp* OR mire OR grassland* OR meadow* OR pasture* OR heath* OR shrubland* OR forest* OR woodland* OR tundra OR taiga OR savanna* OR marsh* OR steppe OR desert* OR aquatic* OR limn* *bird* OR avian OR insect* OR butterfly* OR beetle* OR arthropod* OR moth* OR amphibian* OR reptile* OR mollusc* OR mollusk* OR vertebrate* OR *invertebrate* OR mammal*
AND in "Topic"	climat* change* OR global change* OR climat* warming
NOT in "Topic"	palaeo* OR paleo* OR pleistocene OR holocene

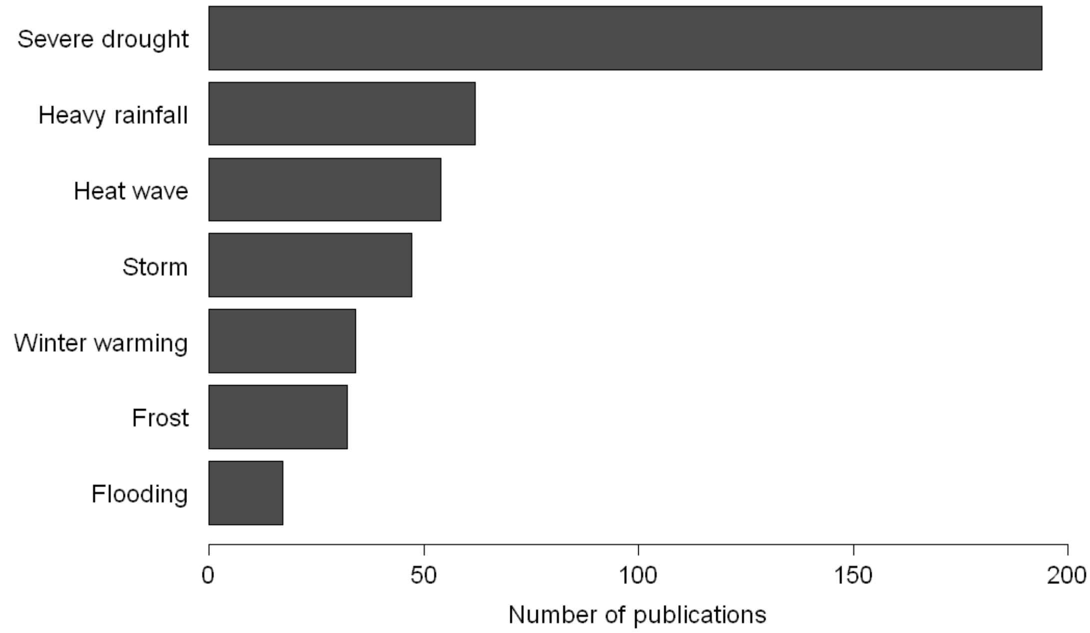
Table S3: Sampling years of all response parameters presented in Figure 1. Given are data from years with maximum drought effect

Ecosystem service	Ecosystem property	Year of sampling with maximum drought effect
Primary production	Above-ground production (ANPP)	2005
	Nitrogen fixing plants	2009
	Plant cover	2009
	Below-ground biomass	2007
	shoot / root - ratio	2006
Gas exchange	Maximum carbon uptake capacity	2005
	Photosynthetic performance	2008
	Leaf gas exchange	2007
	Soil respiration	2010
Nutrient cycling	Decomposition rate	2007
	Mycorrhization rate	2008
	Soil microbial biomass	2008
	Soil enzyme activity	2006
	Plant available NO3-	2008
	Plant available soil NH_4	2008
	soil microbial N	2009
	Leaf C/N-ratio	2009
	Leaf protein content	2009
	Leaf carbohydrate content	2009
	Leaf nitrogen isotope signal	2007
	1° consumer abundance	2008
Water regulation	Soil moisture	2009
	Leaf water potential	2008
	Leaf carbon isotope signal	2007
Community responses	Invasibility	2006
	Plant compositional change	2005
	Senescence	2006
	Variability in length of flowering	2006
	Variability in flower phenology	2008
	Resistance to herbivory	2009
	Competitive effect	2007
	Facilitative effect	2007

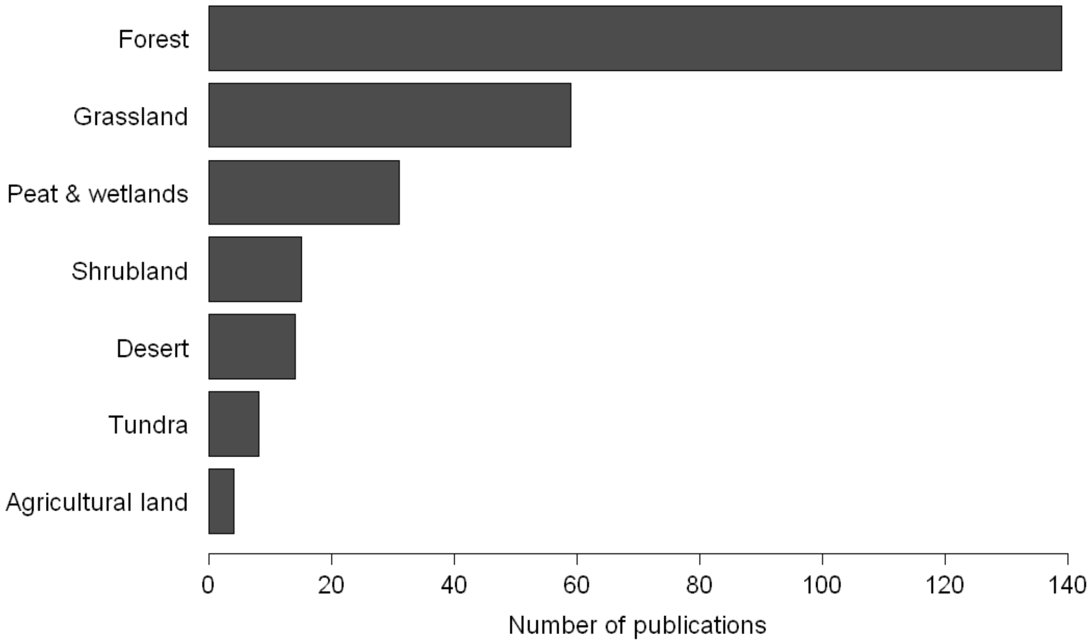
(a)


Extreme weather events
Gradual climate trends
Number of publications
0
50
100
150
200
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
Year

(b)


Severe drought
Heavy rainfall
Heat wave
Storm
Winter warming
Frost
Flooding
0
50
100
150
200
Number of publications

(c)


Forest
Grassland
Peat & wetlands
Shrubland
Desert
Tundra
Agricultural land
0
20
40
60
80
100
120
140
Number of publications

(d)

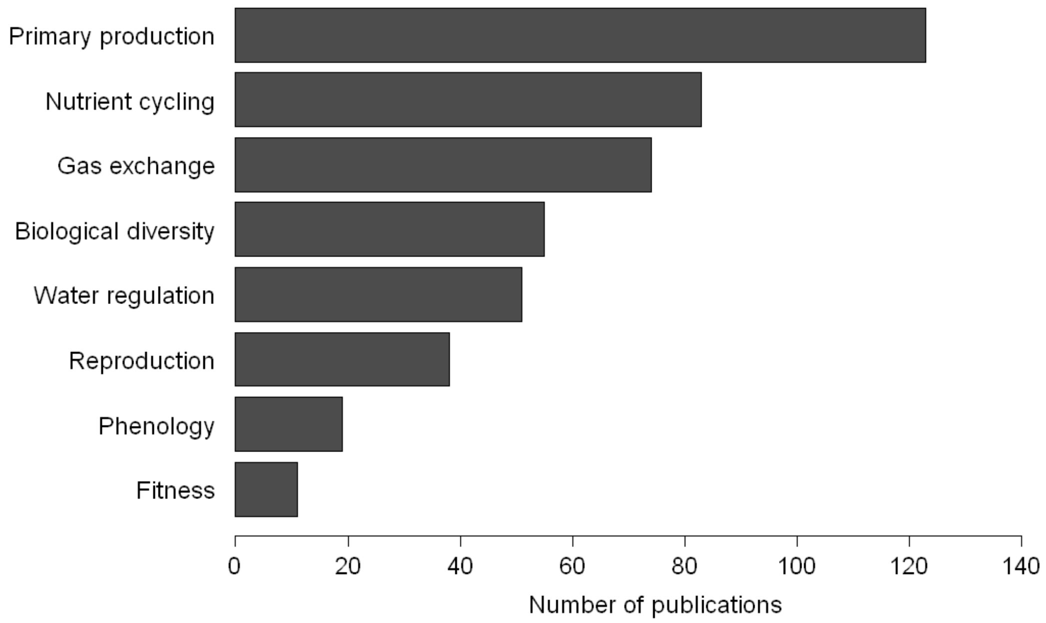


Figure S1: Research on ecological effects of climate extremes and weather events based on publications found in the ISI Web of Science (for search details see Table 2) (a) Temporal development of the number of publications on climate extremes (n=380) in the last two decade (shown is only the last decade); total yield 1134 peer-reviewed papers (b) Studied extreme weather events (n=464 inlcuding double or triple assignments) of the relevant peer-reviewed papers (n=380) yielded by the literature study. 24 publications did not specify the event. (c) Research activity in the three main biomes by proportion of publications based on 380 peer-reviewed papers particularly studying effects of climate extremes on ecosystem functions. Grassland includes deserts, peat and wetlands. Shrubland includes tundra. Any one paper may have been assigned to multiple subject areas. (d) Studied effects of extreme weather events on ecosystem properties arranged by ecosystem services and functions based on 380 peer-reviewed papers particularly studying effects of climate extremes on ecosystem functions

Manuscript 2: How do extreme drought and plant community composition affect host plant metabolites and herbivore performance?

Arthropod-Plant Interactions, 2012, **6**: 15-25.

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Abstract

Water availability and plant community composition alter plant nutrient availability and the accumulation of plant defence compounds therefore having an impact on herbivore performance. Combined effects of drought stress and plant community composition on leaf chemicals and herbivore performance are largely unexplored. The objective of our study was, therefore, to find out the impact of extreme drought and of plant community composition on plant-herbivore interactions. Larvae of the generalist butterfly *Spodoptera littoralis* were reared on leaves of the grass *Holcus lanatus* which was grown in experimental communities, differing in species- and functional group-richness. These communities were either subjected to extreme drought or remained under ambient climatic conditions. Drought decreased relative water content, soluble protein content, nitrogen and total phenol content and increased the content of carbohydrates in the grass. As a consequence, the larvae feeding on drought-exposed plants revealed a longer larval stage, increased pupal weight and higher adult eclosion rates. Plant community composition mainly caused changes to the defensive compounds of the grass, but also marginally affected protein and carbohydrate content. Larvae feeding on species-richest communities without legumes showed the highest mortality. Our findings imply that climate change that is projected to increase the frequency

of severe droughts, as well as alter plant community compositions, is likely to affect arthropod-plant interactions through an alteration of leaf chemicals.

Keywords: climate change; EVENT-experiments; drought; diversity; legume

Introduction

Under global climate change, the variability of precipitation regimes is projected to increase, and is likely to increase the frequency and severity of droughts (Trenberth et al. 2003; IPCC 2007). For the 21st century, droughts are projected to occur more frequently, and more regions, also within Europe, will be affected by severe dry spells (Beniston et al. 2007; IPCC 2007; Li et al. 2009; Wang et al. 2010). Droughts can reduce productivity, but may also alter forage quality in mesic grassland (Heisler-White et al. 2009).

Several studies tested the effect of drought on plant-herbivore interactions (EnglishLoeb et al. 1997; Inbar et al. 2001; de Bruyn et al. 2002; Scheirs and de Bruyn 2005; Nguyen et al. 2007). Drought often resulted in decreased performance of the herbivore (growth, survival), mainly due to an increase in defensive compounds and a decrease in leaf nitrogen (Herms and Mattson 1992; EnglishLoeb et al. 1997; Inbar et al. 2001; de Bruyn et al. 2002). Such studies are in accordance with the so called “plant vigour hypothesis” (Price 1991), as it predicts a better performance of herbivores on vigorously growing plants, compared to stressed plants.

Up until now, most studies investigating the influence of drought on plant tissue quality and herbivore performance have been conducted on potted plants and not under realistic plant-growing conditions (EnglishLoeb et al. 1997; Inbar et al. 2001; de Bruyn et al. 2002; Showler and Moran 2003; Scheirs and de Bruyn 2005). Under field conditions, soil moisture levels do not drop as quickly as in potted plants. Thus, plants might have more time to acclimate, which will result in a different leaf tissue quality compared to plants growing on very quickly drying soils.

However, not only abiotic conditions, like drought, affect water- and nutrient availability for plants. Also biotic factors, like plant community composition, e.g. the specific species assembly and, as a consequence, competitive or facilitative interactions, affect resource availability. Both, abiotic conditions and plant community composition are therefore key determinants for plant survival, plant growth and tissue quality, and might thus also affect herbivores (Schädler et al. 2007). A very important factor, which has been widely neglected in studies on single potted plants, is the influence of plant community composition on plant metabolites and therefore herbivores: species richness as well as functional group richness alter resource partitioning among plants. Higher species diversity might buffer the adverse effects of abiotic stress (Loreau and Hector 2001), but could also change competitive pressure and facilitative interactions under extreme abiotic conditions (Callaway and Walker 1997). It is very likely that it is not the sheer richness of species, but more so the presence of certain functional types that has an effect. The presence of legumes in particular, seems to play a key role in nutrient availability and ecosystem functioning in many studies, by increasing the protein and nitrogen contents of neighbouring species (Spehn et al. 2002; Temperton et al. 2007; Dybzinski et al. 2008).

The combined effects of plant community composition and drought conditions on plant tissue quality and herbivores have rarely been studied to date. Thus, the objectives of our study were twofold: Firstly, we investigated how an extreme drought, applied under field conditions, alters plant metabolites and thus affects the development of herbivores. And secondly, we determined the influence of community composition and diversity on plant metabolite quantity and, consequently on herbivore performance.

The target host grass *Holcus lanatus* is grown under field conditions in communities differing in their number of species and functional types and is exposed to extreme drought. Larvae of the generalist herbivore *Spodoptera littoralis* were reared on leaves of the host grass under constant climatic conditions in a climate chamber.

We hypothesized that i) the extreme drought would lead to an increase in defensive compounds, such as phenols and to a decrease in nitrogen availability, therefore negatively affecting the development of the herbivore; and that ii) the negative effects of drought would be amplified in communities with more species, due to an increase in interspecific competition for water. However, the presence of a legume species would buffer such adverse effects, as it may enhance the nitrogen status of neighbouring plants.

Materials & Methods

Study organisms

Spodoptera littoralis (Boisduval) (African cotton leafworm; Lepidoptera: Noctuidae) is a generalist herbivore that is widely used in laboratories and feeds on over 40 plant families worldwide (Brown and Dewhurst 1975). It can therefore be seen as a model organism for a generalist herbivore. Eggs of this species from laboratory strains were provided by the Max-Planck-Institute for Chemical Ecology, Jena, Germany.

Holcus lanatus L. (Yorkshire Fog; Poales: Poaceae) is a very common, perennial, tufted grass growing on various soils and on all kinds of grasslands across Europe. *H. lanatus* is predominantly found on wet and boggy, relatively fertile and moderate acidic soils (Grime et al. 1988; Wurst and van Beersum 2009). It was chosen due to its wide distribution throughout Europe and its relative importance as a forage plant.

Host-plant treatments and chemical analyses

Our study was conducted in 2009, as part of the EVENT I experiment, which investigates the effects of simulated extreme weather events on plants and ecosystem functions (Jentsch et al. 2007). The experimental design consists of two crossed factors: Extreme weather manipulation and community composition with five replicates of each factorial combination. The factors were applied in a split-plot design, with community diversity nested within weather treatments.

Extreme drought (D) was induced using transparent foil rain-out shelters, starting from a height of 80 cm to avoid strong green-house effects. The intensity of the drought in 2009 was based on the local 1000-year extreme weather event. Vegetation periods (March to September) from the years 1961-2000 acted as a reference period to calculate the length of the drought. A Gumbel I distribution was fitted to the annual extremes, and a 1000-year recurrence was calculated (Gumbel 1958). Drought was defined as the number of consecutive days with a daily amount of less than 1 mm precipitation. This resulted in a drought period with a length of 42 days starting on May 20th and ending on June 30th in 2009. The control treatment (C) remained under natural conditions without any manipulation.

Plant communities were planted in 2 m x 2 m plots except for the monocultures that were grown in 1 m x 1 m plots. The target species *H. lanatus* grows in four community compositions differing by the number of species (one to four) and the number of functional groups (one to three): *Holcus lanatus* monocultures (1-), *H. lanatus* growing together with one grass species (2-), with one grass species and two forbs (4-) or with one grass species and two forbs, including a legume species (4+) (Table 1). Original community composition was maintained by weeding four times per year. All mixed plant communities consist of 100 individuals and monocultures consist of 25 individuals per plot.

Table 1 Community compositions in which the host grass *Holcus lanatus* was grown, differing in species- and functional group number

community	functional groups	species
1-	one (grass only)	Holcus lanatus (L.)
2-	one (grass only)	Holcus lanatus (L.) Arrhenatherum elatius (L.)
4-	two (grass and herb)	Holcus lanatus (L.) Arrhenatherum elatius (L.) Plantago lanceolata (L.) Geranium pratense (L.)
4+	three (grass, herb, legume)	Holcus lanatus (L.) Arrhenatherum elatius (L.) Plantago lanceolata (L.) Lotus corniculatus (L.)

All plants were pre-grown from seeds in autumn 2004 and planted outside in a regular grid 20 cm apart from neighbouring individuals in April 2005 the year, when experimental weather manipulations started (100-year recurrent drought from 2005 until 2007; 1000-year recurrent drought since 2008). Monocultures were established in autumn 2006, also from plants pregrown from seeds in autumn 2004 and planted outside in April 2005.

Soil moisture was logged every hour in 4- communities (n=5) using FD-sensors (ECH$_2$O, Decagon devices, Pullman, USA). Each sensor measured the soil moisture between -2 and -7 cm. According to root length data assessed in previous years, the majority of root biomass is located within the upper 5 cm of the soil (Kreyling et al. 2008). Average daily values were calculated for analysis. Figure 1 shows the course of soil moisture during the drought manipulations.

Leaf chemical analyses of host plant

The relative leaf water content (RWC) towards the end of the drought period was analysed according to Barrs and Weatherley (1962). Leaves were cut and their fresh weight (FW) was immediately determined using a micro-balance. The turgid weight (TW) was determined after placing the leaves in distilled water at 4° C over night. Afterwards leaves were dried to a constant weight at 70° C to determine their dry weight (DW). RWC was calculated as:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} * 100$$ (Barrs and Weatherley 1962)

To analyse the total soluble proteins in fresh plant material, one mixed sample of plant material was sampled per plot at the end of the drought period, immediately frozen in liquid nitrogen and stored at -80° C. Frozen material was mortared, soluble proteins of 100 mg plant material were extracted using 50 mM sodiumphospate-buffer with 1 µm PMSF and 0.5 mM DTT and determined according to Bradford (1976). We used known concentrations of Bovine Serum Albumine as a standard.

To analyse the carbon and nitrogen content, mixed samples of three leaves per plot were taken, dried at 35°C for four days, ground in a ball mill and analyzed with an elemental analyser (Thermo Quest Flash EA 1112).

To analyse the total soluble carbohydrates, total phenolics, and condensed tannins, three mixed samples of at least two plants per plot were taken at the end of the drought period, immediately frozen in liquid nitrogen and lyophilized. 20 mg of leaf material were extracted in 50 % methanol. Total soluble carbohydrates were analyzed using the anthrone method with glucose as a standard (Kleber et al. 1987). Extinction was measured at 620 nm. Total phenols were analyzed using Folin-Ciocalteus reagens and Catechin as a standard and by measuring extinction at 750 nm (Swain and Hillis 1959). Condensed tannins were analyzed by adding 4 % vanillin and concentrated HCl to methanolic plant extracts and by measuring extinction at 500 nm (Broadhurst and Jones 1978). Catechin was used as a standard.

Rearing experiment

For the feeding experiment, 320 freshly hatched larvae of *S. littoralis* were placed on moist filter paper in 160 petri dishes, 10 cm in diameter, closed with parafilm. Thus, each petri dish contained two larvae. To ensure that plants were already stressed by environmental conditions at the start of the rearing experiment, it started ten days before the end of the drought period. Thus, only the first ten days of their development (on average 25 days from hatching to pupation) fell under the period of severe stress of their host plant, while the second part fell under the stress recovery phase of the host, with a lower stress level as soil moisture reached normal levels. Larvae were kept in a climate chamber at 25° C on a 15/9 light/dark cycle. Petri dishes were randomly assigned to four levels in the climate chamber and the levels were shifted every second day, putting the lowest level to the highest place and all other levels one level lower. The plant leaves from one plot were fed to the larvae in four petri dishes (8 larvae in total). Caterpillars were able to eat *ad libitum*, as special care was taken that the grass inside one petri dish was never totally consumed. Leaves were replaced at least every second day. We recorded the mortality of the larvae, the development time until pupation, the weight of the pupae one day after pupation and pupal mortality. Through the isolation in climate chambers we were able to attribute the responses in herbivore performance to differences in plant compounds, as opposed to when feeding is conducted under field conditions and it is impossible to disentangle the effects of plant nutrients, compensatory feeding and direct weather effects on herbivores (Goverde et al. 2002).

Statistical analysis

Chemical leaf traits were analyzed using a two-way ANOVA with weather treatment and community composition as fixed factors. Additionally, we included the number of columns and rows as random factors. This automatically implements the nesting of composition within treatment-blocks in the mixed effect model (Faraway 2006; Dormann and Kühn 2008). If several samples per plot were taken, as was the case with phenolics, carbohydrates and condensed tannins, then the plot number was additionally included as a random factor in the mixed model, to avoid any pseudo-replication.

Developmental time and pupal weight were analyzed using linear mixed effect models with the petri dish nested within the plot nested within the treatment block as a random factor, in addition to the row and the column of the treatment blocks as random factors. Larval and pupal mortality were analyzed using generalized mixed effect models with binomial distribution and otherwise the same model formula as for other developmental traits.

The significance levels in the mixed effect models were evaluated by Markov Chain Monte Carlo sampling of 1000 permutations (Baayen 2009; package language R).The significance of the fixed factors for the generalized mixed effect models was determined by comparing the null model, without any factors, to the simplest factorial model, in which non-significant terms had been removed by backwards stepwise selection. Prior to all analyses, data were transformed accordingly, if the assumptions of ANOVA, homogeneity of variances and normality, were not met (C/N ratio and nitrogen content: log-transformed; RWC: arcsin-squareroot-transformed).

To determine the relationship between chemical leaf traits and development traits, we applied hierarchical partitioning, as leaf chemical traits are often collinear (Schädler et al.

2003). In hierarchical partitioning, the independent influence and the joint influence (the influence from being correlated to another explanatory variable) of explanatory variables is calculated by comparing the model-fits of models with and without the particular variable (Mac Nally 2002; Dormann and Kühn 2008). To determine correlations between the different development traits, a correlation analysis was used to correlate pupal weight with developmental time, and logistic regression was used to investigate the relationship between pupal mortality and pupal weight.

All statistical analyses were performed using R 2.11.0 (R Development Core Team 2010). For mixed effect models we used the software package lme4 (Bates & Mechler 2010), and for multiple post-hoc comparisons the package multcomp was used (Hothorn et al. 2008).

Results

Soil moisture

The vegetation period for the year 2009 (April 1st – October 31st) with a total sum of 459 mm of precipitation was slightly wetter than the long-term average precipitation sum of 437 mm for the time period 1971-2000 (Data: German Weather Service). Soil moisture fell more quickly during the first half of the drought period compared to the second half, but rose quickly again after the drought period was over (Fig.1).

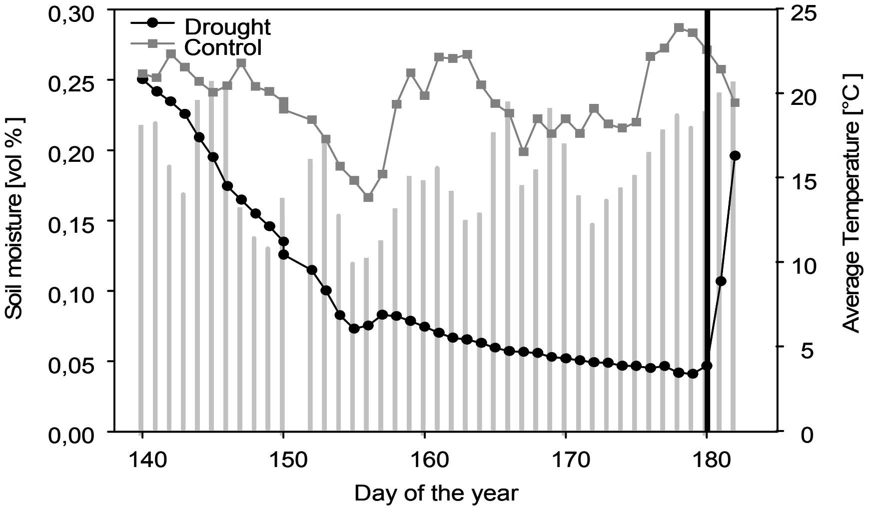

Fig. 1 Course of soil moisture in drought-exposed plots (black circles) and control plots (dark-grey squares), and average daily temperatures, assessed at a height of 1, 20 m (light grey bars). Data are shown from the first day of the drought manipulation (day of the year 140=May 20th, 2009) until two days after the extreme drought ended, indicated by the black vertical line (day of the year 182=July 1st, 2009). The average of hourly readings from five sensors per treatment were taken here (n=5)

The effect of drought and community composition on chemical leaf traits

Drought significantly decreased RWC by 8 % (Fig. 2a), protein content by 23 % (Fig. 2b), nitrogen concentrations by 26 % (Table 3) and phenols by 7 %, when compared to the control treatment (Fig. 2c) (see Table 2 for statistical details). Furthermore, drought significantly increased the C/N ratio by 24 % and the soluble carbohydrates by 32 % (Fig. 2d, e). Condensed tannins however were not altered by drought manipulation (Table 2).

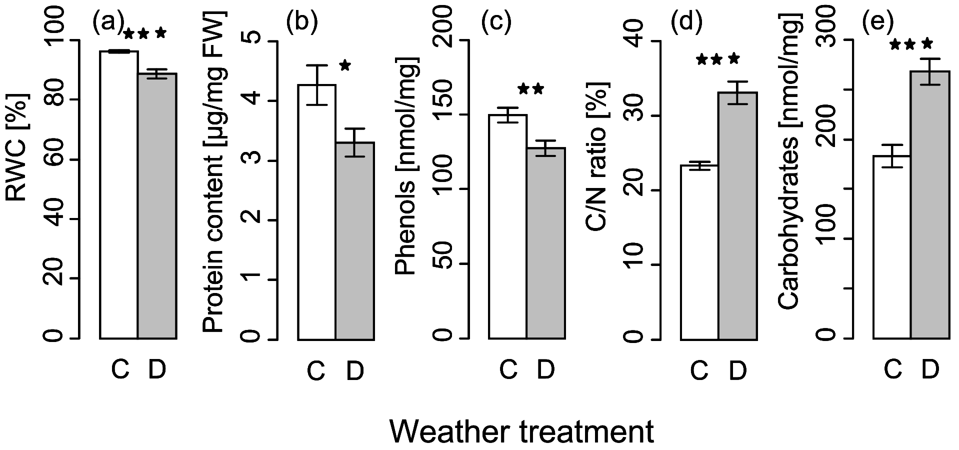

Fig. 2 Differences in a) RWC (n=5), b) the protein content (n=5), c) the total soluble phenol content (n=15), d) the C/N ratio (n=5) and e) the total soluble carbohydrates (n=15) in leaves of *Holcus lanatus* under drought (light grey bars, D) compared to control (white bars, C). Asterisks indicate the level of significance: * p<0.05; **p<0.01; ***p<0.001. Means ± 1 SE are shown

Community composition significantly affected the content of condensed tannins and phenols (Table 2) and marginally significantly affected the content of soluble carbohydrates (p=0.07) and proteins (p=0.01).

Table 2 ANOVA results of the effects of drought treatment, community composition and, if significant, their interaction on RWC, total soluble protein content, nitrogen concentrations, C/N ratio (n=5, respectively), total soluble carbohydrates, total soluble phenols and condensed tannins (n=15, respectively). Values for interaction are only shown, when significant, as otherwise, the interaction term was excluded from the mixed model (df=degrees of freedom, MS=mean sum of squares). Asterisks indicate level of significance: `p<0.01; * p<0.05; **p<0.01; ***p<0.001

	RWC			protein			nitrogen			C/N			carbohydrates			phenols			cond. tannins		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F	df	MS	F
drought	1	0.18	28.4***	1	7.51	7.4*	1	1.02	65.1***	1	1.13	76.6***	1	106533	68.3***	1	5871	11.3**	1	76.4	0.6
composition	3	0.01	1.4	3	2.29	2.26`	3	0.01	0.7	3	0.01	0.8	3	3755	2.4`	3	2547	4.9**	3	516.6	4.2**
interaction	-	-	-	-	-	-	3	0.06	4.1*	3	0.04	2.6`	-	-	-	-	-	-	-	-	-
residuals	30	0.01		32	1.01		32	0.02		32	0.01		97	1558		97	518		97	124.5	

The condensed tannins in *H. lanatus* from 4+ communities were significantly lower than the condensed tannins in leaves from 2- and 4- communities (Fig. 3a). Phenols were reduced in *H. lanatus* growing in legume communities (4+), when compared to *H. lanatus* from monocultures (1-) (Fig. 3b). Carbohydrates were marginally significantly increased in legume communities (4+) when compared to two-species communities without legume (2-) (Fig. 3c), while protein content was marginally significantly lower in four-species communities without legume (4-) when compared to legume communities (4+)(Fig. 3d).

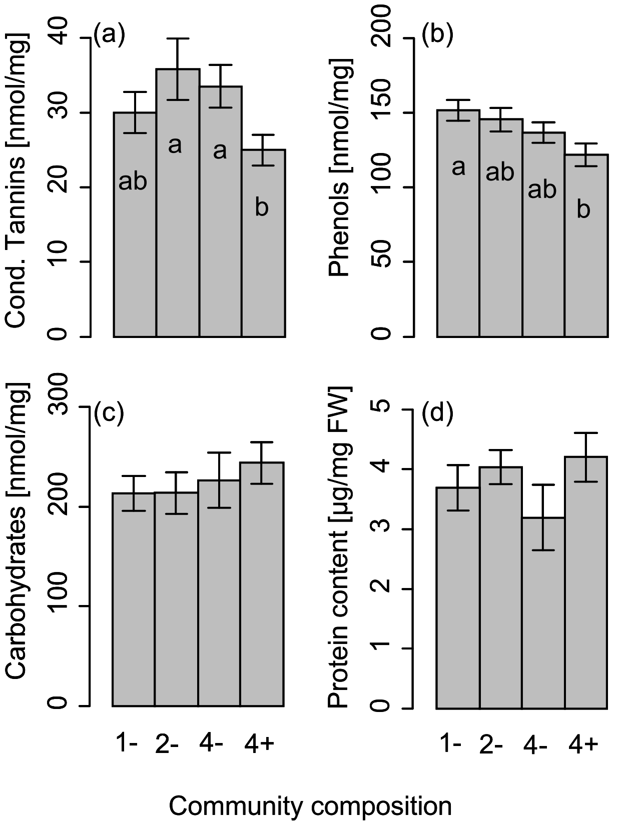

Fig. 3 Effects of plant community composition on a) the content of condensed tannins (n=15), b) the total soluble phenol content (n=15), c) the total soluble carbohydrates (n=15) and d) the total soluble proteins (n=5) in leaves of *Holcus lanatus* (1-: monoculture, 2-: two grasses, 4-: 2 grasses, 2 herbs; 4+:two grasses, 1 herb, 1 legume). Different letters indicate significant differences between the communities (p<0.05). Effects for carbohydrate and protein content were only marginally significant (p<0.1)

As far as leaf nitrogen concentration was concerned, community composition significantly interacted with the drought treatment, as nitrogen concentration was reduced in all communities except for the 4+ communities in response to drought (Table 3).

Table 3 Interactive effects of community composition and the drought treatment on the nitrogen concentration in leaves of *H. lanatus* (n=5). Means ± 1 standard deviation are given. Asterisks behind the community label indicate a significant drought effect in the respective community (p<0.05)

	composition			
	1- *	2- *	4- *	4+
control	1.99 ± 0.31	1.90 ± 0.31	1.88 ± 0.31	1.69 ± 0.30
drought	1.26 ± 0.32	1.45 ± 0.32	1.26 ± 0.32	1.51 ± 0.31

Development traits of S .littoralis and their relation to leaf chemical traits

The community composition of the target grass was found to have a highly significant effect on larval mortality, with larvae feeding from 4- plots showing a significantly higher mortality than in all other communities (Fig. 4). (P=0.007; Chisq= 12.2). The drought treatment did not affect larval mortality.

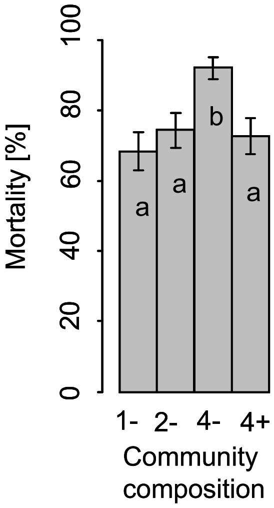

Fig. 4 Effect of the plant community composition, in which *H. lanatus* grows, on the mortality of the 320 S. littoralis larvae. Different letters indicate significant differences between the communities (p<0.05) (n=20/treatment combination)

The development time until pupation was highly significantly increased in those larvae that were reared on leaves subjected to drought (p=0.0003; F= 14.7; df=1). Furthermore, the drought treatment was found to significantly interact with community composition regarding development time until pupation (p=0.015; F= 3.8; df=3), as the larvae feeding on *H. lanatus* from the 2- communities reacted with the greatest increase during the larval stage under drought (Table 4).

Table 4 Differences in the duration of larval stage for 320 *S. littoralis* larvae feeding on *H. lanatus* leaves under the drought (light grey bars, D) and the control (white bars, C) treatments from four different community compositions. Drought significantly increased developmental time with strongest effects in 2- communities (n=20/treatment combination). Means ± 1 standard deviation are given

	composition			
	1-	2-	4-	4+
control	25 ± 2.5	22 ± 2.4	25 ± 2.3	24 ± 2.3
drought	25 ± 2.3	27 ± 2.4	27 ± 2.5	26 ± 2.4

The larvae feeding on drought plants were also found to have significantly heavier pupae (p=0.033; F= 4.7; df=1) (Fig. 5a) and revealed a significantly lower pupal mortality (p=0.007; Chisq= 7.2) (Fig 5b), irrespective of community composition.

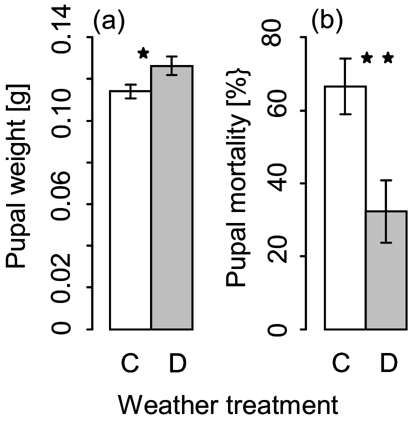

Fig. 5 Differences in (a) the pupal weight and (b) the pupal mortality for *S. littoralis* larvae fed with *H. lanatus* leaves out of the drought (light grey bars, D) and the control (white bars, C) treatments. Asterisks indicate the level of significance: *P\0.05; **P\0.01 (n = 20/treatment combination)

Hierarchical partitioning showed that protein content had the greatest positive influence on the survival of larvae (Table 5).

Table 5 Hierarchical partitions of the effects of leaf chemical traits on development duration, pupal weight, larval mortality and pupal mortality of 320 larvae. The total explained variance (R^2), the individual effect on the explained variance, and the joint effect on explained variance are given. The latter quantifies the effect that can be explained by the correlation of a specific independent variable with other independent variables. + or – behind the most important partitions for one parameter indicate whether the parameters were negatively or positively correlated to the leaf chemical. RWC=Relative water content; C/N=C/N ratio; nitrogen=nitrogen concentration; carbon=carbon concentration; protein=total soluble proteins ; phenols= total soluble phenols ; tannins= condensed tannins ; carbos=total soluble carbohydrates

		RWC	C/N	nitrogen	carbon	protein	phenols	tannins	carbos
development time	total	0.143	0.259+	0.210-	0.014	0.001	0.036	0.029	0.078
	independent	0.113	0.187	0.118	0.019	0.085	0.013	0.014	0.03
	joint	0.03	0.071	0.091	-0.005	-0.084	0.024	0.015	0.048
pupal weight	total	0.229-	0.024	0.04	0.031	0.079	0.094	0.077	0.04
	independent	0.178	0.025	0.025	0.035	0.068	0.047	0.048	0.02
	joint	0.051	-0.001	0.015	-0.003	0.012	0.047	0.029	0.021
larval mortality	total	0.01	0.003	0.001	0.001	0.047-	0.006	0.005	0.009
	independent	0.009	0.009	0.005	0.004	0.065	0.007	0.01	0.007
	joint	0.001	-0.006	-0.004	-0.002	-0.018	-0.001	-0.005	0.002
pupal mortality	total	0.031	0.097-	0.086	0.017	0	0.049	0.096-	0.042
	independent	0.014	0.034	0.028	0.013	0.01	0.023	0.072	0.015
	joint	0.017	0.063	0.058	0.004	-0.009	0.026	0.023	0.027

The development time was mainly positively influenced by the C/N- ratio, and thus negatively by the nitrogen content, indicating that the development took longer, the less nitrogen was in the leaves (Table 5). Pupal weight was found to be negatively affected by RWC. Pupal survival, and thus adult eclosion was positively affected by content of condensed tannins and by C/N ratio. Pupal weight and development time were not correlated (Pearsons correlation coefficient: -0.019; p=0.88). Pupal survival was positively related with pupal weight (p=0.023; logistic regression).

Discussion

The extreme 42-day drought did not only affect the water content of the target grass species, but also resulted in changes to almost all of the leaf chemical traits that we assessed. These changes to the leaf chemicals also clearly affected the development traits of the herbivore caterpillar. However, in contrast to our expectations, drought did not increase defensive compounds in the grass. Furthermore, the drought did not result in a worse overall herbivore performance, as adult eclosion and pupal weight were even higher for larvae fed from drought treated plants. The community composition of the target grass also affected some aspects of the leaf chemical composition, but changes cannot clearly be linked to increased competition or higher stability in more diverse communities. Differences mainly occurred in legume communities: *H. lanatus* growing in legume communities showed no effects of drought on the leaf nitrogen concentration and had the lowest content of condensed tannins and phenols. The community composition of the target grass affected the mortality of the herbivore, with highest mortality in larvae feeding on the grass growing in four-species communities without legume, in which also a trend towards lower protein content was apparent (4-).

Soil moisture and plant stress

The extreme drought conditions were accompanied by a marked decrease in soil moisture over the drought period, and this in turn clearly caused plant stress. In the year 2009 we did not directly quantify plant stress levels, e.g. by determining chlorophyll content or maximum quantum yield. However, a reduction in leaf relative water content in our target grass in all communities, along with marked changes in leaf chemicals, indicate acclimation processes and stress reactions, showing that the plants experienced stress (Sinclair and Ludlow 1985; Chaves et al. 2002).

Effect of drought and community composition on chemical leaf traits

A decrease in proteins and nitrogen, along with an increase in the C/N ratio under severe drought conditions has also been observed in other studies investigating drought effects on leaf chemicals (Shure et al. 1998; Liu et al. 2008). However, this is not in accordance with the so-called "plant stress hypothesis", which assumes available nitrogen to increase under plant stress (White 1984). Nevertheless, as nitrogen uptake is linked to water uptake, a decrease in nitrogen uptake, and therefore also protein content under drought, is not surprising. An increase in carbohydrates under drought could be attributed to osmotic adjustment in the course of drought acclimation. Soluble carbohydrates from starch degradation act as compatible solutes to prevent turgor loss in plant cells (Chaves et al. 2002; Regier et al. 2009). The decrease in total phenolics under drought does not support the idea that plants under stress use the surplus from carbohydrates (due to restricted growth while photosynthesis is still assimilating carbon) to accumulate more C-based defence compounds (Herms and Mattson 1992). However, a reduction of phenols in response to drought has also been reported by Shure et al. (1998). In our study, the need for osmotic adjustment under extreme drought might have been a reason not to accumulate phenols, but rather invest the surplus of carbon-based compounds in soluble carbohydrates.

As community composition can alter resource partitioning between plants, it might also change the forage quality of leaf tissue. Both phenols and condensed tannins were found to be lowest in the communities that included one legume species. This kind of reduction in defence compounds can be explained by the potentially higher nitrogen availability in legume communities caused by N_2-fixing in root-nodules, enabling higher growth rates at the expense of lower defence by C-based compounds (Herms and Mattson 1992). The trend towards higher protein content in leaves from legume communities compared to four-species communities without legumes support the idea of an increase in nitrogen availability in legume communities and increased competition for nitrogen in communities containing four species, but no legume. Moreover, labelling studies indicate a direct uptake of legume-derived nitrogen by grasses (Gubsch et al 2011). Overall, the nitrogen concentration of grasses growing in legume communities (4+) was not affected by drought, but showed changes in response to drought in the other communities. The higher stability in this communities and the possible fertilization effect of the legume support other studies which have shown an enhancement of the nitrogen availability for plants growing in legume communities (Spehn et al. 2002; Temperton et al. 2007; Dybzinski et al. 2008).

Developmental traits of S. littoralis and their relation to leaf chemical traits

The mortality of larvae was high, as the experiment was started immediately after hatching of the larvae, when they are quite vulnerable. Furthermore, *H. lanatus* seemed to be a sub-optimal food source for *S. littoralis*, as larvae the same age from the same egg strain grew better when feeding on *Plantago lanzeolata* and *Trifolium pratense*, under otherwise similar conditions. Nevertheless, as we expected mortality to be high, due to the results from a prior trial experiment, we included enough replicates in order to obtain a substantial data set for statistical analysis.

The reported changes in leaf metabolites, related to the drought treatment and differences in the community compositions, clearly had an effect on herbivore performance. Mortality of larvae was higher in four-species communities without a legume compared to the other communities. Hierarchical partitioning showed that the mortality rate depended mostly on the protein content. These data hint towards a central role of proteins for the survival of the early instars. Other studies, too, showed poor larval survival under low nitrogen concentrations (Myers and Post, 1981; Cates 1987; de Bruyn et al. 2002).

Larval development up to pupation was significantly longer for larvae fed on drought-stressed plants, which might increase predation risk and thus mortality under natural conditions (Benrey and Denno 1997). In accordance with Fischer and Fiedler (2000) and with Morehouse and Rutowski (2010), the prolonged developmental time of the larvae was linked to a reduced N-availability in drought-stressed leaves. It might be that larvae fed longer on N-limited grass to reach a certain "growth target" (Raubenheimer and Simpson 1997). Such compensatory feeding on low quality tissue may be a common phenomenon (Schädler et al. 2007b).

Drought also had positive effects on herbivore performance, irrespective of community composition: The increased pupal weight might be explained by a higher uptake of energy, as carbohydrate contents in leaves increased under drought and as larvae fed longer on drought plants, possibly caused by lowered nitrogen contents. Thus, the "imbalanced diet" when feeding on drought stressed plants caused firstly compensatory longer feeding, to reach a certain nitrogen level necessary for development, and secondly led to a higher energy uptake and higher pupal weights (Raubenheimer and Simpson 1997). RWC was negatively related to pupal weight, as presumably water dilutes nutrients or carbohydrates in well-watered plants. The increased pupal weight was correlated with a lower pupal mortality of the larvae that had been fed from drought-stressed plants, which is in accordance to other studies (Fischer and Fiedler 2000).

It should be reiterated that the samples for the leaf chemical analysis were taken on the last two days of the drought treatment, whereas the larval development lasted from approximately ten days before to ten days after the drought. Thus, larvae fed on leaves that experienced milder stress levels in their late stages, which might have alleviated the effects of extreme drought on leaf chemical traits (Huberty and Denno 2004). Further studies with a higher temporal resolution would make it possible to investigate the course of changes in leaf traits over the whole range of plant stress responses.

Nevertheless, the present study reveals that different development parameters can be differently influenced by extreme drought, even though these only lasted for around half of the duration of their larval development. Furthermore we show that plant community composition (and therefore also competition and the presence of specific plant functional traits) alters leaf metabolism and thereby affects herbivores. Concerning the debate on the hypotheses on plant-stress (White1984) versus plant-vigour (Price 1991) we confirm other studies which show the dependence of herbivore performance on plant-stress level (Scheirs and de Bruyn 2005), investigated traits (Cornelissen et al. 2008) and insect-feeding guild (Larsson 1989; Koricheva et al. 1998; Huberty and Denno 2004), as our results differ from other drought experiments using different feeding guilds (EnglishLoeb et al. 1997; de Bruyn et al. 2002). Differences to other studies may also be attributed to our specific experimental conditions: Plants were grown in experimental communities in the field, not in isolated pots in the greenhouse. This ensures more realistic plant growth conditions. In addition, the herbivores in our study have not been grown on the plants, but were fed in climate chambers, to more clearly relate the obtained results to changes in plant metabolites. Another reason for differences to other studies and prominent hypotheses might be that the plants were severly stressed, but also released from stress and recovered during the feeding experiment. According to Huberty and Denno (2004) this might have caused differences in herbivore performance when compared to feeding experiments under constant stress. Our study provides additional evidence that the nitrogen limitation hypothesis, stating that high nitrogen contents are beneficial for herbivore performance (White1984) does not seem to be widely applicable over all development traits and stages (Fischer and Fiedler 2000). Similar to Fischer & Fiedler (2000) our data provide hints that higher protein or nitrogen contents are beneficial for a faster rate of development and higher larval survival, although they might reduce adult eclosion.

Conclusion

Our findings suggest that extreme droughts, which are projected to increase in frequency with climate change, can also affect the development of herbivores. This is primarily caused by a direct alteration of leaf chemicals under severe drought conditions. However, our study also shows that community composition alters leaf chemicals and might therefore also influence the development of herbivores. Climate change and severe dry spells are likely to alter plant community composition, due to species-specific survival strategies, phenotypic plasticity and niches of plant species, and such alterations in turn might also affect leaf chemicals and thus herbivore performance, as our study shows. Such changes may support desynchronisation processes and thus might destabilize established food-webs and ecosystems. Furthermore, we show that leaf chemicals can influence different development stages in a complex way, making in difficult to draw any direct connections between leaf nutrients and accordingly defence compounds and herbivore performance.

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Manuscript 3: Ecological stress memory and cross stress tolerance in plants in the face of climate extremes

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Highlights

1. We define the concept of an ecological stress memory.
2. Some studies hint towards the existence of an ecological stress memory after climate extremes (drought, heat, frost).
3. Possible mechanisms are e.g. epigenetic modifications.
4. Further work and co-operations between ecologist and molecular biologist are urgently needed.

Abstract

Under climate change, not only the magnitude, but also the frequency of extreme weather events is predicted to increase. Such repeated climate stress events may cause fundamental shifts in species compositions or ecosystem functioning. Yet, few studies document such shifts. One reason for higher stability of ecosystems than previously expected may be ecological stress memory at the single plant level. Ecological stress memory is defined here as any response of a single plant after a stress experience that modifies the response of the plant towards future stress events including the mode of interaction with other ecological units. Ecological stress memory is assessed on a whole plant level in ecological relevant parameters. It is therefore one important aspect of the broader concept of ecological memory that refers to whole communities and ecosystems. Here, we present studies which indicate the existence of ecological stress memory within single plants after drought, frost or heat stress. Possible mechanisms underlying an ecological stress memory are the accumulation of proteins, transcription factors or protective metabolites, as well as epigenetic modifications or morphological changes. In order to evaluate the importance of stress memory for stabilizing whole ecosystems and communities in times of climate change, cooperation between ecologists and molecular biologists is urgently needed, as well as more studies investigating stress memory on a single plant level. Only then the potential of plant stress memory for stabilizing ecosystems in times of intensifying climatic extremes can be evaluated and taken into account for measures of mitigation and adaptation to climate change.

Keywords: global warming; ecophysiology; ecological memory; extreme events; heat stress; frost stress; drought stress

1. Introduction

The increase of climatic variability due to global climate change exerts climatic stress on plants that is novel in magnitude and frequency (IPCC 2007; Hegerl et al., 2011; Min et al., 2011). Extreme weather events such as drought, heat waves, heavy rainfall, or frost spells differ from continuous climatic trends (e.g. warming or rising CO_2-levels), as their ecological consequences are expected to be out of proportion to their relatively short duration (Easterling et al., 2000; Jentsch et al., 2007; Smith, 2011). Thus, extreme weather events may cause stronger effects on plants and plant communities than gradual shifts in means, as their abruptness gives little time for acclimation processes and as their magnitude may be impossible for single plants to cope with.

Collapsing populations of key species have been reported as direct responses to extreme climatic events (Allen and Breshears, 1998). However, there is also increasing incidence for stabilizing processes of climatic fluctuations for instance stimulated by reduced precipitation or recurrent drought events (Fay et al., 2000; Kahmen et al., 2005; Jentsch et al., 2011; Lloret et al. 2011). Such stabilizing mechanisms may occur already at the species and single plant level and are not yet fully appreciated. Ecological stress memory, dealt with in this article, might be one aspect leading to more stable community compositions in the face of an increasing frequency of extreme climatic events.

An ecological stress memory might emerge as plants reveal modifications, like acclimation, upon stress exposure that might persist after the stress stopped. Thus, when stress frequency increases, plants may not have returned to their previous reference state in the time lag between two stress events, thus affecting the stress response to repeated stress: Such a stress memory that the plant keeps after a stress event may lead to a faster stress response and increased stress tolerance upon a following stress event (Bruce et al., 2007; Walter et al., 2011).

Here, we (1) introduce the concept of an ecological stress memory and (2) present studies indicating the existence of an ecological stress memory after drought, frost or heat stress. We further (3) discuss possible mechanisms underlying an ecological stress memory and we (4) consider research challenges in the field of ecological stress memory research in times of rapid climate change.

2. Ecological stress memory vs. ecological memory vs. lagged stress effects

The term “memory“ is used ambiguously in ecology (Rensing et al., 2009). Here, we focus on ecological stress memory, which we see as one important aspect of the broader concept of “ecological memory“. Ecological stress memory differs from lagged stress effects.

Ecological memory refers to whole communities or ecosystems and addresses the capacity of past states or experiences to influence present or future responses of ecosystems (Padisak, 1992). It is composed of species, their interactions, soil properties and the site history and determines the replacement of communities after severe disturbances of the ecosystem (Bengtsson et al., 2003; Schaefer, 2009; Sun, 2011).

Table 1 Levels of mechanisms for ecological stress memory (first four levels may be part of an ecological stress memory) and ecological memory and their effects

Level	**Examples**	**Mechanisms of ecological memory after climatic disturbance events**	**Effect**
Genetic	Genes	Epigenetic processes	Modified inheritance of traits
Cell	Vacuoles	Molecular responses	Accumulation of secondary metabolites
Organ	Sleeping buds	Stimulation of resprouting after drought	Regeneration of leaves
	Flowers	Stimulation of flowering after drought	Enhanced reproduction success
Individual	Habitus	Increased root-shoot ratio	enhanced water supply
Population	Populations	Recruitment events	Cohorts
		Selection of best adapted genotypes	Shifts in genotypic diversity within populations
		Retarded reproduction	Change in abundance
Interspecific	Symbiotic partners	Responses of species-specific microbial activity	Establishment or loss of microbial interaction partners
		Modified pollination	Reduced seed production
Community	Plant community	Modified competitive ability of species after stress	Shifts in plant species dominance, cover and biomass

Important aspects are not only the persistence of species and substrate, but also the possibilities for species to recolonize (Nystrom and Folke, 2001). An overview on the various understandings of ecological memory is provided by Golinski et al. (2008). The complexity of the processes involved in the broad definition of ecological memory including temporal patterns up to evolutionary scales and spatial patterns up to landscape levels (Thompson et al., 2001), may limit its applicability in ecology as well as the possibility to assess and measure such a memory. It thus appears necessary to apply a reductionist framework to foster our understanding of the importance of ecological memory in times of global change (see Table 1 for examples and levels of ecological memory after disturbances). In the following, we focus on ecological stress memory of single individuals as a starting point.

Ecological stress memory is defined here as any response of a single plant after a stress experience that improves the response of the plant towards future stress experience and which is assessed on a whole plant level (Fig. 1).

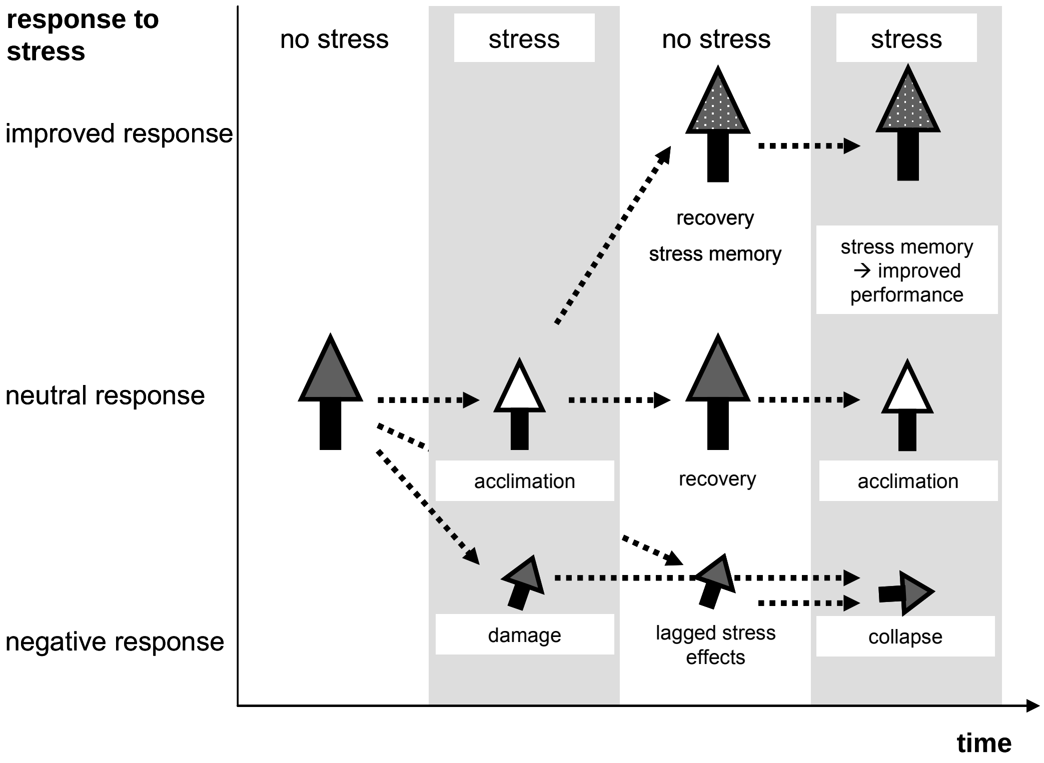

Fig.1 Plant stress response under single and repeated stress without acclimation leads to stress damage (negative response), exhibiting acclimation (neutral response) and exhibiting an additional ecological stress memory (improved response). Acclimation helps to prevent stress damage and to promote recovery, despite often leading to reduced growth during stress. An ecological stress memory exists, when the plant keeps a sort of "stress imprint" after stress exposure that improves plant response to recurrent stress compared to plants without stress memory. Lagged stress effects are detrimental effects that occur some time after the stress occurred. Stress damage may lead to even greater damage or complete collapse when recurrent stress is applied

An ecological stress memory might involve the persistence of acclimation mechanisms and protective substances. However, it is not acclimation per se, as such a memory remains active long after the stress has been applied, and enables the plant to respond quicker and more adequate to a recurrent stress event. It thus requires a persisting “imprint” modifying future stress response. For acclimation, the plant does not need to experience real stress, as, e.g. for frost acclimation, certain environmental cues are sufficient to trigger acclimation within the seasonal life cycle performance. Ecological stress memory has a temporal dimension and, in this sense can only be studied after the stress stopped and the plant recovered, e.g. took up its pre-stress metabolism again and repaired or compensated damage. After applying recurrent stress, ecological stress memory should lead to an improved performance when compared to plants without a persisting stress memory.

Contrastingly, lagged or delayed stress effects are detrimental effects of single stress events that become clearly apparent only after some time, e.g. when the plant dies or a community collapses (Fig. 1). The response of trees to drought, for instance, is often expressed in increased mortality but this may happen even several years after the drought event (Bigler et al., 2007). Likewise, alterations in soil frost events can lead to increased mortality of dwarf-shrubs after more than one year, but with no apparent effects in the first year after the stress event (Kreyling et al., 2010). Such lagged responses clearly indicate carry-over effects in fitness which are not easily detectable directly after the event and which may explain the findings of reduced resilience upon repeated stress events. For example, Lloret et al. (2004) investigated the impact of recurrent drought (1985 and 1995) on resprouting and die-back in *Quercus ilex*. They found a progressive loss of individual resilience upon recurrent drought, as the ability to survive and resprout was reduced compared to the first drought. Mueller et al. (2005) examined *Pinus edulis* and *Juniperus monosperma* mortality after extreme drought episodes and also found a reduction of resilience and a higher mortality rate for a recurrent drought in 2002 compared to the previous drought in 1996. It may have been that detrimental effects have persisted even after many years and plants may not have been recovered before the following stress exposure: Starch stocks in lignotubers of *Q. ilex* were found not to be restored to their pre-stress values even 10 years after an extreme drought (Lopez et al., 2009). Thus, to clearly distinguish such lagged stress effects from an ecological stress memory it may be necessary for experiments to apply a recurrent stress event and to compare the answer of recurrently stressed plants to single stressed plants. Only when the performance to recurrent stress is improved when compared to single stressed plants, the definition allows calling this an ecological stress memory.

In the following, we review mechanisms of drought tolerance and evidences for drought memory, of frost tolerance and frost memory as well as of heat stress tolerance and heat stress memory.

3. Drought tolerance and drought memory

Plants are able to acclimate to drought stress, thereby increasing their drought tolerance. Mechanisms of acclimation include the accumulation of osmoprotective proteins, like dehydrins (Bohnert, 2000; Lambers et al., 2008), the accumulation of soluble sugars (Lambers et al., 2008; Walter et al., 2012), a reduction of the photosynthetic apparatus along with additional mechanisms to prevent damage by reactive oxygen species (Munne-Bosch and Alegre, 2000) and the accumulation of compatible solutes (proline, betaine) (Bohnert, 2000). Changes in gene expression that accompany drought acclimation are often ABA-mediated, and upregulated genes include genes of the LEA family (late embryogenesis abundant) (Bohnert, 2000; Lambers et al., 2008). In addition to physiological changes, phenotypic and morphological responses can be initiated during drought stress, such as an increased root to shoot ratio or the development of roots in deeper soil layers (Newman et al., 2006).

Some recent findings indicate the existence of an ecological drought memory: Walter et al. (2011) found an increase in photoprotection in single grass plants under repeated drought when compared to plants that were not subjected to drought previously, even several weeks after the first drought was applied and after the plants were completely cut and regrown (Fig. 2A).

Similarly, Onate et al. (2011) showed that *Urtica dioica* subjected to combined drought and nutrient deficiency in their juvenile phase revealed improved drought stress tolerance in mature leaves, especially in reproductive shoots.

Under laboratory conditions, Goh et al. (2003) found that *Arabidopsis thaliana* repeatedly subjected to high levels of abscisic acid (ABA), also involved in drought stress signaling and response, led to a formation of ecological stress memory, as gene expression was changed in response to following stress events compared to non-treated plants. Knight et al. (1998) observed changes in drought stress-induced calcium-signaling after plants had encountered either osmotic or oxidative stress previously.

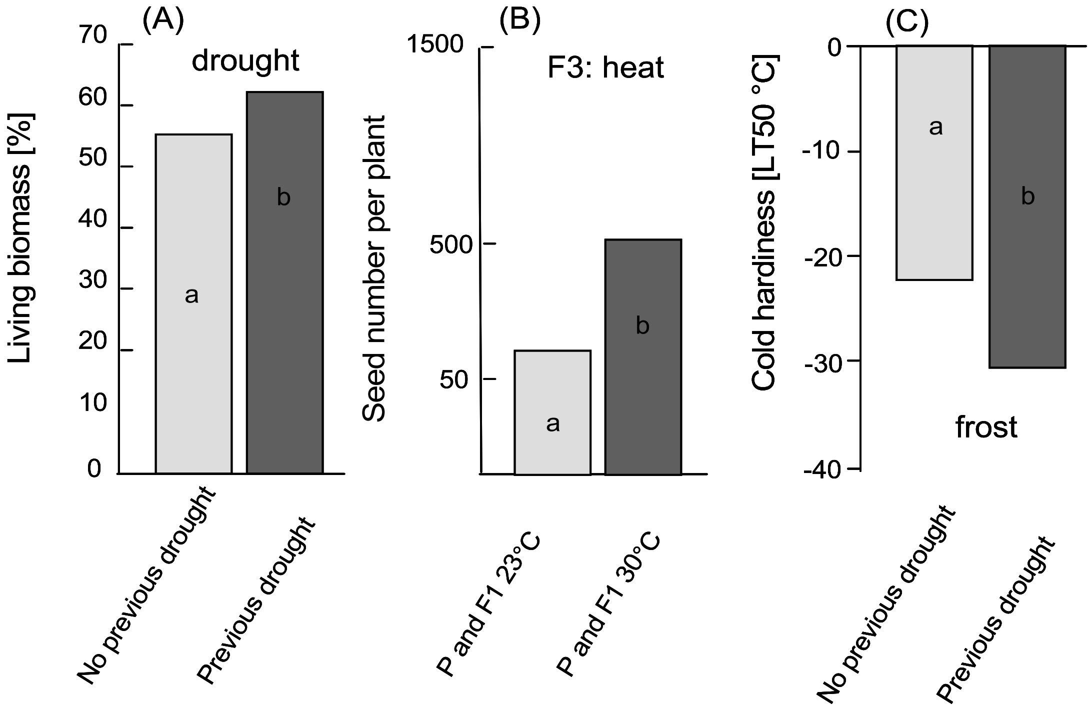

Fig. 2 Examples for (A) ecological drought memory (adapted from Walter et al. 2011), (B) ecological heat memory (adapted from Whittle et al. 2009) and (C) for an ecological cross-stress memory (adapted from Kreyling et al. 2012b). Plants were either unstressed (light gray bars) or subjected to drought stress (A, C) or heat stress (B) (dark gray bars; pretreatment indicated on the y-axis) and performance was measured under subsequent drought (A), heat (B) or frost (C) stress (as indicated by inserts) in the same plants (A, C) or in the F 3 generation (B). Different letters indicate significant differences between plants with and without pre-stress according to the sources

Cuk et al. (2010) showed that modified activity of antioxidative enzymes (catalase, ascorbate peroxidase), which are often also upregulated under drought, is inherited to the next generation of *A. thaliana*. It seems likely that maternal plants inheriting stress tolerance would reveal an ecological stress memory themselves.

To sum up, there is evidence that certain physiological processes in plants are modified by former stress events. These modifications can be decisive in face of repeated events and may stimulate a faster start of protective mechanisms and increased stress tolerance and compensation. However, studies investigating this topic are rare and more studies investigating drought memory in different species and also under more natural conditions and studies comparing the response to recurrent drought manipulations to the response to single drought manipulations are needed in the future.

4. Frost tolerance and frost memory

In regions where subzero temperatures are reached, perennial plants show the potential to acclimate to frost to reduce frost damage, caused by intracellular ice crystals and dehydration. As apoplastic ice formation leads to cell dehydration, drought acclimation and frost acclimation often involve the same mechanisms, like accumulation of soluble sugars or transcription of dehydrins and LEA-genes (Lambers et al., 2008; Janska et al., 2010). Frost acclimation is triggered by low temperature and by changes in the photoperiod (Thomashow, 1999; Janska et al., 2010). Hardening usually takes several weeks, while dehardening, i.e. the loss of frost hardiness, can occur within hours after temperature increase (Rapacz et al., 2000), leaving the plants vulnerable to short-term late frost events during the growing season or after winter warming events.

The frequency of frost days and nights is expected to decrease in various biomes under global climate warming (IPCC 2007), yet, an increase in minimum temperature over winter is unlikely (Kodra et al., 2011). Observed and projected reduction in snow cover, which acts as insulation for many plants, in combination with more variable air temperatures may further exacerbate the frequency of frost stress in many northern regions (Kreyling, 2010). As global warming may regionally lead to an earlier dehardening and onset of the growing season, the risk of late frost damage is likely to increase, when the timing of late frost events is not changing (Rigby and Porporato, 2008;Woldendorp et al., 2008). Furthermore, global warming might lead to more frequent freeze-thaw cycles during winter, possibly associated with (partial) dehardening after especially warm winter days, leading to frost damage at further sub-zero temperatures (Bokhorst et al., 2009).

It is well established that plants are able to “remember“ low temperatures over a certain time span, as vernalization, the promotion of spring flowering favored by low temperatures requires some sort of winter-memory (Sung and Amasino, 2005). Here, we argue that cold tolerance acclimation is no ecological stress memory itself, as the plants harden under low temperatures but without experiencing frost stress. Cold acclimation rather is an evolutionary response to avoid frost stress. Experience of frost stress, for example during freeze-thaw cycles or under late frost events, could enable the plant to react differently to the next frost spell, even without prior acclimation.

Tahkokorpi et al. (2007) found increased anthocyanin levels in spring in newly grown stems of *Vaccinium myrtillus* after plants had been subjected to frost stress in winter, compared to plants not experiencing frost stress before. This strongly indicates an ecological frost stress memory on a whole plant level.

Under laboratory conditions, Knight et al. (1996) found a modified calcium signature in *A. thaliana* after plants had experienced a cold shock before. Calcium acts as a second messenger in low temperature signaling and may therefore trigger altered response to repeated frost.

However, Polle et al. (1996) showed that spruce needles surviving a spring frost event revealed less antioxidative enzymes and pigments in the following fall, thus probably showing reduced frost protection despite a transient increase in antioxidative enzymes after spring frost was applied. This shows that frost stress may lead to a loss of resilience rather than to the formation of a “positive” ecological frost memory.

To sum up, few studies hint towards the existence of an ecological frost stress memory (Tahkokorpi et al. 2007; Knight et al. 1996), although the response to repeated frost stress has not yet been investigated. It remains to be elucidated if frost stress experience helps to survive subsequent frost stress, as one study also indicates a decrease in frost resistance after frost stress (Polle et al. 1996).

5. Heat stress tolerance and heat stress memory

On a cell-level, heat stress acclimation is rather well understood: Upon exposure to extremely high temperatures, expression of normal housekeeping genes is stopped and heat shock proteins (HSP), which act to prevent protein damage or photo-oxidation and which repair already denaturated proteins (chaperones) are increasingly synthesized (Parcellier et al., 2003; Baniwal et al., 2004; Kotak et al., 2007). Furthermore, compatible solutes like prolin or betaine act to stabilize proteins (Schulze et al., 2005).

Under global warming it is very likely that heat waves will increase in frequency and magnitude over most land areas, as indicated, e.g., by the European mega-heatwaves in 2003 and 2010 (Schär et al., 2004; Barriopedro et al., 2011; IPCC 2011). Yet, studies investigating an ecological heat stress memory are widely lacking in plant ecology.

Interestingly, a transgenerational ecological stress memory was found for mild heat stress: Plants from the F3 generation showed a heat-specific fitness improvement when the parental and F1 generation had been treated with mild heat (30°C), even when the F2 generation was grown under normal conditions (Whittle et al., 2009) (Fig. 2B). As for all transgenerational studies, the existence of ecological stress memory within one plant generation was not tested, but is implied by the inheritance of stress tolerance.

To conclude, we found no study investigating an ecological heat stress memory in single plants. Nevertheless, the results by Whittle et al. (2009) indicate the existence of a heat stress memory improving heat tolerance after heat stress was already experienced in the past.

6. Cross-stress memory

As frost, heat and drought stress all involve cell dehydration, acclimation mechanisms are partly the same (Beck et al., 2007). It is thus possible that acclimation and formation of a stress memory to one kind of stress also prevents damage by other stressors, providing cross-stress memory and tolerance. For instance, frost tolerance of local populations or ecotypes, respectively, is related to drought tolerance (Blodner et al., 2005).

More specifically, exposure to an extreme drought event in the preceding year was found to support late frost tolerance in grass species (Kreyling et al., 2012a) and maximum frost hardiness in juvenile *Pinus nigra* (Kreyling et al., 2012b) (Fig. 2C).

Another form of cross-stress tolerance is increased herbivore resistance after the experience of abiotic stress like drought, caused by an increase in C-based secondary metabolites upon growth restriction due to abiotic stress (Herms and Mattson, 1992). It is unclear yet, how long such modifications of secondary compounds are maintained. However, Agrawal (2002) showed that the progeny of maternal plants attacked by herbivores also revealed higher induced resistance towards herbivores. This implies heritability and thus a form of stress memory of induced defense, although it is unclear if this could also act to prevent drought, heat or frost damage.

7. Possible mechanisms behind an ecological stress memory

As possible mechanisms for an ecological stress memory, Bruce et al. (2007) suggest the accumulation of transcription factors or proteins to facilitate a fast response upon repeated stress exposure as well as epigenetic mechanisms, such as histone modifications or chemical changes at the DNA (methylation, acetylation) that are inherited through mitotic or even meiotic cell divisions (Bossdorf et al., 2008; Chinnusamy et al., 2008; Boyko and Kovalchuk, 2011). Another possibility is the accumulation of protective substances. However, this is not likely to be very important, as synthesis of protective substances is costly and often prevents normal growth (Herms and Mattson, 1992). Today, it is well established that plant stress induces epigenetic changes (Goh et al., 2003; Chinnusamy et al., 2008). It was shown that epigenetic changes upon UV-C and flagellin exposure (Molinier et al., 2006), upon TMV (tobacco-mosaic-virus)-exposure (Boyko et al., 2007), upon pathogen or herbivore attack or low nutrients (Verhoeven et al., 2010) and upon nitrogen deficiency (Kou et al., 2011) are inherited. Furthermore, inherited DNA-hypomethylation in rice seedlings increased pathogen resistance (Akimoto et al., 2007) and inherited epigenetic changes upon TMV exposure increased pathogen resistance in progenies (Kathiria et al., 2010) However, no general heritability of epigenetic changes upon stress exposure was found in *A. thaliana,* implying that transgenerational epigenetic memory seems to be restricted to special conditions (Pecinka et al., 2009). The results of Tahkokorpi et al. (2007) hint towards epigenetic changes as underlying mechanisms of an ecological frost memory, as the new stems growing in spring never experienced frost stress themselves but revealed modifications (see Section 4). Thus, information had to be conveyed through mitotic divisions.

A further possibility to retain an ecological stress memory are changes in phenology or morphology of the plant that remain stable over longer time scales than mere changes in the accumulation of protective substances. Shifts in root to shoot ratio in response to drought (Kalapos et al., 1996; Kahmen et al., 2005) or winter warming pulses (Kreyling et al., 2008) are one obvious morphological response with implications for future drought tolerance. Furthermore, specific leaf area can be adapted to drought conditions (Kalapos et al., 1996), thereby reducing water loss of this tissue also for future drought events (see Table 1 for examples of mechanisms of an ecological stress memory).

8. Research challenges

Studies investigating ecological stress memory are rare. Most studies on the duration and heritability of plant stress are conducted on a cellular level and focus on genetic or epigenetic aspects. In such studies, time spans between the initial and the repeated stress is usually restricted to only several hours to days (Bruce et al., 2007). More ecologically relevant research and assessments of stress tolerance and ecological stress memory are needed. Furthermore, multigenerational epigenetic studies should consider consequences of extreme weather events more prominently.

In order to evaluate the ecological relevance of ecological stress memory, assessing ecologically meaningful parameters at the plant level in controlled lab experiments needs to be accompanied by field experiments and observations after naturally occurring extremes. Here, long-term field- and monitoring studies investigating the response to naturally occurring extremes in natural plant communities might be very valuable. To simultaneously elucidate underlying processes and mechanisms, cooperation between ecologists and molecular biologists are urgently needed. For instance, it is still unclear whether unseasonable frost damage in spring influences acclimation in the following fall and if this might be detrimental or beneficial.

In particular the absolute degree and the temporal stability of ecological stress memory requires attention because ecological stress memory could play an important role in stabilizing ecological communities in the face of more frequent extreme climatic events. Here, we suggest that ecological stress memory can, at least partly, explain the surprisingly weak effects of repeated extreme drought events on the productivity of grassland communities (Jentsch et al. 2011). Heritability of beneficial stress memory to following generations, potentially by epigenetic processes, is another highly relevant aspect for our understanding of ecological response to more frequent extreme climatic events.

To conclude, there are evidences for the existence of an ecological stress memory. However, mechanisms and consequences are not yet well investigated. A stress memory of single plants might act to stabilize plant communities under frequent climatic extremes and might increase resilience. It might be even possible to mitigate detrimental effects of extreme events by artificially applying milder stress on a small scale, e.g. for agriculture. However, it is not yet clear if, on a field and landscape scale, lagged detrimental effects might lead to a reduction of resilience under repeated extreme weather events that outweigh possible positive effects of an ecological stress memory. Furthermore, increased acclimation towards recurring stressors might reduce mortality of plants, but might as well reduce agricultural yield if plants reduce their photosynthetic activity to prevent damage. Hopefully, future research will contribute to elucidate mechanisms and consequences of an ecological stress memory.

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Manuscript 4: Do plants remember drought? Hints towards a drought-memory in grasses

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Abbreviations

F_m: maximum fluorescence yield of the dark adapted leaf
F_0: steady state fluorescence yield of the dark adapted leaf
F_v: variable fluorescence yield of the dark adapted leaf
F_v/F_m: potential maximum quantum yield of photosystem II
Pn [μmol CO_2 $m^{-2}s^{-1}$]: Net photosynthesis
PPFD [μmol $m^{-2}s^{-1}$]: photosynthetically active photon flux density
RWC [%]: Relative leaf water content

Highlights

1. Grasses react differently to recurrent drought when compared to a single drought.
2. Results indicate improved photoprotection of recurrently droughted plants.
3. “Stress imprints” after stress preexposure can lead to improved performance under recurrent stress exposure.
4. Differences in reaction to a recurrent drought are obtained after several weeks and harvest.

Abstract

The frequency of extreme drought events is projected to increase under global climate change, causing damage to plants and crop yield despite potential acclimation. We investigated whether grasses remain acclimated to drought even after a harvest and remember early summer drought exposure over a whole vegetation period. For this, we compared the response of *Arrhenatherum elatius* plants under a second, late, drought (they were pre-exposed to an early drought before), to plants exposed to a single, only late, extreme drought. Surprisingly, the percentage of living biomass after a late drought increased for plants that were exposed to drought earlier in the growing season compared to single-stressed plants, even after harvest and resprouting after the first drought. Relative leaf water content did not differ between the two treatments. Net photosynthesis was non-significantly reduced by 25% in recurrent drought treatment. Maximum quantum efficiency ($F_v/F_{m)}$ and maximum fluorescence (F_m) were reduced in plants that were exposed to recurrent drought. These findings indicated improved photoprotection in double-stressed plants. Our results provide first hints towards a “drought memory” over an entire vegetation period, even after harvest and resprouting. However, the advantage of improved photoprotection might also cause reductions in photosynthesis that could have adverse effects on crop yield under more severe or longer droughts.

Keywords: recurrent drought stress, climate warming, memory-effect, water deficit, Chlorophyll *a* fluorescence, phenotypic plasticity

1. Introduction

Droughts are often regarded as major threats to ecosystems under global climate change, as water stress limits crop yield more than all other biotic and abiotic factors combined (Lambers et al., 2008). The frequency and magnitude of regional drought periods have been increasing since the 1970s, with an exacerbation of the situation projected for many parts of the world (Schar et al., 2004; Trenberth et al., 2003). In Europe, the Mediterranean and mid-continental regions are expected to experience increased drought periods, accompanied by heat waves, as witnessed during the summer drought of 2003 (Schar et al., 2004).

Recent studies have investigated the impact of single drought events on ecosystems (Noormets et al., 2008), plant communities (van Peer et al., 2004; Kreyling et al., 2008) or single species (Galle et al., 2007; Jentsch et al., 2009). However, the consequences of recurrent drought events when compared to a single drought event on stress response and post-stress recovery are still not well understood.

Theory predicts that abiotic stress reduces resilience of ecosystems as a response to a single stress event, resulting in deteriorated performance and a total loss of resilience, when the system is affected by recurrent stressful events (Scheffer et al., 2001). On the other hand, plants are able to acclimate when faced with abiotic stress (Lambers et al., 2008; Bruce et al., 2007), revealing phenotypic plasticity as a response to environmental variability (Aubin-Horth and Renn, 2009). Short-term drought acclimation includes modifications to pigment content and the xanthophyll cycle (enhanced non-photochemical quenching) in order to prevent photodamage (Munne-Bosch and Alegre, 2000; Jiang et al., 2005). Phenotypic plasticity can cause a “stress imprint” that might facilitate a fast and protective response to a recurrent stressful event. Bruce et al. (2007) mention epigenetic changes and accumulation of signalling proteins or transcription factors as mechanisms for such a “memory effect”. Persistent changes in gene expression have been documented following a stressful event (Aubin-Horth and Renn, 2009). Epigenetic modifications can be inherited through mitosis or even meiosis (Goh et al., 2003; Bird, 2007; Bruce et al., 2007; Verhoeven et al., 2010). Up to now, experiments investigating these stress imprints have been mostly restricted to small time spans of less than one week (Bruce et al., 2007). Furthermore, studies investigating plant performance and functional consequences, and not only underlying molecular mechanisms in response to recurrent stress are rather rare. In woody communities, drought was found to reduce resilience, rendering plants more vulnerable to a recurrent disturbance (Lloret et al., 2004; Mueller et al., 2005). In grassland communities, mild droughts and warming did not lead to an enhanced resistance or recovery to an extreme follow-up drought but rather to a larger decrease in green vegetation cover in communities experiencing recurrent drought (Zavalloni et al., 2008). It has also been shown that plants can reveal memory effects after frost events, leading to a deteriorated performance long after frost stress was applied (Polle et al. 1996; Tahkokorpi et al. 2007; Kreyling et al. 2010).

The objective of our study was to identify differences in the effects of recurrent drought compared to a single drought on plant productivity and the photosynthetic performance of potted individuals of *Arrhenatherum elatius* (L.). *A. elatius* is a widely distributed and agriculturally important European perennial grass. It occurs mainly in moist, but not waterlogged habitats (Grime et al., 1988). Our explicit interest was on agriculturally important performance parameters (living biomass, photosynthesis, photoprotection) of a potential long lasting drought memory, and not on elucidating underlying genetic or chemical mechanisms. We investigated the presence, and potential photosynthetic mechanisms, related to a "long-term memory-effect" in grasses by imposing a recurrent drought eight weeks after an early drought. Between the first and the second drought, plants had been harvested and resprouted. As a control we compared those double-stressed plants to plants that received a single drought only. We aimed at quantifying not only resistance to recurrent drought but also recovery of ecophysiological parameters in the early post-stress phase. In order to show the generality of our results we included a variety of European provenances as a random factor in our experiment. Plants from these provenances have been found to be genetically distinct and thus potentially differing in phenotypic plasticity (Michalski et al., 2010).

We hypothesized that plants experiencing recurrent drought will not react differently to water deficit than plants experiencing a single drought. Furthermore, grasses after recurrent drought will not recover faster or slower than grasses after single drought. This is because drought acclimation that could buffer the adverse effects of a recurrent drought is unlikely to persist after total aboveground harvest and resprouting.

2. Materials and Methods

2.1. Plant material and experimental setup

A. elatius plants from six different provenances from Germany, Poland and Hungary were grown from seeds at the IPK Genebank, Satellite Collections North on the Island of Poel in Germany (Table 1).

Table 1 Geographical details on the six provenances used in the study

accesion-no.	country	lat [° WGS84]	long [° WGS84]
GR 331	Germany	51,8	13,7
GR 339	Poland	50,6	21,7
GR 357	Germany	51,1	11
GR 364	Poland	50	22,5
GR 7260	Germany	51,3	12,4
RCAT041661	Hungary	47,5	18,1

In April 2007, three-month-old individuals were planted into 6l bottomless tubes (20cm in diameter) at the Ecological Botanical Garden of the University of Bayreuth, Germany (49°55´19´´N,11°3455``E, 365 m asl). Tubes were embedded into the homogenized soil (loamy sand consisting of 82 % sand, 13 % silt, 5 % clay to a depth of 80cm). Plants were kept under natural ambient conditions for two years and harvested twice per year. Furthermore, tubes were periodically weeded. In April 2009, tubes, including the soil, were arranged under a rain-out-shelter for the experiment. To avoid the lateral flow of water into the pots, they were placed on plates on a plastic sheet. The transparent rain-out shelter was left open at the side up to 80 cm, allowing air exchange near to the surface and thus avoiding greenhouse-effects. Plants were subjected to two different treatments and arranged in a completely randomized design: 28 plants in the recurrent drought treatment (two plants died in the two years before the experiment started) were subjected to an early drought in June 2009 (D1), whereby water was completely withheld for 16 days from June 3rd until June 18th. The same plants were subjected to a later drought (D2), whereby water was withheld for 16 days from September 4th to September 19th. The recurrent drought treatment was compared to a single drought treatment: 27 replicates (three plants died prior to the experiment) were watered regularly every third day with 300ml rain water (C1) while the first drought period was applied to double-stressed plants. C1 plants were exposed to their first drought in September (C2), concomitant to the second drought of the recurrent drought treatment (See Fig. 1 for an overview).

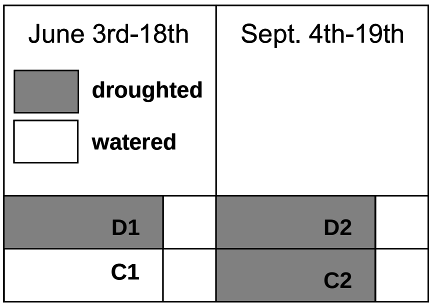

Fig. 1 Overview on the experimental time course and on applied treatments. In June, drought was applied for 16 days to D1 plants, while C1 plants were watered regularly. All plants were watered after the 16th day until the onset of the late drought in September. In September all plants (D2 and C2) were subjected to the drought for 16 days and were watered after that.

Only in comparing plants subjected to a second, late drought (D2) to plants experiencing their first drought (C2) at the same point in time, we could prevent confounding of potential drought memory effects with seasonality or timing effects. A comparison between the response to the first drought in June and the response to the second drought in September is thus not valid to investigate potential drought memory effects. We also did not have a well-watered control in September, as we were interested in a potential “drought-memory”, which can only be investigated by comparing single-stressed with double-stressed plants. To quantify effects of a single, early drought (D1), drought plants were compared to well-watered plants during the first drought period (C1). All plants were watered with the same amount of water (300 ml, every third day) until the onset of the experiment and in between the two drought treatments.

2.2. Aboveground biomass

Aboveground biomass was harvested on July 5th, 17 days after the first drought (D1 and C1) ended, and on October 9th, 21 days after the second drought ended (D2 and C2). After this time, reversible drought damages should have been recovered. As we only compare D2 with C2 plants and D1 with C1 plants, the difference of four days in recovery time after drought does not have an effect on the results. Plants were cut 4 cm above the ground in order to simulate common management techniques in meadows, sorted into living (green) and dead biomass. Dead biomass was defined as wilted, brown plant parts that lost chlorophyll. Biomass was dried at 70° C for 72 hours and weighed. Percentage of dead biomass was calculated as percentage of oven-dried, dead biomass in relation to overall oven-dried biomass of individual plants.

2.3. Relative leaf water content (RWC)

Relative leaf water content was determined in the afternoon of the 13th day of the first and second drought treatment (June 15th and September 16th), according to Barrs and Weatherley (1962).The second lowest leaf of each plant was cut, stored in a moistened plastic bag for transport, and immediately weighed to determine fresh weight (FW). Leaves were stored in distilled water at 4° C over night and weighed the next morning to determine turgid weight (TW). Afterwards leaves were dried at 70° C and the dry weight (DW) was determined.

RWC was calculated as:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} * 100$$

2.4. Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was recorded using a pulse-amplitude-modulated photosynthesis yield analyzer (PAM 2000 and Mini-PAM) (Waltz, Effeltrich, Germany) with a leaf clip holder as described by Bilger et al. (1995). The second or third fully-expanded leaves were measured on four different blades of one individual. Four measurements per plant were averaged for further analysis. We obtained predawn fluorescence values (between 2:00 and 4:00) at the end of the first drought treatment, throughout the second drought period and throughout the early recovery phase after the second drought. The maximum quantum efficiency of photosystem II was calculated as F_v/F_m. Variable fluorescence (F_v) and maximum fluorescence (F_m) were measured before dawn. F_v was calculated as F_m-F_0, F_m being the maximum fluorescence of the dark adapted leaf after applying a saturating light pulse and F_0 being the steady state fluorescence yield of the dark adapted leaf (Maxwell and Johnson, 2000).

To enable a comparison between absolute fluorescence values, a fluorescence standard material was measured before and after each measuring cycle. Standard measurements were used to normalize the fluorescence values obtained and to calibrate the two different PAMs in use. Predawn measurements of fluorescence at the dark adapted leaf allow drawing conclusions about underlying processes which alter plant photosynthetic performance and about photoinhibitory damage and non-photochemical quenching (Maxwell and Johnson, 2000). Absolute F_0 and F_m values were taken to separate the effects of photodamage, becoming apparent with an increase of F_0, from the effects of photoprotection related to enhanced non-photochemical quenching, becoming apparent with a decrease in F_m (Osmond et al., 1993; Araus et al., 1998; Maxwell and Johnson, 2000).

2.5. Leaf gas exchange

The net CO_2 assimilation rate (Pn) and transpiration were measured at midday (between 11:30 and 13:30), when drought stress should be at its maximum, due to high temperature and irradiance. It was measured on the second, fully developed leaf of each plant using a gas-exchange system (Li-6400, Li-Cor, Lincoln, NE, USA) equipped with a CO_2 cartridge to adjust and maintain constant CO_2 of 400 μmol mol^{-1} air within the leaf cuvette. Gas exchange measurements were conducted on clear days without clouds to maintain constant PPFD. After reaching steady-state photosynthesis, data were logged. The leaf area was estimated simultaneously by measuring the leaf width and later on used to correct values for net photosynthesis, as leaf blades did not fill the whole leaf cuvette. Gas exchange was measured under ambient light conditions at the end of the first drought period, in the early and late drought period and in the early recovery phase after the drought.

2.6. Statistical analysis

To determine significant differences between single and recurrent drought treatments, analyses of variance were performed for all variables for each sampling date. We defined “treatment” as a fixed factor. “Provenance” was a random factor in this experiment, as provenances were chosen randomly out of a larger population of provenances, and as we were not interested in the specific provenances, but in the whole population of our plants (Dormann and Kühn, 2009). We examined the residuals against fitted plots and normal qq-plots prior to each analysis to test whether the assumptions for ANOVA, homogeneity of variances and normality, could be met (Faraway, 2006). If this was not the case, data were powertransformed (fluorescence data), log-transformed (absolute biomass data) or arcsin-transformed (relative water content) accordingly.

All statistical analyses were performed using R 2.11.0 (R Development Core Team 2010). For mixed effect models we used the software package nlme (Pinheiro et al., 2008).

3. Results

3.1. Effects of the first drought (D1)

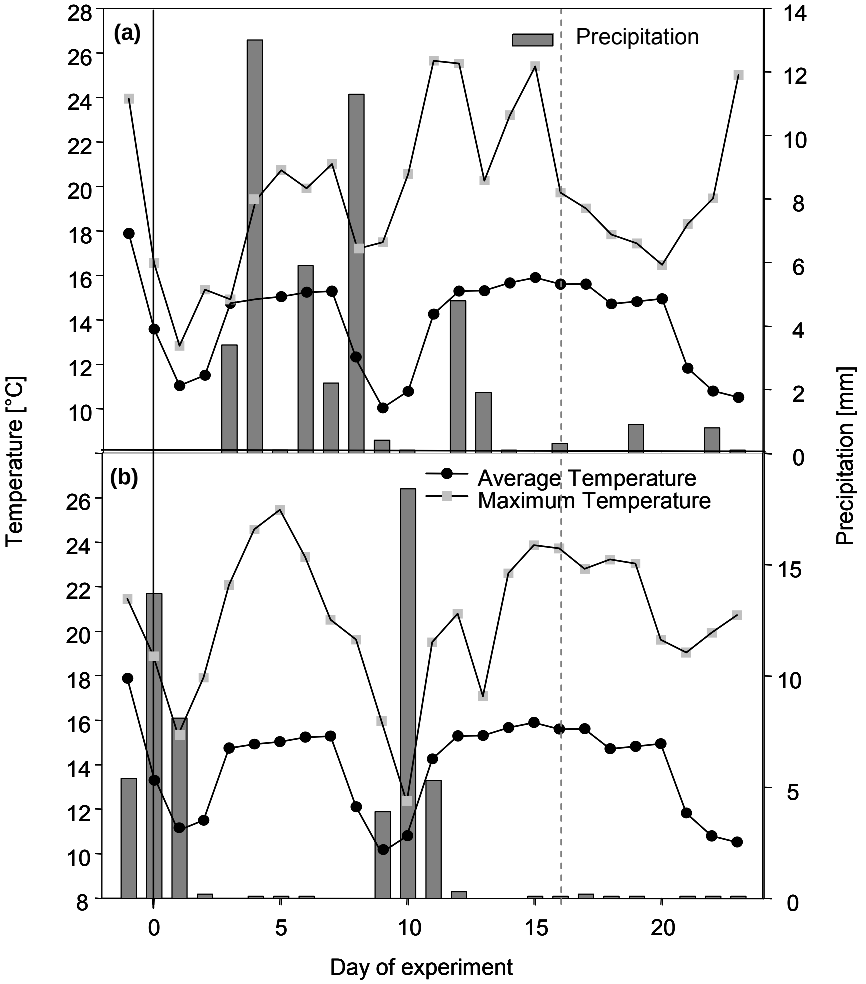

Fig. 2 Course of daily maximum temperature (grey squares), daily average temperature (black circles) and precipitation (dark grey bars) at the study site during the experimental periods in June (a) and September (b). Vertical black line indicates the start of the drought treatment, grey dashed line indicates when rewatering started.

Temperature and precipitation data during the experimental period in June are shown in Figure 2.

Early drought treatment (D1) in June significantly reduced the relative leaf water content measured at the end of the drought treatment compared to the well-watered control (C1) by around 22% (P<0.001; Fig. 3a).

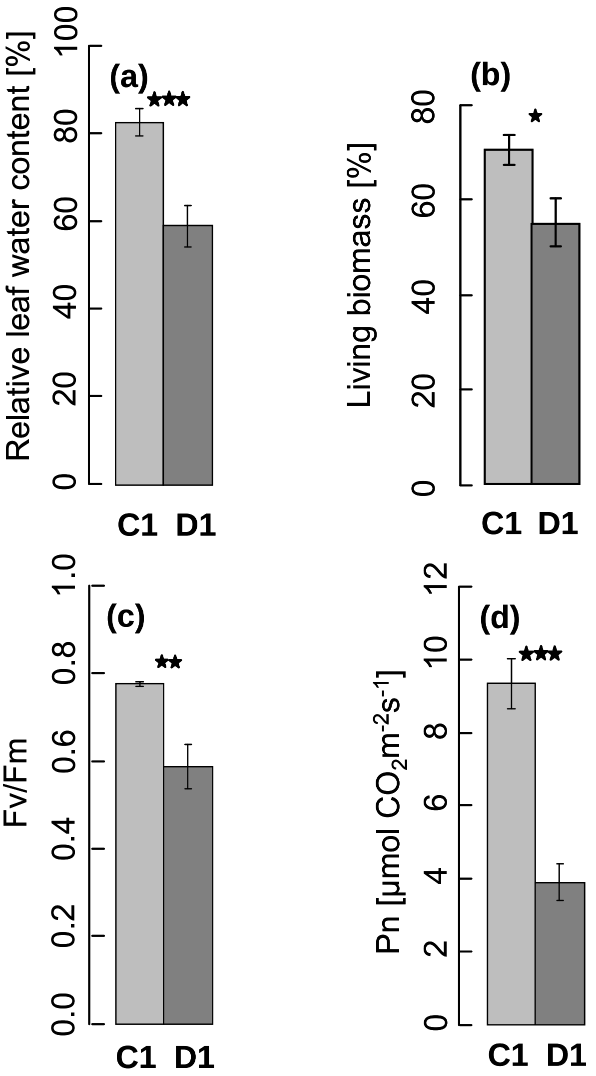

Fig. 3 Effects of the first drought (D1) in June (dark grey) compared to well-watered control plants (C1) (light grey) on relative water content of leaves (a), percentage of living biomass (b), maximum quantum efficiency Fv/Fm, measured predawn (c), and net photosynthesis Pn, measured during midday (d). Relative leaf water content (a) was measured three days before the end of the drought treatment, maximum quantum efficiency Fv/Fm (c) and net photosynthesis Pn (d) were recorded on the last day of the drought treatment. Total aboveground harvest was conducted 17 days after the end of the first drought treatment and the percentage of dead biomass (dry weight) (b) was calculated. Means and SE are shown, asterisks indicate significance (*, P≤ 0.05; **, P≤ 0.01; ***, P≤ 0.001).

Early drought treatment (D1) had no effect on the total aboveground biomass when compared with the well-watered control treatment (C1) (data not shown). However, drought reduced the percentage of living biomass significantly by around 15% (P=0.03; Fig. 3b). On the last day of early drought treatment (D1) the photochemical efficiency (F_v/F_m) of plants under drought was significantly reduced compared to the well-watered control (P=0.004; Fig. 3c).

Plants under early drought treatment (D1) exhibited reduced net photosynthesis by 58% on the last day of the early drought treatment (P<0.001; Fig. 3d).

3.2. Effects of recurrent drought events (D2) compared to a single drought event (C2) in September

3.2.1. Temperature, Relative leaf water content and productivity in the second drought period

Average daily temperatures during the experimental period in September ranged between 10°C and 20°C (Fig. 2b). The maximum temperature was exceptionally high with 25.5° C on the 6th day of the experiment in September and lowest on the 11th day of the experiment with 12.3° C. The withholding of water was reflected in the relative water content of the leaves, which was reduced to around 65% but did not reveal any differences between single and recurrently dried plants (D2 vs. C2: P=0.38, Fig. 4a).

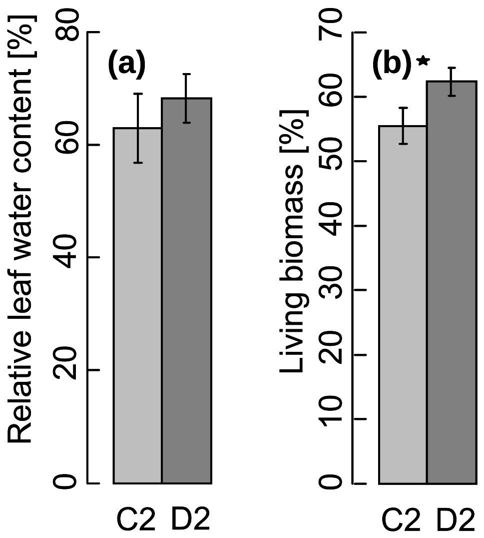

Fig. 4 Leaf water status at the end of the second drought period 13 days after water had been withheld (a), and percentage of living biomass for the second harvest, three weeks after the end of the drought treatment (b). Means +/- 1 SE are shown, asterisk indicates significance of difference (*, P≤ 0.05).

Plants in single and recurrent drought treatments produced an almost equal amount of overall (living and dead) aboveground biomass (1.47g in recurrent drought treatment (D2) and 1.49g in single drought treatment (C2)). However, the percentage of living biomass, was increased significantly by 7% in plants subjected to recurrent drought (D2) compared to plants experiencing their first drought (C2) (P=0.048) (Fig. 4b). Total living biomass was increased by 10% in recurrent drought treatment, although this effect did not prove to be significant (0.91g in recurrent drought treatment and 0.82g in single drought treatment, P=0.18).

3.2.2. Photosynthetic parameters

With progressive drought stress, F_v/F_m in the single and recurrent drought treatment declined, reaching a minimum for the double-stressed plants (D2) on the 14th day of the experiment, two days before rewatering (Fig. 5a). Single-stressed plants (C2) already reached minimal quantum efficiency on the 11th day of the drought and F_v/F_m values rose again after that. The loss of leaf water and photochemical efficiency under extreme drought was reflected in a decline of net photosynthesis by more than 60% compared to net photosynthesis before the drought treatment started (D2 and C2) (Fig. 5b).

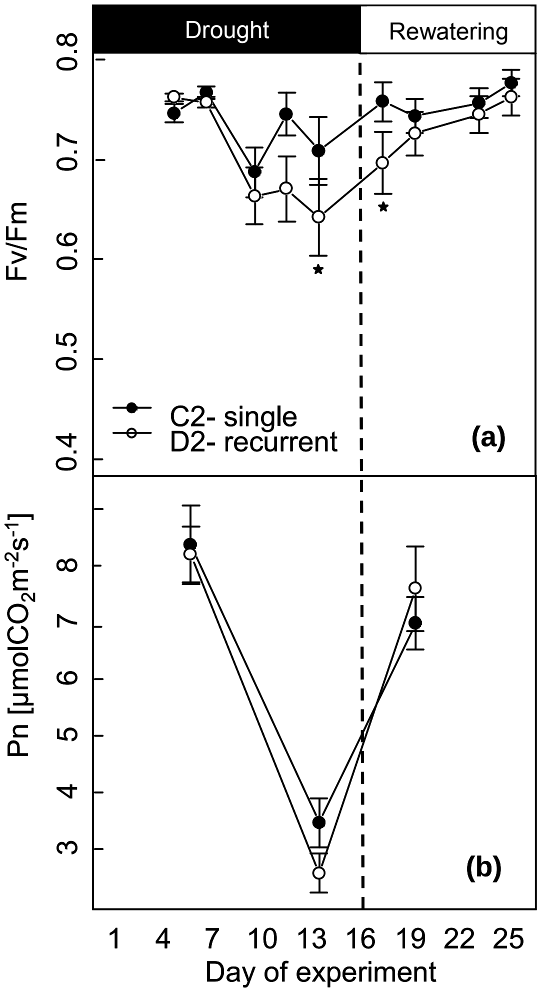

Fig. 5 Course of maximum quantum efficiency Fv/Fm, measured predawn (a) and net photosynthesis, measured during midday (b) in A. elatius subjected to recurrent or single drought before and during the drought phase and rewatering in September. Dark grey dashed line indicates the start of the drought, black dashed line indicates the end of the drought and the start of rewatering in September. Means +/- 1 SE are shown, asterisk indicates significance of difference between single and recurrent drought treatments on single days (*, P≤ 0.05).

Grasses under recurrent drought (D2) showed lower maximum quantum efficiency compared to plants exposed to a single drought (C2), from the eleventh day of the experiment until the end of measurements, 10 days after the onset of rewatering (Fig. 5a). This reduction was significant on the last day of measurements under the drought (14th day of the experiment, P=0.05) and on the first day after rewetting (17th day of the experiment, P=0.02) (Fig. 5a). During drought and in the post-drought recovery phase, plants subjected to single (C2) and recurrent drought (D2) did neither differ significantly regarding net photosynthesis, nor transpiration (transpiration data not shown). However, on the 14th day of the experiment, net photosynthesis of grasses subjected to recurrent drought (D2) was 25% lower compared to grasses being subjected to their first drought (C2), but this effect was not significant (P=0.11). A closer look at fluorescence parameters on both of these days with significant reductions in F_v/F_m (14th and 17th day of experiment) revealed that the decrease of F_v/F_m in plants subjected to recurrent drought can be explained by a decrease in F_m rather than by an increase in F_0 (Table 2). Two days before rewatering, F_m was reduced by around 20% (14th day of the experiment, P=0.07). F_0 was non-significantly reduced by around 6% (P=0.57). F_m was reduced by around 10% on the first day of measurements after rewetting (17th day of the experiment, P=0.3) as opposed to F_0, which was reduced by only 0.5% in plants subjected to recurrent drought (P=0.94) (Table 2).

Table 2 Maximum fluorescence (F_m) and steady state fluorescence (F_0) two days before rewatering (14) and one day after rewatering (17) in the recurrent and single drought treatment during the experimental period in September. Means +/- 1 SE are shown (n=5).

		day of experiment	
		14	17
F_m			
	single	0.696 ± 0.0031	0.793 ± 0.0026
	recurrent	0.553 ± 0.0030	0.718 ± 0.0025
F_0			
	single	0.157 ± 0.0001	0.170 ± 0.0001
	recurrent	0.148 ± 0.0001	0.169 ± 9.9e-5

F_v/F_m of both treatments recovered gradually after the drought treatment ended, reaching pre-drought values ten days after rewatering had started, on the 26th day of the experiment (Fig. 5a). One week after rewetting, net photosynthesis had been almost completely restored, showing reductions of only 9% compared to pre-drought values.

4. Discussion

This study investigated, whether *A. elatius* plants of six mid- and eastern European provenances can remember drought stress over an entire vegetation period even after a harvest. We hypothesized that plants would not show different performance under recurrent drought. This hypothesis was not confirmed, as grasses responded consistently different in recurrent drought as compared to a single drought, indicating enhanced photoprotection.

Surprisingly, this effect persisted after total aboveground biomass harvest and regrowth. The observed changes in reaction to recurrent drought are not in accordance with findings indicating reduced resistance or resilience after having already been exposed to drought stress before (Lloret et al., 2004; Zavalloni et al., 2008). However, these were conducted under field conditions and Zavalloni et al. (2008) investigated community responses. Thus, different outcome of the experiments are not surprising. The findings are in accordance with reported stress imprint effects or stress memory (Bruce et al., 2007). However, to our knowledge, no study has already provided evidence that grasses do remember drought stress even after a harvest and can exhibit improved performance in the face of repeated abiotic stress over such a long duration. Stress imprint and acclimation were previously mostly reported to last for "several days" (Bruce et al., 2007).

Under severe drought, grasses experiencing recurrent drought showed reduced maximum quantum efficiency F_v/F_m (Fig. 5a). This was mainly related to reductions in maximum fluorescence, indicating enhanced dissipation of light energy to prevent photodamage (Maxwell and Johnson, 2000). Osmond et al. (Osmond et al., 1993) and Araus et al. (Araus et al., 1998) suggest that a correlation of reduced F_v/F_m with an increase of F_0 can be interpreted as chronic photoinhibitory damage due to the degradation of the D1 protein in the reaction centers. By contrast, a constant F_0 and decreasing F_m values, as in our study, point towards photoinhibition related to enhanced non-photochemical quenching via the Xanthophyll cycle and thus indicate photoprotection (Araus et al., 1998). Another possible explanation of decreased F_v/F_m caused by reduced F_m values might be a reduction of chlorophyll. Unfortunately, we did not measure chlorophyll content in our study. However, a reduction of chlorophyll can be considered as a feature of acclimation, as it reduces the possibility of photodamage because of an excess of energy (Munne-Bosch and Alegre, 2000). Net photosynthesis did not reveal any differences between grasses under recurrent (D2) and single drought (C2), but showed a trend towards lower photosynthesis in plants receiving recurrent stress under extreme drought (Fig. 5b). This is in accordance with the reduced photochemical efficiency in plants recurrently experiencing drought (D2).

The results of the aboveground biomass support the hypothesis of enhanced photoprotection of double-stressed plants, as the percentage of living biomass was significantly increased in plants experiencing their second drought, although total aboveground biomass or total living biomass were not significantly altered.

Plants can adapt to drought by enhancing root growth. However, relative water content of the leaves was not significantly enhanced in plants experiencing recurrent drought, indicating that the observed results can not be explained by changes in root biomass or improved water uptake mechanisms.

Ecophysiological measurements did not reveal any consistent differences between recurring (D2) and single drought (C2) in the post drought recovery phase. Maximum quantum efficiency two days after rewetting in grasses subjected to recurring drought was significantly lower compared to grasses subjected to their first drought. This was more likely related to increased stress levels under drought rather than a lower recovery rate. Some studies indicated that recovery rate depends on experienced stress level (Miyashita et al., 2005; Resco et al., 2009). The significantly lower percentage of dead biomass in plants subjected to recurring drought can be a sign of quicker recovery, but is more likely to be a sign of improved photoprotection, as discussed above. In accordance to other studies, the recovery of ecophysiological parameters was quite fast, almost reaching pre-stress levels after about one week (Galle et al., 2007; Galmes et al., 2007).

We did not elucidate underlying molecular or biochemical mechanisms for acclimation in this study, as we were interested in the effects or recurrent drought on agricultural relevant performance parameters. Thus, we can only hypothesize about potential long-lasting acclimation processes in the grasses. The observed phenotypic plasticity could be either explained by belowground dynamics or by long-lasting changes in gene expression, rendering the plants more permissive to react quickly to recurrent stress, e.g. epigenetic processes (Aubin-Horth and Renn, 2009;Molinier et al., 2006; Bird, 2007; Bossdorf et al., 2008). Verhoeven et al. (Verhoeven et al., 2010) recently showed that stress induces changes in methylation patterns and that these patterns are heritable. An investigation of the changes in methylation patterns as a response to drought and a link of observed methylation patterns to stress response are very promising. Furthermore, we could only investigate six provenances of *A. elatius* plants, which originated mainly from areas in Europe with quite similar climatic conditions. Other, more different provenances were not surviving in sufficient replicates for our study. Nevertheless, an extension of our experiment to other provenances and plant groups seems promising, as they may reveal different acclimation patterns and therefore also different responses to recurrent drought.

5. Conclusion

To conclude, our study indicates that grasses under drought retain a long-lasting stress imprint that facilitates a faster and more protective response towards a recurrent drought.

Grasses being subjected to recurring drought showed improved photoprotection. However, under more intense or frequent drought events, the reduction of photochemical efficiency and thus photosynthesis, could lead to a loss of productivity (Murchie et al., 2009). This is indicated in our study by a trend towards a lower photosynthetic rate under extreme drought. However, further studies have to elucidate acclimation processes on a molecular and biochemical level more deeply, and relate them to functional parameters. Long-term acclimation mechanisms still have to be scrutinized for a better understanding of plant responses to a changing climate, and to be able to make projections and recommendations for adaptation to climate change.

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Manuscript 5: Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought

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Highlights:

1. Cold hardiness of *Pinus nigra* shows local adaptation to climate at its geographic origin
2. Winter cold hardiness increases with summer drought and summer warming
3. Cold hardiness is related to the content of soluble carbohydrates and composition of fatty acids and alkanes
4. *Pinus nigra* shows similar cold hardiness as the native Central European forest trees

Abstract

Adaptation to the adverse effects of climate change is being investigated more and more through the introduction of species from warmer and drier climates, such as the (sub-) mediterranean *Pinus nigra* to dry sites in temperate Central Europe. Winter survival, however, may pose a serious threat to this strategy as cold extremes, which naturally determine the poleward range limits of forest trees, are not expected to follow the general warming trend in the near future.

Here, juveniles of *P. nigra* from eight provenances throughout Europe were exposed to different climate change scenarios (factorial combinations of 42 days of drought and warming by 1.6°C) in a common garden experiment in Bayreuth, Germany. Cold hardiness (LT50) was determined by the Relative Electrolyte Leakage method (REL) in two consecutive winters.

Cold hardiness of foliage differed by 10°C between the provenances studied and a local adaptation to minimum temperature was found. Cold hardiness was further affected by extreme summer drought, increasing cold hardiness by 3.9°C on average in the subsequent winter, and by summer warming, increasing cold hardiness by 3.4°C. Year-round warming had no significant effect on cold hardiness. Cold hardiness was related to the content of soluble carbohydrates and to the composition of fatty acids and alkanes in the needles. Juveniles of *P. nigra* exhibited a comparable cold hardiness as juveniles of species native to Central Europe (*P. sylvestris, Picea abies, Fagus sylvatica* and *Quercus petraea*) under the same climatic conditions. Cold hardiness of the fine roots of *P. nigra* averaged -16.5°C compared to -23.8°C on average for needles.

Our results imply that the cold hardiness of the foliage is adaptive to both long-term growing conditions at the seed origin (genetic heritage) and short-term alterations of these conditions (individual plasticity), while first hints suggest that cold hardiness of the roots is high and might not be adaptive. For *P. nigra*, below- and above-ground cold hardiness of selected provenances in mid-winter appear suitable for cultivation in temperate regions.

Keywords: frost hardiness, black pine, ecotype, cold tolerance, global warming, winter ecology

1. Introduction

Species respond to climate change by poleward range shifts (Parmesan and Yohe, 2003). The speed of warming, however, is expected to exceed natural migration rates in many cases (Thomas et al., 2004). In forestry in particular, human-assisted range shifts are proposed to counter long generation cycles and modest dispersal abilities of forest trees (Schaberg et al., 2008b; McKenney et al., 2009). Yet, the importance of winter conditions is often overlooked, especially in the ecology of temperate regions (Kreyling, 2010). Absolute minimum temperatures have strong implications for species distributions by often determining their poleward range limits (Sakai and Weiser, 1973; Repo et al., 2008). A single cold extreme can offset any distributional adaptations to the general warming trend (Jalili et al., 2010) and in spite of the mean warming and their decreased frequency of occurrence, both the intensity and the duration of such cold extremes may even increase regionally within this century due to atmospheric circulation changes and internal atmospheric variability which counteract the warming trend from greenhouse forcing (Vavrus et al., 2006; Kodra et al., 2011).

Phenotypic plasticity and the adaptive potential of forest trees are determined by their high genetic diversity, allowing forest trees to develop local adaptations to environmental stressors (Hosius et al., 2006; Schaberg et al., 2008b). The cold hardiness of *Pinus devoniana*, for instance, increases with increasing frost risk along an altitudinal gradient (Saenz-Romero and Tapia-Olivares, 2008). Similarly, changes to the cold hardiness of *Fagus sylvatica* indicate local adaptation to the prevailing minimum winter temperatures (Visnjic and Dohrenbusch, 2004) and to late spring frost risk (Kreyling et al., 2011b) across Europe, and the frost tolerance of *Tsuga heterophylla* is adapted to frost risk along latitudinal and altitudinal gradients in North America (Kuser and Ching, 1980). Provenance trials demonstrate a differential performance between the provenances of different geographic origins of *Pinus nigra* (Varelides et al., 2001), which is the target species of this study. *P. nigra* was selected because it is discussed in forestry as target species for translocations to Central Europe (Kölling, 2007; Huber 2011) and because of its high genetic diversity (Nkongolo et al., 2002; Jagielska et al., 2007). Based on its fragmented submediterranean range, one could assume that it lacks adaptation to winter frost, at least in some provenances. Provenance trials suggest that frost damage occurs around -20°C and in particular those provenances from Corsica do not survive -25°C (summarized in Huber, 2011).

The cold hardiness of evergreen tree species fluctuates over the course of the year. During acclimation in autumn, the plant organs become increasingly tolerant to the damaging effects of tissue freezing, particularly protecting cellular membranes which are a prime place of freezing injury (Bigras et al., 2001). Even though the genetic controls of the protective processes in conifers are complex and not yet sufficiently understood (Holliday et al., 2008), data has been summarized on the chemical components that are involved (Thomashow, 1999). During acclimation, lipid composition in the plasma membrane shifts towards more unsaturated lipids (Bakht et al., 2006) in addition to accumulation of soluble carbohydrates, hydrophilic polypeptides, antioxidants and chaperones in the membranes (Thomashow, 1999). Increased concentrations of all these chemical components serve the general purpose of preventing intra-cellular ice crystallization (Bigras et al., 2001).

Plants grown under generally warmer conditions, however, may lose their functional adaptations to frost (Eccel et al., 2009). Plants can further cope with different environmental stressors by similar responses at the cellular and molecular level when these stressors trigger similar signal chains. Drought and frost, for instance, lead to similar physiological responses in a coniferous forest tree - aiming to prevent cellular dehydration (Blodner et al., 2005). More frequent drought events may therefore make up for diminished acclimation due to warming.

The (sub-) mediterranean distribution of our target species *P. nigra* is reflected in high drought tolerance (Isajev et al., 2004) relative to temperate species such as *Pinus sylvestris* or *Fagus sylvatica*. Therefore, translocation of *P. nigra* is discussed as one adaptation strategy against the adverse effects of climate change at dry sites in Central Europe (Kölling, 2007). The minimum temperature in winter, however, is one of the most important factors setting the northern boundaries of the natural ranges of forest tree species (Sakai and Weiser, 1973; Koerner and Paulsen, 2004). The cold hardiness of one single provenance of *P. nigra* was lowest among eight *Pinus* species (with *P. nigra* showing the southernmost native range of the tested species) in a common garden experiment in Trondheim, Norway (Strimbeck et al., 2007). As tree species are generally well adapted to the minimum temperatures of their environment (Sakai and Weiser, 1973), the range of frost tolerance of *P. nigra* across provenances needs to be examined in detail before translocations to other climates are undertaken. This holds particularly true because climate modelling implies that cold extremes will remain stable in their magnitude throughout this century in spite of climate warming (Vavrus et al., 2006; Kodra et al., 2011). With regard to the life span of trees, the expected decrease in frequency of cold spells (e.g. Vavrus et al., 2006; Kodra et al., 2011) is clearly less important than magnitude and duration of individual cold spells, as even with decreased frequency the likelihood of experiencing at least one cold spell is still close to 100%. Furthermore, forests grow slowly and management action aiming at stable and productive forests in future need to be started now. Target species for translocations need therefore not only be adapted to future conditions, but also survive current conditions with prevailing occurrences of cold extremes.

Plant organs differ in their cold hardiness. Generally, roots are the least frost tolerant (Mancuso, 2000; Bigras et al., 2001). The on-going decline of *Chamaecyparis nootkatensis* in the Pacific Northwest of North America, for instance, has been linked to root frost damage due to climate change-induced reductions in the insulating snow cover (Schaberg et al., 2008a). A similar reduction in snow cover is also projected for Central Europe (Kreyling and Henry, 2011). In addition to shoot cold hardiness, root freezing tolerance should therefore be investigated.

Here, eight provenances of *P. nigra* from autochthonous origins and from southern Germany were tested for their cold hardiness in a common garden experiment in southern Germany. We hypothesized that (1) cold hardiness differs between provenances, with provenances from colder origins displaying superior cold hardiness, and that (2) cold hardiness is affected by climatic experiences of the individuals with drought increasing cold hardiness and warming decreasing cold hardiness. We further expected that (3) differences in cold hardiness between provenances are physiologically-related to the content of soluble carbohydrates and lipid composition of the needles, and that (4) the (sub-) mediterranean species *P. nigra* is less frost-tolerant than tree species native to Central Europe, while (5) cold hardiness of the fine roots of *P. nigra* is high compared to cold hardiness of its foliage as it naturally occurs in regions without continuous snow cover.

2. Material and methods

Juveniles of *P. nigra* from eight provenances throughout Europe were exposed to different climate change scenarios (warming and extreme drought) in a common garden experiment. Cold hardiness was determined by the Relative Electrolyte Leakage method (REL) in two consecutive winters. The experiment was established in Bayreuth, Germany (49°55’19” N, 11°34’55” E) in March 2009. The long-term mean annual temperature for the site is 8.2°C, whereas long-term mean annual precipitation is 724 mm.

2.1. Experimental design

Eight provenances of *P. nigra* (Figure 1; Table 1) were obtained as seeds and cultivated at the *Bavarian Institute for Forest Seeding and Planting (ASP)* in Teisendorf, Germany from April 2008 to April 2009.

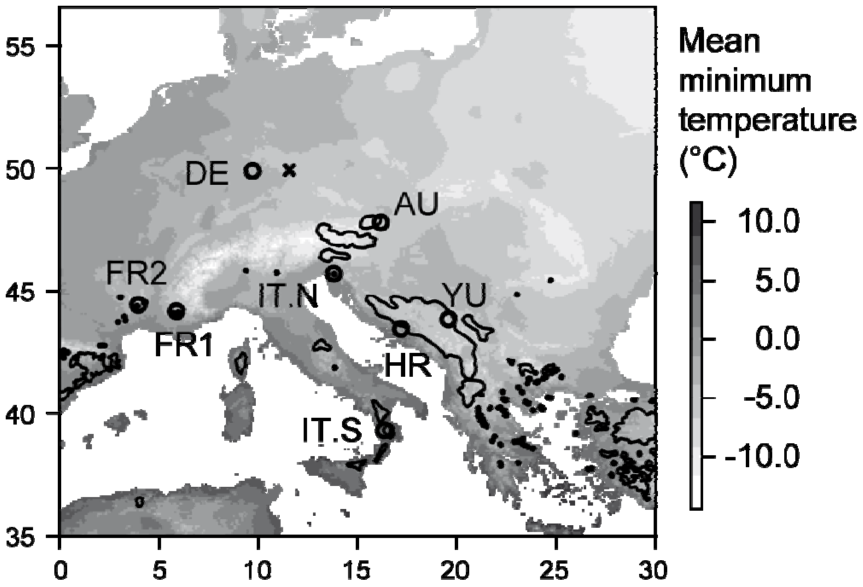


Figure 1: Origins of the target provenances (open circles) within the distribution of P. nigra (black lines and dots for fragmented populations Isajev et al., 2004). X indicates the experimental site. Abbreviations of provenances are specified in Table 1. Grey scales display the mean minimum temperature for the period 1950 to 2000 with a 5' spatial resolution (Hijmans et al., 2005).

These provenances are part of an international long-term provenance trial which started in 2009 (Huber, 2011). The provenances stem from autochthonous populations of *P. nigra* except for the provenance from Zellingen, Germany, which was introduced from Austrian sources in 1909. Subspecies identities of the provenances are assigned geographically and morphologically (Table 1), as genetic analyses are not yet available (Huber, 2011).

Table 1 Origins of target provenances used in the experiment with corresponding climatic information. Skie: Identification number in an international provenance trial (Huber, 2011). MAT: Mean Annual Temperature; MinT: Mean Minimum Temperature; MAP: Mean Annual Precipitation; Precip. Seasonality: Coefficient of variation in mean monthly precipitation sum. All climate data for the period 1950 to 2000 from worldclim (Hijmans et al., 2005).

Provenance	Country	Subspecies	Skie	North	East	Altitude (m)	MAT (°C)	MinT (°C)	MAP (mm)	Precip. Seasonality
DE	Germany	*nigra*	01	49°53'17"	09°43'16"	290	9.2	-3.1	587	18
AU	Austria	*nigra*	07	47°46'00"	16°11'00"	369	8.4	-4.9	712	33
YU	Serbia	*nigra*	12	43°49'39"	19°35'22"	866	8.7	-5.6	964	17
HR	Croatia	*nigra/ dalmatica*	14	43°26'00"	17°13'00"	256	13.2	1.2	1108	33
IT.N	Italy	*nigra*	17	45°42'00"	13°49'00"	372	11.4	-1.2	1212	17
IT.S	Italy	*laricio*	19	39°18'08"	16°20'22"	1500	9.0	2.2	1300	48
FR1	France	*nigra*	23	44°09'10"	05°52'30"	549	10.7	-2.9	789	16
FR2	France	*laricio*	24	44°24'18"	03°58'39"	581	10.8	-0.9	745	19

The Croatian provenance stems from a location very close to one of the few autochthonous stands of *P. nigra* subspecies *dalmatica* and its assignment to the subspecies *nigra* is somewhat questionable. The seedlings were transported to Bayreuth and individually planted into 4-litre plastic pots filled with sandy silt (pH 7.3, total C 1.9%, total N 0.15%, plant available NO_3^--N 22.5 mg l^{-1}; plant available NH_4^+-N 1.8 mg l^{-1}). Selection of the plants occurred randomly for each provenance from all those plants alive at the planting date. The mean plant size at the start of the experiment was 12.2 cm ± 2.5 cm SD.

The potted individuals were exposed to the fully crossed threefold factorial combination of (1) a drought manipulation (drought and control) and (2) a continuous warming manipulation (warming and reference) and (3) the provenance treatment (eight provenances). The two climate treatments were crossed resulting in four climate manipulations (control, drought, warming, warming & drought), that were replicated three times, resulting in 12 experimental units in total. The provenance treatment was nested within each experimental unit. Each provenance was further replicated with seven plants per experimental unit (nested replicates), resulting in 21 plants per factorial combination of the three-factorial design and 672 plants overall. Each experimental unit was covered by a single rain-out shelter (11 m by 7 m, 3.8 m high) constructed of a steel frame (GlasMetall Riemer GmbH) and covered with a transparent polyethylene sheet (0.2 mm, SPR5, Hermann Meyer GmbH) enabling an almost 90% penetration of photosynthetically-active radiation. The edge of the rain-out shelters was at a height of 80 cm.

The control irrigation regime simulated the local daily 30-year average precipitation. The application was carried out twice a week with collected rain water. The drought treatment consisted of 42 days without precipitation, which represents the local statistical 1000-year recurrence drought event. Drought duration was not *a priori* set before the manipulations. We monitored plant performance during the treatment and would have stopped the treatment when either 66% of the plants showed water stress symptoms (discoloration of foliage) or when 33% of the plants exhibited lethal stress, or when the local 1000-year extreme would be reached. The latter condition was set because we assume that events with more than 1000 year recurrence time are not too realistic even when changing frequencies of extremes due to climate change are acknowledged (Schär et al., 2004). The same protocol was applied in a parallel experiment with four grass species (Beierkuhnlein et al., 2011), which all showed severe drought symptoms after about 20 days, emphasizing the high drought tolerance of *Pinus nigra*. The drought treatment started on May 27th 2009 and resulted in the soil moisture falling below the permanent wilting point (pF = 4.2) of the soil approximately three weeks after the start of the treatment (Figure 2). In the re-wetting phase each individual in the drought treatments received 240, 280 and 300 ml on three days within one week (in total 820 ml or 36 mm). Following that, the pots were irrigated according to the control precipitation treatment. Total amount of precipitation in the drought treatment was 13% lower than in the control over the year. The drought was simulated in the first year of the experiment only. Throughout the second year, all plants received control irrigation.

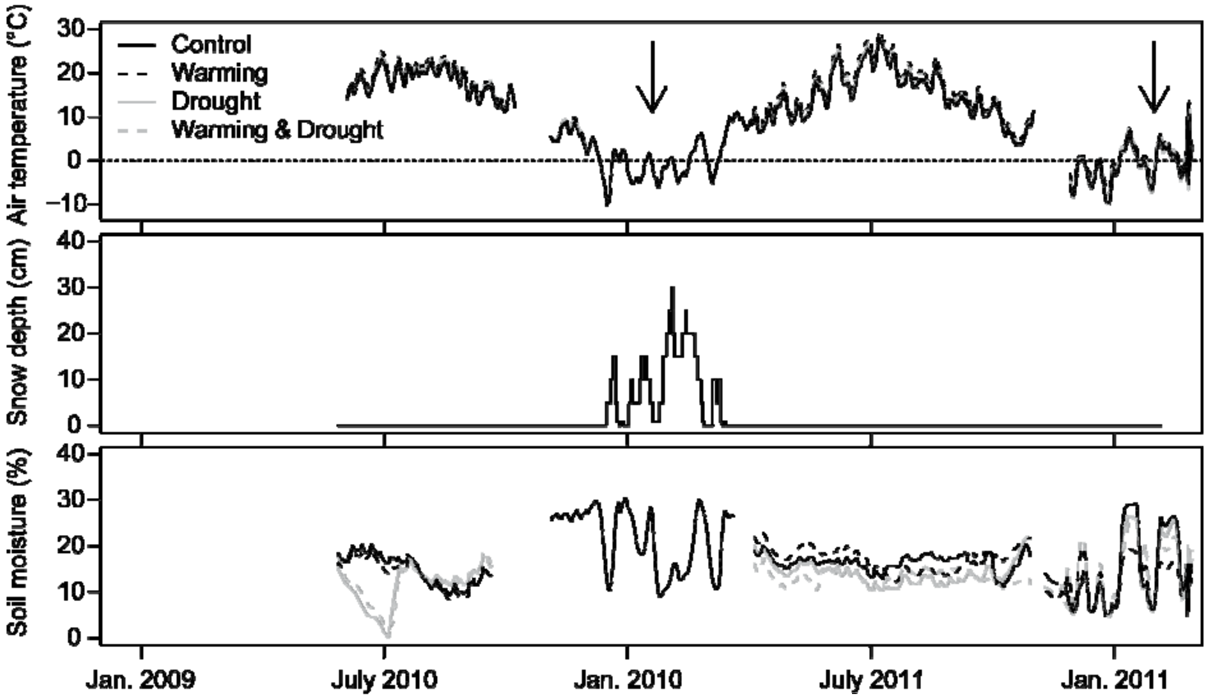


Figure 2: Temperature at mean plant height, snow cover and soil moisture (-2.5 to -7.5 cm) over the course of the experiment. Sampling dates are indicated by arrows.

The warming treatment was performed continuously until October in the first year of the experiment and from April to the end of the experiment in January of the second year. The warming manipulation took place both passively (wind-shelters which reduced the wind speed by 70 % and black floor-covers versus white floor covers) and actively (IR-radiation with approximately 30 W per m²), which increased the air temperature at plant height by 1.6°C on average when the warming treatment was affected (Figure 2). Maximum differences were 5.2°C (single measurements) or 3.6°C for daily mean temperature. The fourth treatment was a combination of drought and warming. The warming increased the drought effect, reducing the soil moisture by another 1.5% on average (Figure 2).

During the first winter of the experiment, plants were kept outside the shelters in a sand bed from October to April. Figure 2 illustrates that the plants were covered by snow during the coldest parts of the first winter. For the second winter, plants were kept inside the shelters with the warming treatment ongoing.

2.2. Response parameters

Cold hardiness was quantified by a slightly modified version of the relative electrolyte leakage method (REL) of ex-situ samples according to Strimbeck et al. (2007): Pre-tests revealed no differences in absolute values when the samples were frozen with or without 1ml solution containing an ice nucleator, presumably because the surface of the samples was wet and froze at around 0°C anyhow. Furthermore, higher freezing rates were applied. At a rate of 0.6°C/h (Strimbeck et al. 2007) it would have taken 3.5 days to reach our minimum temperature, whereas commonly the rate of 6°C/h is applied (e.g. Sutinen 1992, Schaberg 2008). Two needles from the current year were sampled per individual in mid-winter of both years (January 20th in 2010 and January 31st in 2011), rinsed with de-ionized water, and cut to 0.5 cm. Samples from the seven nested replicates per provenance and experimental unit were combined to form one mixed sample, homogenized and subsequently divided into seven subsamples subjected to different temperature levels for one hour (+4.5°C, -7.5°C, -14.5°C, -23°C, -33°C; -40°C, -196°C (liquid N)) using a controlled environment chamber (Licht-Thermostate Typ 1301, RUMED) and a manually controlled chain of freezers sequentially at the lowest temperatures. Initial electrolyte leakage was determined in 16 ml 0.1% v/v Triton X-100_Bidest after 24 h and the final electrolyte leakage was determined after autoclavation of the samples. Electrolyte leakage was quantified by the conductivity of the solution at 25°C measured with a WTW inolab pH/Cond 720. Cold hardiness is expressed as the LT50 for each mixed sample, estimated by non-linear regression of the REL versus the temperature levels using the formula by Anderson et al. (1988):

$$Y_T = Y_{min} + \frac{Y_{max} - Y_{min}}{1 + e^{k(T_m - T)}} \qquad (1)$$

Y_T is the REL at temperature T, Y_{min} is the asymptotic value of the response variable in uninjured tissue, Y_{max} is the asymptotic value at maximum low-temperature stress, k represents the steepness of the response curve, and T_m is the midpoint of the symmetrical curve (an estimate of LT50). Curve fitting was carried out using a quantile regression and the function “nlrq()” from the software package “quantreg” (Koenker, 2006).

The multitude of different technical protocols for REL used in the literature (freezing with or without additional solution, various freezing rates and durations, etc.) limits the comparability between studies strongly. However, the relative differences within a protocol should be robust and more or less independent of e.g. freezing rates (Sutinen et al 1992). Therefore, we stick to the interpretation of relative differences within our study and minimize the discussion of absolute values.

Cold hardiness of needles from the current year or terminal buds was additionally determined for juvenile and adult individuals of the most important local tree species (*P. sylvestris, Picea abies, Fagus sylvatica* and *Quercus petraea*). Samples were taken on 26th of January in the first winter. Three mixed samples of seven individuals each were obtained from a nearby forest (lowland site: 350 m asl) and, for the juvenile stage of the two conifers, from a highland site (Waldstein, Fichtelgebirge, 760 m asl) about 50 km northwest of the experimental site.

In the second winter, carefully excavated fine roots of two provenances (FR1 and IT.S) exhibiting low and high cold hardiness in their foliage in the first year were analyzed for their cold hardiness by applying the same protocol as for the needles.

Mean annual minimum temperatures for the period 1950 to 2000 (mean temperature of the coldest day for the years 1950-2000) for each geographic origin of the provenances were retrieved from worldclim (Hijmans et al., 2005) and used as indicators for minimum temperatures (Table 1). We assume that the relative differences between geographic origins have been suitably reflected, although these values exceed the absolute minimum temperatures due to daily averaging (for our experimental site the minimum temperature based on worldclim is -3.5°C while the absolute annual minimum temperatures between 1998 and 2011 at an hourly resolution ranged between -10.8 and -25.5°C). An ordinary least squares regression between this indicator and cold hardiness was applied for the control treatment in order to detect local adaptation to late frost events.

Soluble carbohydrates were quantified in the first winter for two provenances exhibiting low and high cold hardiness, respectively (FR1 and IT.S). Mixed samples of one needle from the seven plants per experimental unit were taken, immediately frozen in liquid nitrogen and stored at -30°C. Frozen material was ground in a ball mill; soluble carbohydrates of 20 mg of plant material were extracted in 50 % methanol and analyzed using the anthrone method (Kleber et al., 1997). Extinction was measured at 620 nm. We used known concentrations of Glucose as a standard.

Lipid composition was obtained for the same two provenances (FR1 and IT.S). Epicuticular wax lipids including alkanes as the most abundant wax component were recovered by rinsing needles for 60 seconds in dichloromethane (DCM), which resembles standard techniques using chloroform (Radler and Horn, 1965). After removal of the epicuticular wax lipids, needles were ground to a fine powder using a ball mill (Retsch M200). Internal waxes were recovered by standard Soxhlet extraction using a mixture of DCM/Methanol (93:7) (Wiesenberg et al., 2010). Extracts of internal and epicuticular waxes were dried and sequentially separated using solid-phase extraction into lipid fractions including fatty acid and alkane fractions (Wiesenberg et al., 2010). The following section only discusses the results of the alkane fraction as a representative component of epicuticular wax lipids and the fatty acids of internal lipids as the dominant compound class of cell membranes. Other fractions were also analyzed, but no significant differences in their distribution patterns were obtained. Aliquots of deuteriated standards ($D_{39}C_{20}$ acids and $D_{50}C_{24}$ alkane, respectively) were added to the lipid fractions for compound identification and quantification. Fatty acid fractions were derivatized using BSTFA (N,O- Bis (trimethylsilyl) trifluoroacetamide) for 1h at 80°C, whereas alkanes did not require any further preparation. All fractions were measured using gas chromatography coupled with flame ionization detection (Agilent 7890).

In addition to lipid distribution patterns, molecular proxies were also determined to evaluate the differences between provenances and climate manipulations. The average chain length (ACL) of lipids is influenced by lipid biosynthesis and regulates the water repellency of hydrophobic hydrocarbon chains of fatty acids in cell membranes as well as fatty acids and alkanes in epicuticular waxes (Kolattukudy et al., 1976). Initially, the ACL was used to differentiate plant and microbial sources of organic matter in terrestrial sediments (Bray and Evans, 1961):

$$\mathrm{ACL} = \Sigma\,(z_n * n) / \Sigma\,(z_n) \qquad (2)$$

where n is the number of carbons and z_n the amount of fatty acids or alkanes with n carbons. Another parameter to obtain the differences in the lipid biosynthesis as affected by environmental stress is the predominance of odd versus even alkanes, the so-called carbon preference index (CPI: Kolattukudy et al., 1976):

$$\mathrm{CPI} = [(\Sigma\, n\text{-}C_{25\text{-}33\ \mathrm{odd}} / \Sigma\, n\text{-}C_{24\text{-}32\ \mathrm{even}}) + (\Sigma\, n\text{-}C_{25\text{-}33\ \mathrm{odd}} / \Sigma\, n\text{-}C_{26\text{-}34\ \mathrm{even}})]/2 \qquad (3)$$

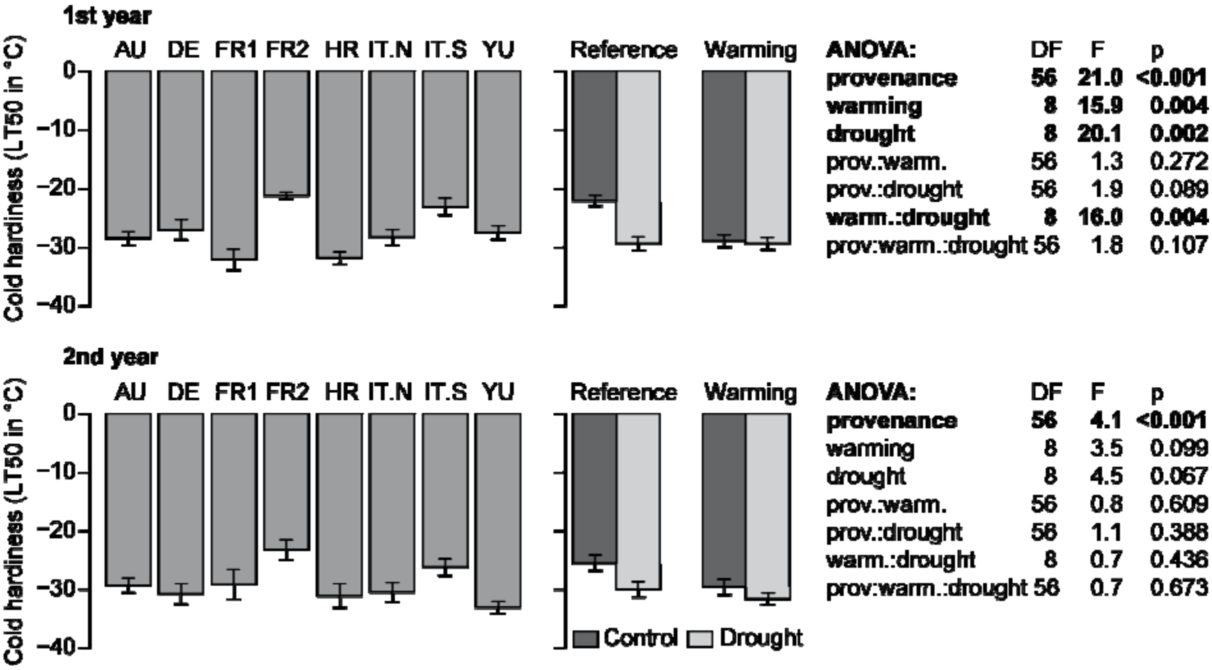
The degradation of alkanes and a less effective synthesis of the predominant odd alkanes in waxes lead to a reduction of the CPI under environmental stress (Wiesenberg et al., 2008).

An analysis of variance (ANOVA) combined with linear mixed effect models were applied to test for the main and interactive effects of the three factors: provenance, drought, and warming on cold hardiness (LT50), soluble carbohydrate content and lipid composition. Including the experimental unit as a random factor accounted for the split-plot design (Pinheiro and Bates, 2004). Data were log transformed to improve the homogeneity of variances and the normality of residuals if necessary. All statistical analyses were conducted with the software R 2.11.1 (R Development Core Team, 2010) and the additional packages "nlme" and "quantreg".

3. Results

3.1. Local adaptation in cold hardiness

Mean cold hardiness differed between the provenances by about 10°C in both winters (Figure 3).



1st year

ANOVA:	DF	F	p
provenance	**56**	**21.0**	**<0.001**
warming	**8**	**15.9**	**0.004**
drought	**8**	**20.1**	**0.002**
prov.:warm.	56	1.3	0.272
prov.:drought	56	1.9	0.089
warm.:drought	**8**	**16.0**	**0.004**
prov:warm.:drought	56	1.8	0.107

2nd year

ANOVA:	DF	F	p
provenance	**56**	**4.1**	**<0.001**
warming	8	3.5	0.099
drought	8	4.5	0.067
prov.:warm.	56	0.8	0.609
prov.:drought	56	1.1	0.388
warm.:drought	8	0.7	0.436
prov:warm.:drought	56	0.7	0.673

Figure 3: Cold hardiness (LT50) as affected by geographic origin (provenances; left) and preceding climatic conditions (drought and warming in interaction; right) in the first (upper panel) and second (lower panel) year of the experiment. ANOVA-results are provided per year with significant effects in bold. Mean values and standard errors are shown for 84 individuals per bar for the provenances and 168 individuals per bar for the climate treatments. Note that the drought manipulation only took place in the first summer of the experiment. The warming treatment stopped three months before sampling in the first year while running throughout sampling in the second year.

The LT50 values ranged between a minimum of -21.2°C for provenance FR2 and a maximum of -32.1°C for provenance FR1 in the first winter; and between a minimum of -23.2°C for provenance FR2 and a maximum of -33.1°C for the provenance from Serbia (YU) in the second winter. Provenances from colder origins generally displayed superior cold hardiness (Figure 4). Significant correlations ($r^2 = 0.77$ in the first winter and $r^2 = 0.80$ in the second winter) between cold hardiness and mean minimum winter temperature at the origins were found for the autochthonous provenances in both years if the provenance from Croatia was excluded from the analyses. When included, no significant correlation was found in the first winter, while the correlation was weaker ($r^2 = 0.59$) while still remaining significant in the second winter.

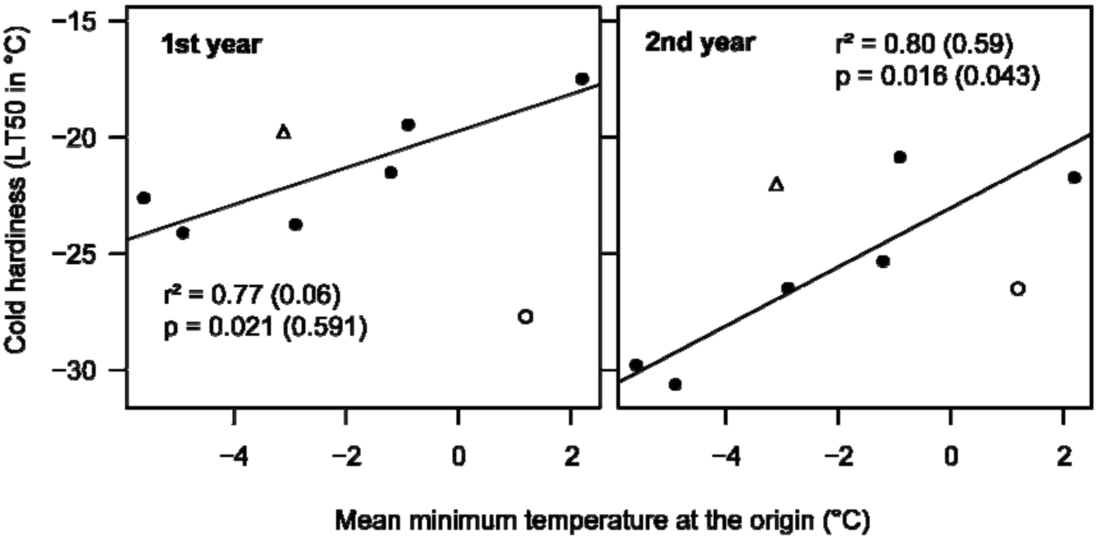


Figure 4: Local adaptation in cold hardiness depending on the mean minimum temperature at the origin. Linear regressions are shown for all autochthonous provenances (DE, open triangle, not included) excluding the provenance from Croatia (HR, open circle), as its autochthonous status is questionable. Results of the regression including the provenance from Croatia are given in parentheses. Cold hardiness (LT50) displays the mean of the control treatment per provenance (n = 21).

3.2. Climatic experiences alter cold hardiness

Cold hardiness was affected by the climatic experiences of the individuals. The extreme summer drought increased cold hardiness by 3.9°C on average in the first winter and there was a non-significant trend in the same direction in the second winter after the drought manipulation (Figure 3). Unexpectedly, the summer warming from the first year resulted in increased cold hardiness of 3.4°C on average while the year-round warming of the second year resulted in no significant effect, although the trend followed the same direction as in the first year. Interestingly, the drought and the warming effect in the first year were not additive (ANOVA, interaction between drought and warming: $F = 16.0$; $p = 0.004$), resulting in lower cold hardiness in the untreated variant and comparable, high cold hardiness in the other three climate manipulations (Figure 3).

3.3. Physiological reasons for varying cold hardiness

The amount of soluble carbohydrates in the needles increased by 25.9% in a provenance exhibiting high cold hardiness compared to a provenance showing low cold hardiness (Table 2; ANOVA: F = 15.3; p = 0.004). The drought manipulation had no significant effect on the carbohydrate concentration (F = 0.0; p = 0.889).

Table 2: Comparison of carbohydrate content and average chain length (ACL) of fatty acids of current year needles between two provenances exhibiting low (IT.S) and high (FR1) cold hardiness. Samples taken in the first winter of the experiment, mean ± standard deviation provided, n = 3.

	Cold hardiness of source	Control	Drought
Cold hardiness (LT50 in °C)	high	-25.8 ± 2.3	-36.7 ± 1.0
	low	-17.5 ± 1.6	-26.8 ± 4.5
Soluble carbohydrates (‰TM)	high	118.4 ± 9.4	111.9 ± 9.5
	low	89.0 ± 15.9	93.7 ± 3.0
ACL of epicuticular wax fatty acids	high	18.8 ± 0.2	17.7 ± 0.2
	low	18.1 ± 0.0	17.8 ± 0.1
ACL of internal fatty acids	high	17.8 ± 0.5	17.6 ± 0.1
	low	17.5 ± 0.1	17.1 ± 0.3
ACL of epicuticular wax alkanes	high	27.5 ± 0.1	27.6 ± 0.0
	low	27.5 ± 0.1	27.5 ± 0.1
CPI of epicuticular wax alkanes	high	9.3 ± 0.2	10.4 ± 0.2
	low	12.0 ± 0.6	11.9 ± 0.4

The composition of internal fatty acids (ACL) as main components of cell membranes did not differ significantly between a provenance exhibiting high cold hardiness and a provenance showing low cold hardiness (F = 3.7; p = 0.092). Likewise, no effect of the drought manipulation was found (F = 1.7; p = 0.222). For the epicuticular wax lipids, the provenance with the high cold hardiness was characterized by a slightly higher ACL (3.9 %; F = 5.2; p = 0.051) than that with the low cold hardiness. The drought treatment led to a general decrease in ACL (F = 22.0; p = 0.002), which was stronger for the plants with a high (5.5 %) rather than a low cold hardiness (1.5 %; interaction between provenance and drought manipulation: F = 7.7; p = 0.024).

The greatest differences among lipids were observed for the CPI values of the epicuticular wax alkanes between the two provenances. CPI values were 22.7 % lower for the provenance with high cold hardiness compared to that of the provenance with low cold hardiness (F = 45.8; p < 0.001). Drought led to an increase of the CPI value by 11.5 % in the provenance with high cold hardiness, while no effect of the drought manipulation was observed in the provenance with low cold hardiness, resulting in no significant effects of the drought manipulation (F = 2.3; p = 0.166) and the interaction between provenance and drought (F = 3.4; p = 0.104). Hence, the difference between both provenances decreased after drought, but CPI values were still 15.1 % higher in plants with low cold hardiness.

3.4. Cold hardiness among species

Cold hardiness of the (sub-) mediterranean *P. nigra* reached similar levels to the cold hardiness of tree species native to Central Europe in the vicinity of the experimental site (Figure 5, ANOVA for all juvenile lowland samples: F = 2.0; p = 0.163). Needles of adult conifers, however, showed superior cold hardiness compared to juvenile trees (F = 2.6; p = 0.046; *Picea abies* and *Pinus sylvestris*) and juveniles from highland sites exhibited higher cold hardiness compared to lowland sites (F = 7.4; p = 0.026; *Picea abies* and *Pinus sylvestris*).

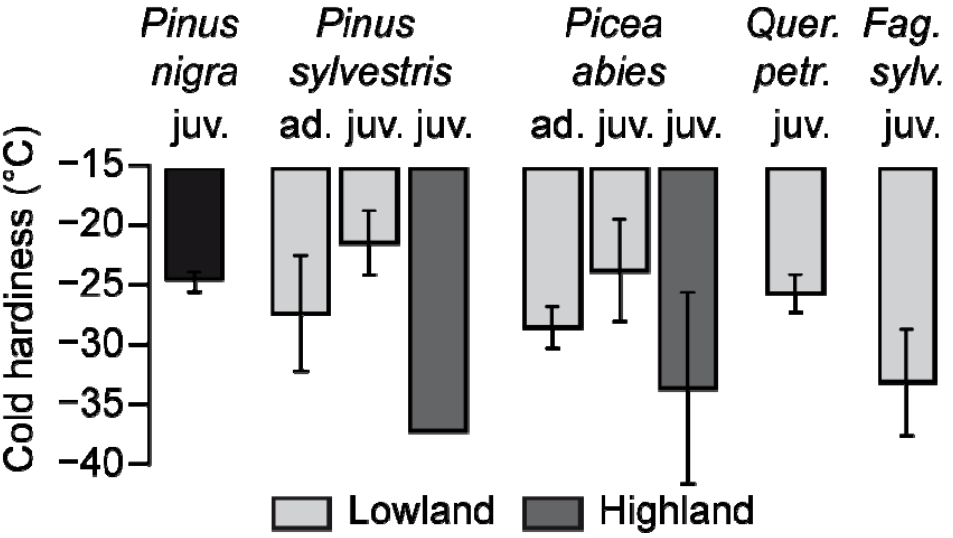


Figure 5: Comparison of the cold hardiness (LT50) of *P. nigra* with common forest tree species in the vicinity of the experimental site (lowland, 350 m asl) and, for the juvenile stage of the other two conifers, from a highland site (760 m asl). juv.: juveniles (2-4 years old); ad.: adults (>30 years old). *Quer. petr.: Quercus petraea*; *Fag. sylv.: Fagus sylvatica*. n = 3 mixed samples of 7 individuals each per bar (mean and SE). For *P.nigra*: provenance DE (Zellingen, Germany) in the control treatment.

3.5. Cold hardiness of roots

Cold hardiness of fine roots of *P. nigra* averaged -16.5°C. The two tested provenances (IT.S and FR1) did not differ significantly (F = 1.6; p = 0.239) in the cold hardiness of their roots in the second winter of the experiment (-15.4 ± 1.9°C and -17.5 ± 1.1°C respectively (±1SE), n = 12). The drought (F = 0.1; p = 0.805) and warming (F = 0.1; p = 0.754) treatments also resulted in no significant effect on LT50 of the fine roots.

4. Discussion

4.1. Local adaptation in cold hardiness

Cold hardiness differed by about 10°C between the studied provenances of *P. nigra*. Local adaptation to minimum temperature regimes was indicated as provenances from colder origins reached superior cold hardiness. These results correspond well with findings from other forest trees such as *P. devoniana* (Saenz-Romero and Tapia-Olivares, 2008), *Fagus sylvatica* (Visnjic and Dohrenbusch, 2004), *Tsuga heterophylla* (Kuser and Ching, 1980), *Fagus crenata* and *Betula ermanii* (Gansert et al., 1999), all showing local adaptation to winter cold extremes. Our data indicates further that minimum temperature does not only determine the northern range limits of species (Sakai and Weiser, 1973), but that within species variability in cold hardiness also needs to be taken into account. The provenance from Croatia (HR), however, did not fit well into the overall pattern. We assume that this provenance is either not autochthonous, i.e. originating from a warmer winter climate, or belongs to the subspecies *dalmatica*, which is described for very restricted areas along the Croatian coast. Genetic characterization of the species and subspecies will shed light on this question.

P. nigra is known for its high genetic diversity (Jagielska et al., 2007) which surpasses that of other pines (Nkongolo et al., 2002). Although no consensus on its taxonomy has been reached (Huber, 2011), six main subspecies are recognized with *P. nigra* ssp *nigra* being the most abundant in Europe (Isajev et al., 2004). Provenances furthermore differ in growth and ecological performance, expressed in local adaptations to soil and mean annual temperature and precipitation in provenance trials (Varelides et al., 2001). The strongly contrasting cold hardiness in our experiment suggests that minimum temperature is another genetically selective parameter, not only for frost sensitive subspecies such as *P. nigra* ssp *laricio* (Varelides et al., 2001), but also for *P. nigra* ssp *nigra*, which is usually considered to be the most frost tolerant among the subspecies (Isajev et al., 2004, Huber, 2011).

4.2. Climatic experiences alter cold hardiness

Cold hardiness was affected by climatic experiences of the individuals with drought increasing cold hardiness by 3.9°C on average in the subsequent winter and no significant carry-over effect to the second winter. This finding can be explained by drought and frost triggering similar responses at the cellular and molecular level to prevent cellular dehydration (Blodner et al., 2005). Without experiencing drought themselves, the newly formed needles in the second year of the experiment lacked significant additional cold hardiness in the drought manipulation. More frequent drought events accompanying climate change may therefore increase cold hardiness in single (dry) years, but not generally.

It has been suggested that trees grown under generally warmer conditions may lose their functional adaptations to frost (Eccel et al., 2009). Surprisingly, our results contradict this expectation with increased cold hardiness by 3.4°C on average in the warming treatment after the first season. The warming, however, was stopped in October and acclimation of the formerly warmed individuals evidently surpassed the control plants when subjected to the same temperature from October on. Responsiveness to current year climates are also reported for deciduous forest trees (Repo et al., 2008). Yet, the year-round warming in the second year of the experiment resulted in no significant difference between the treatments. Clearly, further experiments on interacting climatic drivers are urgently needed, as the response to such interactions might differ considerably from single factor experiments (Shaw et al., 2002; Kreyling et al., 2011b).

Here, we focused on the realized maximum frost hardiness, e.g. the hardiness directly after the coldest days of winter. Much bigger differences than observed between the provenances (10°C) or between the climate manipulations (up to 3.9°C) occur within each needle over the course of the year (more than 60°C in a single provenance of *P. nigra*; Sutinen et al., 1992). Cues which drive this strong seasonality involve photoperiod and minimum temperature experience. Their relative importance, however, is still unresolved, differs between species (Kozlowski and Pallardy, 2002; Holliday et al., 2008) and may even differ between provenances in the same species. Our results add to this discussion by showing that both genetic heritage (differences between the provenances) and preceding climatic experience (here mainly summer drought) can affect the absolute frost hardiness. Potential differences in the temporal pattern of frost hardiness between provenances are of high ecological relevance, especially with regard to early or late frost events and phenological differences within species (Visnjic and Dohrenbusch, 2004; Kreyling et al. 2011b). These points call for more detailed investigations on intra-specific differences in seasonality of frost hardiness.

4.3. Physiological reasons for different cold hardiness

Differential cold hardiness between provenances was related to contents of soluble carbohydrates and fatty acids in the needles. Content of soluble carbohydrates is also reported to be closely related to local adaptations in cold hardiness of different *Quercus* species (Morin et al., 2007). The lipid contents of *P. nigra* have been reported previously for mature trees and needles collected during late summer (Maffei et al., 2004). In contrast to these mature trees, where *n*-C_{29} and *n*-C_{31} alkanes contribute 2.2 % and 37.2 %, respectively, to total alkanes, the juveniles of different provenances in our study were all dominated by *n*-C_{29} alkane (36.3 ± 2.8 %) and lower contents of *n*-C_{31} alkane (9.6 ± 1.0 %). This difference is probably due to different needle and plant age when compared to the literature results, whereas differences between provenances are not likely as they did not differ in their relative contribution of *n*-C_{29} and *n*-C_{31} alkanes in our study. In general, the hydrophobicity of the waxes is improved under water and cold stress to protect plants against water loss by an increased turnover of wax components towards hydrophobic aliphatic compounds which is not necessarily related to shifts in the total amount of waxes (Shepherd and Griffiths, 2006). For trees, such investigations are still scarce and limited to selected tree species (e.g. for different *Picea* species: Cape and Percy, 1993; or *Pinus palustris*: Prior et al., 1997). Our observations of small changes in the lipid composition (ACL values) confirm minor influences of cold and water stress on lipid biosynthesis, as described elsewhere (Cape and Percy, 1993; Shepherd and Griffiths, 2006). The low CPI values of epicuticular wax alkanes of the plants with high cold hardiness, however, indicate a strong biosynthesis rate associated by a production of byproducts and degradation products such as even alkanes. This increased production of wax components indicates the role of alkanes to improve the cold hardiness (Prior et al., 1997). The drought manipulation led to a reduction in the formation rates of alkanes (higher CPI) for the provenance with higher cold hardiness similar as observed for sesame plants (Kim et al., 2007). Hence, biosynthesis of epicuticular wax alkanes is influenced by water stress and appears to be related to cold hardiness in *P. nigra.* Freezing tolerance in plants is accompanied by lipid remodeling at the outer membrane (Moellering et al. 2010), another aspect fitting well to our data and indicating that the effect of changes in the lipid composition might be more important for cold hardening than previously assumed. It should be noted, though, that both the observed differences in cold hardiness and the differences in composition and concentrations of cell membrane compounds could be driven by other factors such as water stress over summer in the provenance with superior frost hardiness. Seasonality of precipitation and mean annual precipitation were three times lower at the origin of this provenance (Table 1). Further causal and functional analyses of frost hardiness and hardening are clearly required (Holliday et al., 2008).

4.4. Cold hardiness among species

Juveniles of the (sub-) mediterranean species *P. nigra* exhibited comparable cold hardiness as juveniles of species native to Central Europe in the vicinity of the experiment, i.e. under the same climatic conditions. Under colder conditions in Norway it has been shown that *P. nigra* is more sensitive to freezing injury than boreal conifers (Strimbeck et al., 2007). Its ability to adjust to prevailing climatic conditions therefore appears limited in comparison to boreal species such as *P. sylvestris* or *Picea abies*. Yet, under the same climatic conditions, these species did not differ from *P. nigra* in our study, implying that realized frost hardiness and potential frost hardiness need to be discussed separately. It should be emphasized here that within-species variation in cold hardiness, i.e. differences among provenances of *P. nigra* and differences between lowland and highland sites or juvenile and adult individuals for the other species clearly exceeded among-species variation at the juvenile stage. Generally, variation among species at the same site and under the same climatic conditions appears less important than commonly assumed. Within-species variation and individual performance might be more relevant for forest ecology (Clark, 2010).

We used juvenile trees in their second to third year in this experiment. Our results concerning *P. sylvestris* and *Picea abies* confirm previous findings that seedlings are more sensitive against frost events than older trees (Bolte et al., 2007). However, the juvenile stage is of high importance for the natural regeneration of forest stands. Moreover, the high selective pressure of single extreme events such as frost or drought can reduce the genetic diversity of future stands (Hosius et al., 2006).

4.5. Cold hardiness of roots

Cold hardiness of fine roots of *P. nigra* averaged -16.5°C over two provenances, which is a high value compared to the cold hardiness of its foliage (-23.8°C on average for the control treatment). This might be an adaptation to the species' natural habitat where soil frost events occur more or less regularly as no snow cover insulates the soil against air temperature fluctuations (Kreyling, 2010). *Chamaecyparis nootkatensis* serves as an example of a forest tree from temperate rain-forests with low root cold hardiness (roots do not survive temperatures below -5°C) in response to deep snow cover in its natural habitat (Schaberg et al., 2008a). Winter climate change, however, is expected to lead to reduced snow cover and, in consequence of the reduced insulation, to colder soils despite the general air warming trend (Groffman et al., 2001). For Central Europe, a reduction in snow cover is already taking place, while minimum temperature of the soil may not decrease (Kreyling and Henry, 2011). Interestingly, no response in cold hardiness of fine roots occurred for the different climate treatments in our experiment. In addition, we investigated root cold hardiness for two provenances with strongly contrasting shoot cold hardiness and did not find significant differences in the roots. This supports Schaberg et al. (2008a) who conclude that no acclimation occurs in cold hardiness of fine roots. More detailed investigations concerning this aspect are clearly needed, especially with respect to the question if cold hardiness of roots lacks adaptive potential to changing climate conditions. For *P. nigra* our results imply that no selective pressure is expected as root cold tolerance is generally high.

4.6. Assisted colonization

P. nigra, based on its ecology and natural distribution (Isajev et al., 2004), is well adapted to warmer and drier conditions expected for parts of Central Europe under climate change (Kölling, 2007, Huber, 2011). Here, we show that cold hardiness, at least of some provenances, is also no limitation for the use of this species in Central Europe even if cold extremes remain constant throughout this century (Vavrus et al., 2006; Kodra et al., 2011). Assisted colonization or transplantations are widely applied in forestry and may serve as adaptation strategy against adverse effects of climate change on ecosystem functioning (McKenney et al., 2009, Schaberg et al., 2008b). Numerous examples of failed transplantations (Zobel et al., 1987), however, warn against rushed action. The assisted colonization of pre-adapted ecotypes of key species within their current range is suggested to contribute to functional integrity of forest stands without the need to introduce exotic species with unknown risks (Kreyling et al., 2011a). Yet, naturally dominating tree species may lack pre-adapted ecotypes at their warm and dry range limits. Here, congeneric species from adjacent climates are preferable over other species. *P. sylvestris* and *P. nigra* may serve as an example, with the latter potentially replacing the former at warmest and driest sites of its range while maintaining ecosystem functioning. Our results suggest that cold hardiness is significantly related to climatic conditions at the origin of the provenances, implying that the selection of frost-tolerant provenances could be based on the current climatic conditions within the species ranges. However, our finding that climatic experiences within the life of single plants alter cold hardiness indicates that provenance trials under control conditions may be misleading under changing climatic mean and extreme conditions. The multitude of possible climatic variables to be selected for and uncertainties concerning future climates imply that the search for best-adapted provenances should not be the only strategy. In addition, management actions which promote genetic diversity (e.g. supporting natural regeneration and addition of genetically diverse material) are crucial as genetic diversity enables organisms to continue adapting and evolving to new conditions within one or several generation cycles (Hosius et al., 2006; Schaberg et al., 2008b). Furthermore, the role of herbivores and diseases under changing climate requires detailed investigations. For instance, a needle blight known as the "red band disease" (*Dothistroma septospora*) is reported to increase in importance over recent years in *P. nigra* (Isajev et al., 2004), a development that may be related to climate change (Watt et al., 2011).

Ultimately, tree species responses should be regarded in the context of populations under competitive pressure. The advantage of common garden experiments is that they can detect the spectrum of possible species-specific responses. Nevertheless, there is a need to test the obtained results in communities where the competitive balance might amplify or buffer responses.

5. Conclusions

Cold hardiness of *Pinus nigra* foliage is highly variable between provenances and shows signs of local adaptation to prevailing minimum temperatures at the origin. Both severe drought events and summer warming can increase cold hardiness, indicating that the interaction of different climate parameters leads to unexpected results and that winter survival can be altered by climatic events during the growing season. Physiologically, cold hardiness is related to soluble carbohydrate content and lipid composition. Interestingly, variation of cold hardiness of the needles within the (sub-) mediterranean species *P. nigra* was higher than between this species and other species common to the temperate zone of Central Europe. Taken together, our results imply that the cold hardiness of the foliage of *P. nigra* is adaptive to long-term growing conditions at the origin (genetic heritage) and to short-term alterations of these conditions (individual plasticity), while first hints suggest that cold hardiness of the roots is high and probably not under selective pressure currently. Our data from mid-winter suggests that below- and above-ground cold hardiness of selected provenances appear to be well adapted to cultivation in temperate regions as an adaptation strategy against the adverse effects of climate change in dry habitats. However, with respect to late spring and early autumn frost events, the temporal pattern of frost hardiness with potential intra-specific differences should be investigated in more detail. Before translocations are recommended, further investigations are required, e.g. exploring the role of biotic interactions under changing climatic conditions. Generally, within-species diversity should be conserved at the species level and improved in anthropogenically founded stands in order to allow for adaption to climate change.

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Manuscript 6: Increased rainfall variability reduces biomass and forage quality of temperate grassland largely independent of mowing frequency

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Highlights

1. Grassland was subjected to increased rainfall variability and mowing frequency.
2. Increased rainfall variability reduces grassland productivity and forage quality.
3. More frequent mowing initially increases and later on decreases productivity.
4. Mowing regime does mostly not interact with rainfall variability manipulations.
5. Sufficient overall rainfall amount is important for grassland resilience.

Abstract

Climate models indicate that global warming will stimulate atmospheric exchange processes and increase rainfall variability, leading to longer dry periods and more intense rainfall events. Recent studies suggest that both the magnitude of the rainfall events and their frequency may be as important for temperate grassland productivity as the annual sum. However, until now interactive effects between land management practice, such as mowing frequency, and rainfall variability on productivity and forage quality have not been studied in detail. Here, we present the data from a field experiment (EVENT II) in which a Central-European grassland was subjected to increased spring rainfall variability (low, intermediate and extreme rainfall variability without any change to the rainfall amount) and increased mowing frequency (four times compared to twice a year). We assessed biomass production,

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[*] authors contributed equally to the publication

forage quality parameters, root-length and shoot-root ratio. Enhanced spring rainfall variability reduced midsummer productivity and the leaf N and protein concentrations of a target species, but did not exert any long-term effects on biomass production and forage quality in late summer. However, the increased spring rainfall variability reduced aboveground net primary productivity by 15 %. More frequent mowing increased productivity in the first year of the study, but decreased productivity at the end of the second year, showing a decline in the potential for overcompensation after a history of more intense mowing. Generally, more frequent mowing decreased the shoot-root ratio and increased the concentration of leaf N. Increased mowing frequency neither buffered, nor amplified the adverse effects of rainfall variability on productivity, but made leaf N concentrations in early summer more responsive to altered rainfall patterns. These results highlight the fact that even relatively small and short-term alterations to rainfall distribution can reduce production and forage quality, with little buffering capacity of altered mowing frequency. Comparisons with productivity data from the first year of the study, in which both, rainfall distribution and rainfall amount were modified, demonstrate the crucial role of sufficient moisture (annual rainfall amount) for grassland resilience: In this first year, negative effects of extreme rainfall variability lasted until the end of the year. To conclude, increased rainfall variability under climate change will likely affect agricultural yield in temperate meadows. Management strategies to buffer these effects have yet to be developed.

Keywords: EVENT II experiment, extreme weather event, rainout-shelter, forage quality, *Alopecurus pratensis, Trifolium pratense*

1. Introduction

Climate change is projected to modify not only annual precipitation sum, but also to result in more extreme rainfall regimes in many parts of the world (IPCC 2007; Jentsch and Beierkuhnlein, 2008). This will cause more severe drought periods as well as an increase in the frequency and magnitude of extreme precipitation events (Trenberth et al., 2003, Min et al., 2011). Evidence is mounting that the frequency and severity of droughts and extreme precipitation events has already increased over recent decades in many regions (Blenkinsop and Fowler, 2007; Haylock and Goodess, 2004; IPCC 2007).

Primary productivity and ecosystem functioning in terrestrial ecosystems are strongly influenced by the annual amount of precipitation (Sala et al., 1988). However, recent research suggests that rainfall variability may exert an even stronger influence on ecosystem functioning, where especially temperate grassland systems seem to be responsive to changes in rainfall variability. In grassland, more extreme rainfall regimes (less, but more intense rainfall events) affect ANPP (aboveground net primary productivity) (Barrett et al., 2002; Fay, 2009; Heisler-White et al., 2009; Knapp et al., 2002), carbon cycling (Chou et al., 2008; Fay, 2009; Harper et al., 2005) and N mineralization (Barrett et al., 2002, Heisler-White et al., 2009). The latter may in turn affect leaf quality in terms of N or protein content. Large reductions in ANPP have been shown in mesic grassland in response to more extreme rainfall patterns (Fay et al., 2003; Heisler-White et al., 2009; Knapp et al., 2008).

In addition to the rainfall amount and variability, land management strategies, such as mowing frequency, can affect productivity and leaf litter quality in managed grassland. More frequent cutting is known to increase leaf N content. However, whether or not mowing increases or decreases the productivity of grassland depends on the mowing intensity, e.g. mowing history, mowing frequency and cutting height (Green and Detling, 2000; McNaughton, 1979; Turner et al., 1993; Weigelt et al., 2009). Mowing or defoliation is likely to alter the response to rainfall variability by altering plant community composition (Swemmer and Knapp, 2008). Furthermore, a reduction of transpirative tissue alters water uptake and consumption and therefore reaction towards rainfall (Heitschmidt et al., 1999; McNaughton, 1979; Yang and Midmore, 2004). Currently, a knowledge gap exists on how land management practices, such as mowing frequency, are interacting with more extreme rainfall regimes: Increased mowing frequency might buffer the effects of rainfall variability on grassland, diminishing the amplitude of the response towards rainfall extremes (Swemmer and Knapp, 2008). A study by Bernhardt-Römermann et al. (2011) indicates that climate parameters get less important for biomass production under intermediate mowing frequencies. However, land management strategies might also amplify the effects of rainfall variability. To our knowledge, this is the first study to experimentally manipulate mowing and rainfall patterns in European managed grassland (meadows) in order to identify any potential interactions between rainfall variability and mowing frequency.

The primary objectives of our study were (1) to investigate the factorially-combined effects of increased spring rainfall variability and increased mowing frequency on the productivity and the forage quality of semi-natural, Central-European temperate grassland and (2) to determine, whether mowing frequency amplifies or buffers the effects of rainfall variability on biomass production and leaf quality of a target species. We conducted a field experiment in which we altered the temporal distribution and the magnitude of the rainfall events, but not the overall rainfall sum. To assess potential interactions between rainfall variability and mowing frequency, we crossed the factor rainfall variability with the factor mowing frequency (two or four times per year). In the previous year, we altered the total rainfall amounts along with the alterations in rainfall variability. This enables a comparison between the effects of the altered total rainfall amounts and distribution and the effects of altered rainfall variability under constant total rainfall amounts.

We hypothesized that

(i) increased rainfall variability negatively affects productivity and leaf quality, as has been shown for other mesic grasslands,

(ii) increased rainfall variability alone can cause changes in productivity that are comparable to changes caused by alterations in both, variability and the annual sum of rainfall together,

(iii) more frequent mowing increases productivity and forage quality, as has been shown for more frequent, but still moderate mowing frequencies,

(iv) more frequent mowing buffers adverse effects of increased rainfall variability on productivity and leaf quality, as growth responses might be synchronized and less responsive to rainfall changes after mowing.

2. Material & Methods

2.1 Study site

The study was conducted within the EVENT II experiment in a semi-natural grassland in the Ecological Botanical Garden of the University of Bayreuth, Germany, Central Europe (49°55´19´´N, 11°3455``E, 365 m asl) (Jentsch & Beierkuhnlein, 2010). Communities are dominated by tall grasses, especially *Alopecurus pratensis* L. (meadow foxtail). The regional climate is temperate and moderately continental, with a mean annual temperature of 8.2 °C (1971–2000), and daily means ranging between -19.6 and 27.6. The mean annual precipitation of 724 mm (1971–2000) has a bimodal distribution with a major peak in June/July and a second peak in December/January (data: GermanWeather Service). The experiment was installed on a semi-natural, established meadow. For more than 20 years prior to the experiment, the meadow was mown twice per year and not fertilized. The rectangularly shaped experimental area has a total height difference of 95 cm within the diagonal from southwest to north east, and about 7 cm from southeast to north west.

The soil of the experiment is classified as Stagnosol with a sandy-loamy Ap-horizon of about 30 cm depth, a strongly loamy Sw-horizon (20 cm) and a sandy-clayey Sd-horizon (>40 cm). Plant roots mainly occur in the upper 15 cm, with almost no roots penetrating below the A-horizon, mean pH-value is 5.9.

2.2 Experimental Design

The EVENT II experiment was established in 2008. The experimental design consists of two factorially-crossed factors: (1) manipulation of the temporal distribution and magnitude of rainfall events in the growing season and (2) manipulation of mowing frequency. We implemented three scenarios of rainfall variability treatments in 2008 and 2009, assigned to the same plots: (1) low rainfall variability with weekly irrigation, ensuring a continuous water supply, (2) intermediate rainfall variability, with natural ambient rainfall variability and (3) extreme rainfall variability, including an extreme spring drought.

Table 1 Average soil moisture [vol %], variation coefficient (CV) of soil moisture [%], number of rainfall events exceeding 1 mm, the sum of the rainfall amount [mm] and the variation coefficient (CV) of daily rainfall amount [%] in 2008 and 2009.

parameter	year	time span	low	mid	extreme	natural
average soil moisture	2008	26/05-30/10 2008 (158 days)	**29**	21	19	
	2009	01/04-31/10 2009 (214 days)	**30**	29	25	
	2009	01/04-17/05 2009 (47 days)	**42**	40	36	
	2009	-29/06 2009 (43 days)	**29**	**29**	21	
	2009	-09/08 2009 (43 days)	**32**	31	27	
	2009	-28/10 2009 (80 days)	23	**24**	22	
CV soil moisture	2008	26/05-30/10 2008 (158 days)	20	31	**38**	
	2009	01/04-31/10 2009 (214 days)	35	35	**37**	
	2009	01/04-17/05 2009 (47 days)	6	8	**13**	
	2009	-29/06 2009 (43 days)	21	24	**30**	
	2009	-09/08 2009 (43 days)	25	**29**	26	
	2009	-28/10 2009 (80 days)	35	**36**	35	
no. of events	2008	26/05-30/10 2008 (158 days)	**61**	53	45	
	2009	01/04-31/10 2009 (214 days)	**80**	71	60	
	2009	01/04-17/05 2009 (47 days)	**19**	16	16	
	2009	-29/06 2009 (43 days)	**17**	13	1	
	2009	-09/08 2009 (43 days)	**17**	16	**17**	
	2009	-28/10 2009 (80 days)	**27**	25	25	
precipitation sum	2008	26/05-30/10 2008 (158 days)	**445.2**	334.8	296.1	334.8
	2009	01/04-31/10 2009 (214 days)	596.8	596.8	596.8	458.5
	2009	01/04-17/05 2009 (47 days)	**130.2**	99.5	99.5	99.5
	2009	-29/06 2009 (43 days)	102.5	**108.1**	36.6	77.4
	2009	-09/08 2009 (43 days)	164.2	152.2	**223.7**	127.1
	2009	-28/10 2009 (80 days)	199.9	**205**	**205**	154.5
CV precipitation	2008	26/05-30/10 2008 (158 days)	164	183	**204**	183
	2009	01/04-31/10 2009 (214 days)	204	256	**297**	227
	2009	01/04-17/05 2009 (47 days)	227	**280**	**280**	**280**
	2009	-29/06 2009 (43 days)	156	248	**656**	183
	2009	-09/08 2009 (43 days)	192	205	**223**	220
		-28/10 2009 (80 days)	203	**275**	**275**	192

[1] Highest values in each category are in bold.

[2] Values for the vegetation period 2008 and 2009 and for the time spans between the compensation irrigation treatments in 2009 for the differing rainfall variability treatments are given. Values for 2009 shown over one time span begin with a compensation irrigation and exclude the following compensation irrigation, as the latter is only effective for soil moisture and biomass for the following period.

In 2008, the first year of the study, total growing season amount of rainfall and variability of rainfall were altered. This made it possible to assess direct drought effects, as the extreme rainfall variability treatment also received least total rainfall (see Table 1 for an overview over soil moisture and rainfall parameters in both years).

In 2009, the main year of the study, we controlled the amount of rainfall over the growing season for all treatments and manipulated only the distribution of rainfall, in order to isolate the effect of rainfall variability. All rainfall variability treatments were adjusted to the total 597 mm of rainfall of the low variability treatment in four compensation irrigations (Table 2). Thus, not only the length of the dry intervals, but also the magnitude of rainfall per event was changed.

The low rainfall variability treatment received at least the 30-year weekly average rainfall each week. The vegetation periods from 1971 to 2000 served as a reference (data: German Weather Service). Missing amounts on natural rainfall were added if the weekly rainfall was less than the long-term average for the same week. This treatment ensured continuous water availability. If weekly rainfall exceeded the long-term sum, it was not subtracted for the next irrigation. For 2008, the overall rainfall amount of 553 mm on the low rainfall variability treatment (natural plus irrigated rainfall) within the vegetation period (April 1st-October 30th) exceeded the 30-year-average by 94 mm. In 2009, the total amount of 597 mm, irrigated on all treatments by applying compensation irrigations (see below), exceeded the 30-year-average by 138 mm. Both years consequently resemble rather wet years.

The intermediate rainfall variability treatment remained under ambient conditions without any treatment, except for the compensation irrigations applied in 2009 which adjusted rainfall sum to the low rainfall variability treatment at four points of time (Table 2). Thus, in 2009 the intermediate rainfall variability treatment received the ambient rainfall plus the compensation irrigations.

Table 2 Amount and timing of compensation irrigation [mm] in 2009 on the extreme and intermediate rainfall variability treatments given to apply the same overall rainfall amount on all treatments over the vegetation period.

	Treatment	
date	extreme	mid
May 18th	36.6	36.6
June 30th	96.5*	25.1
August 10th	60.3	60.3
October 28th	32	32

*applied on two consecutive days

In the extreme variability treatment, rainout shelters excluded natural rainfall in the early growing season for 42 days from May 19th until June 30th in both years, resulting in an extreme spring drought. The tunnel shaped rainout shelters had a base area of 5.5 m by 7.5 m and a height of 2.5 m. A metal frame was covered by low-density polyethylene foil which allowed a nearly 90% penetration of photosynthetically active radiation. The foil started from a height of 80 cm off the ground to allow near-surface air-exchange, thus reducing any microclimatic artefacts, like increased temperatures or reduced wind speed. The rainout shelters have a buffer zone of 1 m around the plots towards the shelter edge and additional plastic sheet pilings around the treatment within the buffer zone reaching down to a depth of 25 cm avoiding rain run-off to flow into the treatment.

Due to the compensation irrigation, the extreme spring drought was followed by two days of heavy rainfall in 2009. Such a scenario resembles future projections of drier growing seasons with more extreme rainfall events for Germany (Jonas et al. 2005; Jacob 2009).

We installed an additional roof-artefact control during the spring drought manipulation of the extreme variability treatment where natural rainfall was applied under rainout shelters, resembling the rainfall of the intermediate rainfall variability treatment. We did not observe any differences in biomass production between the roof-artefact control and the intermediate variability treatment.

Irrigation was applied using portable irrigation systems (Kreyling et al. 2008b). A lateral surface flow was reduced by using plastic sheet pilings around all plots reaching down to a depth of 20 cm - 25 cm.

To determine the interactions between rainfall variability and mowing frequency, two different mowing frequencies were applied and nested within the rainfall variability treatment, thus resulting in a split-plot design (with rainfall variability manipulation being the plot factor, and mowing frequency the subplot factor). Each rainfall manipulation block was split into four plots with different mowing frequencies, each plot 1.5 m x 1.5 m in size: Two plots per block were mown only twice per year and two plots were mown four times per year. Each rainfall variability manipulation block was replicated five times and was restricted to occur just once in each row and each column of the experimental design. Within one rainfall manipulation block, mowing frequency plots were 50 cm apart from each other, the rainfall manipulation blocks were located three meters apart from each other.

2.3 Soil moisture

Soil moisture was logged every hour using frequency domain (FD)-sensors (ECH$_2$O, Decagon devices, Pullman, USA) that had been installed in May 2008 in each plot to capture the dynamics of soil water content in response to rainfall variability (n=5/ treatment). Each sensor measured the soil moisture between -2 and -7 cm. According to the root length data, the majority of root biomass is located within the upper 5 cm of the soil. Average daily values were calculated for analysis.

2.4 Biomass production and ANPP

Primary productivity was estimated based on the total aboveground harvest of all plant material in two 0.1 m^2 rectangles from the core of each plot. Harvesting was conducted in the first week of July and in the second week of September for the plots that were mown twice per year. The plots that were mown four times per year had additional harvests in the third week of May and the first week of August. To compare the results of productivity for July and September, the weight of aboveground dry biomass was summed for the plots that were already mown before (the cumulated biomass of May and July for the July harvest and the cumulated biomass of August and September for the September harvest for the plots mown four times per year). The dry weight of the two rectangles was averaged. To assess ANPP, the total biomass produced over the whole year was calculated. Aboveground biomass was dried at 70° C for 72 hours and weighed to the nearest 0.1 g.

2.5 Root length and shoot-root ratio

Root length was acquired by the minirhizotron-technique in 2009. One clear plastic tube (5 cm in diameter) was installed at a depth of 45 cm at a 45-degree angle at the beginning of 2009. Images of 3.8 cm^2 were taken at 5 cm, 15 cm, 25 cm, and 35 cm depth along each tube by a digital camera mounted on an endoscope. The images in each plot were taken in the week after the first drought period (July 1st-July 4th) and at the end of the vegetation period (September 14th-September 18th). Images were analysed for root length using the line intersection method (Tennant, 1975) within a systematic grid (10 x 10, grid width of 0.2 cm x 0.2 cm). Afterwards, the values for each depth were summed to assess the summed root length over all rooting depths. Shoot-root ratio was evaluated using the ratio between above-ground biomass and the summed root length over all depths (Kreyling et al., 2008a). Both parameters were standardized beforehand to the same mean and standard deviation due to the different measured units of above- and belowground parameter.

2.6 Forage quality

To determine leaf N (N) and carbon (C) concentrations of the dominant tallgrass, *Alopecurus pratensis*, one mixed sample per plot was taken after drying and weighing the biomass in 2009. Samples were ground in a ball mill and analyzed with an elemental analyser (Thermo Quest Flash EA 1112). To provide additional information about the impacts of drought on the forage quality in other species, we include data from another sampling campaign here. In this, we assessed the drought effects on the protein content of the key legume *Trifolium pratense*. We took mixed samples from the leaves of three different plants on the last day of the drought treatment, which were immediately frozen in liquid N. We determined the total soluble proteins according to Bradford (1976). Soluble proteins were extracted using 50mM TRIS-HCl (pH 7.6) and 1µM PMSF.

2.7 Statistical analysis

We performed two-factorial ANOVA in order to test for the significance of the effects of the fixed factors rainfall variability treatment and mowing frequency on the response variables. To account for the split-plot design, we included the row and the column of the weather treatment blocks as random factors in our linear mixed effect model, as each rainfall manipulation was restricted to occur just once in each row and each column of the design. To include row and column number as random effects automatically implements the nesting of mowing frequency within rainfall treatment blocks in the model, as one weather treatment block with its corresponding and unique row and column combination includes four values of the response variable (within one block the two mowing frequencies are represented twice each) (Faraway, 2006). Prior to analyses, we tested whether the assumptions of an ANOVA, homogeneity of variances and normally-distributed errors had been met by visually checking the residuals against the fitted plots and the normal qq-plots (Faraway, 2006). If these assumptions were not fulfilled then the data were square-root (root length) or log-transformed (biomass, N data). All statistical analyses were performed using R 2.11.0 (R Development Core Team, 2010). For mixed effect models we used the software package lme4 (Bates & Maechler, 2010), and the package multcomp (Hothorn et al., 2008) for multiple post-hoc comparisons. Significance levels in mixed effect models were evaluated by Markov Chain Monte Carlo sampling of 1000 permutations, using the software package language R (Baayen, 2009).

3. Results

3.1 Rainfall and soil moisture characteristics

The vegetation period for the year 2008 (April 1st – October 31st) with a total sum of 427 mm of rainfall was slightly drier than the long-term average rainfall sum of 437 mm for the time period 1971-2000, whereas the vegetation period for 2009 was slightly wetter (459 mm). Fig. 1 shows soil moisture dynamics for 2008 and 2009.

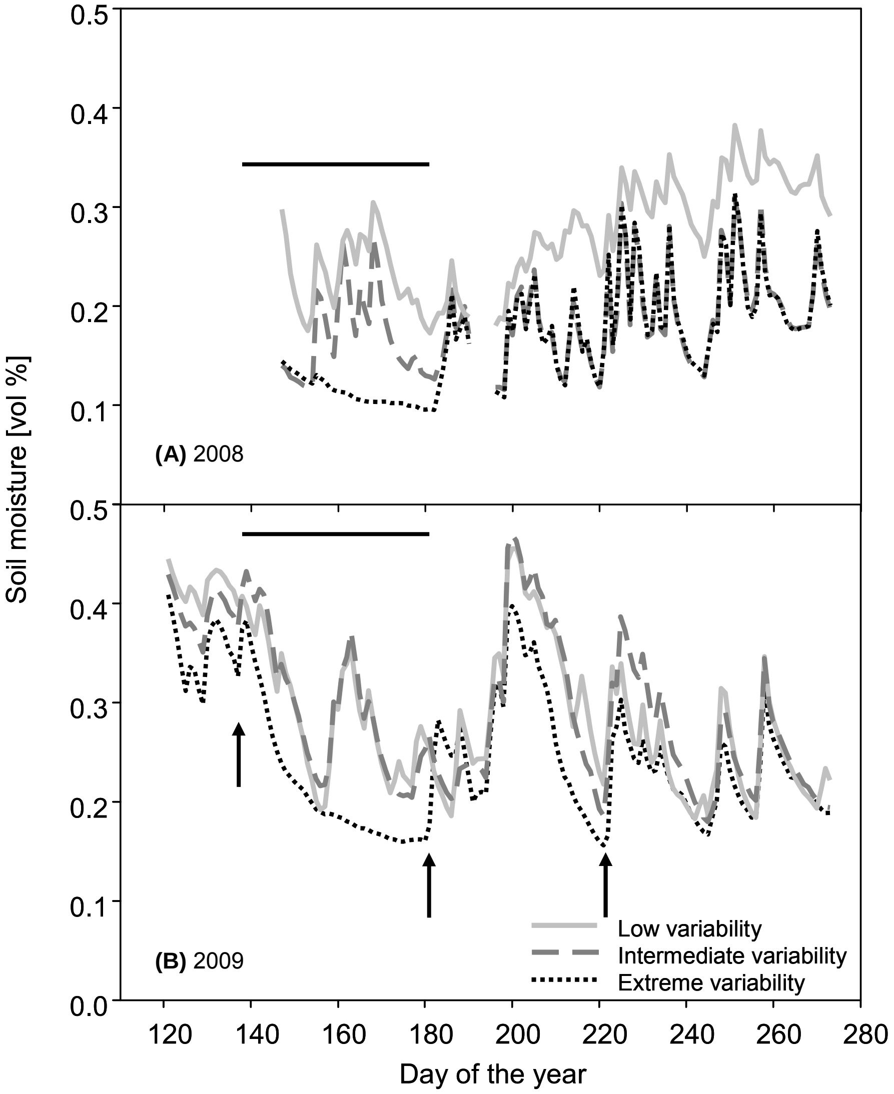

Fig. 1 Soil moisture response to (A) altered rainfall variability and altered rainfall sum (2008) and (B) to altered rainfall variability with constant rainfall sum (2009). Results for low rainfall variability (light grey solid line), intermediate rainfall variability (dark grey dashed line) and extreme rainfall variability (black dotted line) are shown throughout the vegetation period.

In 2008, measurements started on May 26th (day 147 of the year) and results are missing from July 9th until July 13th (days 191-195 of the year) due to a technical error. The black vertical line indicates the length of the drought for the extreme variability treatment (days 138-181 of the year), the black arrows indicate the timing of the first three compensation irrigations in 2009 (amounts of irrigation for each weather treatment given in Table 1). Soil moisture was recorded at a depth of -2 - -7 cm using FD sensors.

In 2008, the overall soil moisture from May 26th until the end of October was greatest and least variable (expressed as CV: coefficient of variation: standard deviation/mean) in the low rainfall variability treatment, followed by the intermediate and then the extreme rainfall variability treatment. Variability of total daily rainfall was most variable in the extreme variability treatment and least variable in the low variability treatment (Table 1).

The rainfall treatments in 2009 caused changes in soil water dynamics in terms of soil moisture and variability in soil moisture (Table 1). Mean soil moisture over the whole vegetation period for medium rainfall variability and low rainfall variability did not greatly differ (difference < 4 %), but was reduced by around 17 % in the extreme rainfall variability treatment compared to the low rainfall variability treatment. In each of the four periods between compensation irrigation, soil moisture was lowest for the most extreme rainfall variability treatment, particularly during the drought period, where it was reduced by 28 % compared to the other two treatments. Variability in soil moisture (CV) in each of the four periods was lowest for the low rainfall variability treatment, although the overall annual CV was almost the same in the intermediate rainfall variability treatment (difference <0.5 %). Variability in soil moisture and daily rainfall amount was overall greatest in the extreme rainfall variability treatment (Table 1).

3.2 Effects of increased rainfall variability, modified rainfall amounts and mowing frequency in 2008

In 2008, diminished rainfall amounts and increased rainfall variability negatively affected biomass production in July (In the following, we give degrees of freedom (df) and residual df in parentheses after F in the following notation: F(df, residual df).)(F(2,56)= 3.44; p=0.039) and September (F(2,56)=22.05; p<0.001), as well as ANPP (F(2,56)=13.52; p<0.001) (Fig. 2a-c). In July, biomass production was reduced by 21 % in the spring drought treatment (extreme variability) compared to the low rainfall variability treatment with the overall highest rainfall amounts (p=0.025) (Fig 2a). In September, biomass production was reduced by 49 % in the extreme variability treatment with the overall lowest rainfall sum and by 35 % under ambient rainfall compared to the low variability treatment that was regularly watered (p<0.001), with marginally significant differences between the intermediate and extreme variability treatments (p=0.087) (Fig. 2b). Overall ANPP was lowered by 33 % in the extreme variability treatment and by 17 % in the intermediate variability treatment compared to the low variability treatment (p<0.001 and p=0.031). ANPP in the extreme variability treatment with the lowest rainfall amounts was lowered by 19 % compared to the intermediate variability treatment (p=0.021) (Fig. 2c).

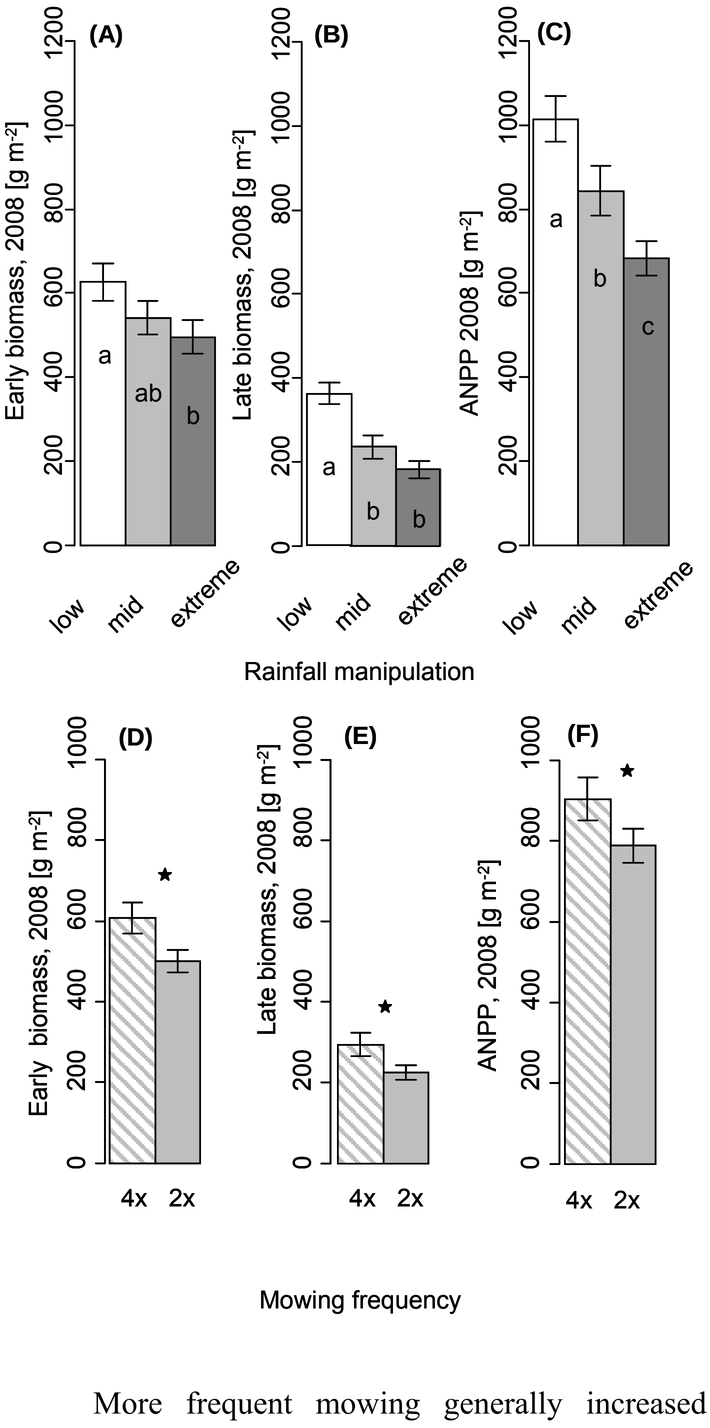

Fig. 2 Effects of altered rainfall variability and rainfall sum (A-C) and of altered mowing frequency (D-F) in 2008. Biomass production in (A) July 2008, (B) September 2008 and (C) ANPP in 2008 in response to altered rainfall variability and rainfall sum and in response to altered mowing frequency (D-F, patterned bars: mown four times per year, shades bars: mown twice per year). In graphs A-C, white bars show the low variability treatment with the highest overall rainfall amounts, light grey bars show the intermediate variability treatment with intermediate rainfall amounts, and dark grey bars show the extreme variability treatment with the lowest overall rainfall amount. Means ±1SE are shown, different letters and asterisks indicate significant differences below p=0.05.

More frequent mowing generally increased biomass production in July 2008 (F(1,56)=5.70; p=0.02) (Fig. 2d) and September 2008 (F(1,56)=5.99; p=0.018) (Fig. 2e), and thus increased ANPP in 2008 (F(1,56)=4.23); p=0.044) (Fig. 2f). There were no significant interactions between mowing frequency and rainfall variability in 2008 (data not shown).

3.3. Effects of increased rainfall variability in 2009

Rainfall variability significantly altered biomass production in the early summer of 2009 ((F(2,56)= 11.19; p<0.001), shortly after extreme drought followed by heavy rainfall was applied to the extreme variability treatments (first week of July) (Fig. 3a). Communities subjected to the extreme rainfall variability revealed 20 % less aboveground biomass compared to the low variability treatment (p<0.001) and 13 % less biomass compared to the intermediate variability treatment (p= 0.002). These strong effects of extreme rainfall variability assessed in July did not persist until September, as then extreme rainfall variability did not significantly affect biomass production (F(2,56)=0.72; p=0.49) (Fig. 3b). Nevertheless, the strong early summer effects of extreme rainfall variability led to negative ANPP responses (F(2,56)=9.11; p<0.001), as communities under extreme rainfall variability produced 15 % less biomass than communities under low variability treatment (p<0.001) and 7 % less biomass than communities under intermediate variability treatment (p= 0.024) (Fig. 3c).

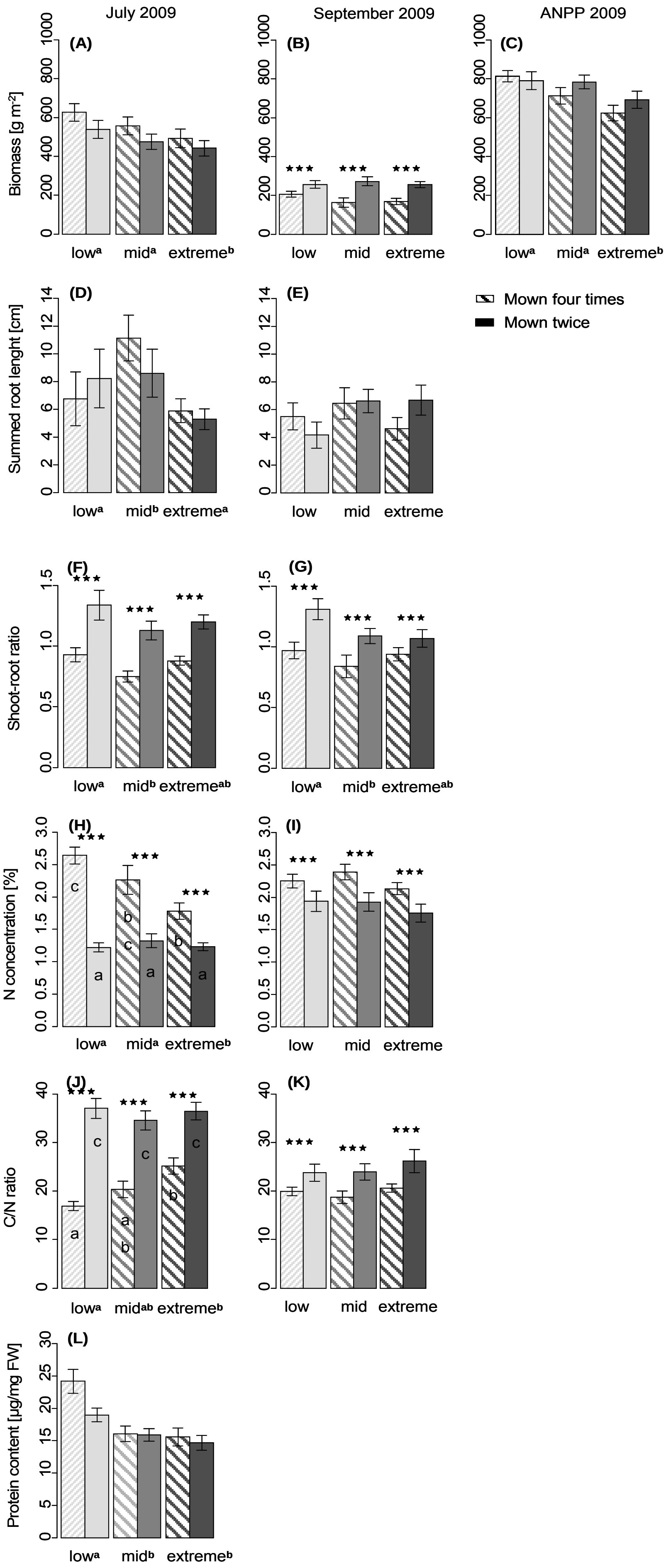

Fig. 3 Plant response to altered rainfall variability with constant rainfall sum and to mowing frequency in 2009. Effects of low rainfall variability (light grey), intermediate rainfall variability (grey) and extreme rainfall variability (dark grey) and of mowing (patterned: four times; shaded: twice) on (A) early aboveground biomass in July, (B) late aboveground biomass in September, (C) aboveground net primary productivity, (D) summed root length in July, (E) summed root length in September, (F) early and (G) late shoot-root ratio, early (H) and late (I) N concentrations in leaves of *Alopecurus pratensis*, early (J) and late (K) C/N ratio in Alopecurus pratensis and (L) protein content in leaves of the legume *Trifolium pratense* on the last day of the drought treatment. Means ±1SE are shown, different superscript letters over the treatment names indicate significant differences below p=0.05 between the rainfall variability manipulations, asterisks indicate level of significance of differences between the two mowing frequencies (*<0.001) and different letters within the bar indicate differences in the post-hoc test between the subgroups, when the interaction between mowing frequency and rainfall variability was significant (only (H) and (J))**

A redistribution of rainfall resulted in changes in the summed root length in July (F(2,56)=4.41; p=0.017) (Fig. 3d). The summed root length was highest in the intermediate rainfall variability treatment and 43 % and 24 % shorter in extreme and low rainfall variability treatments (p= 0.024 and p=0.032, respectively). In September, no differences occurred in the summed root length (F(2,56)=2.17; p=0.12)(Fig. 3e).

The shoot-root ratio in July was affected by extreme rainfall variability (F(2,56)=5.44; p= 0.007), as it was increased by 22 % in the low rainfall variability treatment when compared to the intermediate rainfall variability treatment (p=0.003) (Fig. 3f). The effect of this rainfall variability treatment on shoot-root ratio persisted until September (F(2,56)=4.10; p=0.022), as the shoot-root ratio was still increased in the low rainfall variability treatment compared to the intermediate rainfall variability treatment (p=0.017) (Fig. 3g).

Increased spring variability in rainfall patterns also affected the forage quality in early summer: Leaf N concentration of the target grass *Alopecurus pratensis* was decreased in July under extreme rainfall variability compared to low (p<0.001) and intermediate rainfall variability treatments (p= 0.037; overall rainfall treatment effect F(2,54)=5.03; p=0.01)(Fig. 3h). The C/N ratio in June was marginally significantly affected by rainfall variability (F(2,54)=3.07; p= 0.055), as it was increased under extreme rainfall variability compared to low rainfall variability (p=0.002) (Fig.3j). Rainfall effects on N concentration and C/N ratio interacted with effects of mowing frequency, shown at section 3.5. Extreme rainfall variability did not affect the forage quality in September, as neither leaf N concentration (F(2,56)=1.71; p=0.19) nor the C/N ratio (F(2,56)=1.19; p=0.31) in *Alopecurus pratensis* was altered in response to rainfall treatments (Fig. 3i,k). Protein content in the legume *Trifolium pratense* was also affected by rainfall variability treatments (F(2,54)=12.33; p<0.001), as protein content was increased under a low rainfall variability treatment when compared to intermediate and extreme rainfall variability (p<0.001) (Fig. 3l).

3.4 Effects of the mowing frequency in 2009

In 2009, more frequent mowing caused a marginally significant positive response of productivity during midsummer: The cumulated biomass of the two harvests until July 2009 in the communities that had been mown four times was 12 % greater than the biomass production in those plots that had only been mown twice per year (F(1,56)=11.19); p= 0.057) (Fig. 3a). Contrastingly, more frequent mowing in mid and late summer resulted in a 32 % decrease in biomass compared to those communities that had only been mown twice per year

(F(1,56)=38.66; p<0.001) (Fig. 3b). This reversal of effects resulted in ANPP showing no response to altered mowing frequency over the whole year (F(1,56)=2.00; p=0.16) (Fig. 3c).

The shoot-root ratio decreased consistently by more frequent mowing in early (F(1,56)=58.79; p<0.001) and late (F(1,56)=21.04; p<0.001) summer ((Fig. 3f,g), whereas the root length was not affected at any point in time (Fig. 3d,e).

The C/N ratio in leaves of *Alopecurus pratensis* in July decreased (F(1,54)=122.02; p>0.001)and N concentration increased (F(1,54)=109.81); p<0.001) as a result of more frequent mowing in early summer (Fig. 3h,j), both interacting with rainfall variability (see section 3.5). The same effect of mowing frequency on the leaf N relation was observed in September (F(1,56)=14.49 for N content and 15.99 for C/N ratio; p<0.001) (Fig. 3i,k).

3.5 Interactions between rainfall variability and mowing frequency

Mowing frequency did not interact with rainfall variability in terms of productivity, root length or shoot-root ratio in any year. There was a significant interaction for leaf N concentration and consequently C/N ratio in July 2009 (F(2,54)=5.34; p=0.011 and F(2,54)=3.54; p=0.036, respectively): Differences in N concentrations caused by rainfall variability were comparatively greater for more frequently mown communities (Fig. 3h), which caused the significant interaction between mowing frequency and rainfall variability. Accordingly, the differences in the C/N ratio in July caused by rainfall variability were comparatively greater in those plants that were mown more frequently and was hardly noticeable in plants mown only twice per year (Fig. 3j).

4. Discussion

Climate change is projected not only to alter the annual amount of rainfall, but also rainfall variability, resulting in more intense rainfall events and longer dry periods in between. Our results emphasize that overall rainfall amount is a strong driver of grassland productivity and forage quality. However, we demonstrate that this is not only caused by mere differences in amount, but also by differences in rainfall variability which usually accompany shifts in rainfall amounts: We show that altered rainfall variability even under constant rainfall amounts affected community productivity and also the leaf quality of our target species. Mowing frequency strongly affected biomass production and quality, but neither buffered nor amplified the effects of rainfall variability on productivity.

4.1 Effects of increased rainfall variability in 2009, compared to the effects of increased rainfall and modified rainfall amounts in 2008

In the dry year of 2008, soil moisture for the low variability treatment was always higher compared to the other two treatments, according to the highest total rainfall amount.
The effects from the rainfall treatments (altered amount and variability) on productivity were generally greater in 2008 and lasted longer than in 2009. We did not expect such great effects in the first year of the study, as other studies often show weaker, lagged or even no effects of grassland productivity towards drought (Bloor et al., 2010; Gilgen and Buchmann, 2009; Kreyling et al., 2008b). These results highlight the important role of the overall rainfall amount for grassland productivity. In 2008 there were also differences in biomass production between the low rainfall variability treatment with the highest rainfall amounts and the intermediate rainfall variability treatment with the lower rainfall amounts, whereas in 2009, when both treatments received the same amount of rainfall, there were no differences between the low and intermediate rainfall variability treatments. Nevertheless, the results from 2009 showed that changes in rainfall variability can only affect productivity, when the variability is extreme. However, we show that it is not only rainfall amount that influences productivity and forage quality of temperate grassland, but also the rainfall variability and intervals, in which a given rainfall amount is applied. The relatively short-lived effects of extreme spring rainfall variability in 2009, compared to the long-lasting effects of the treatments in 2008, highlight the importance of sufficient water availability and thus the total rainfall amounts for grassland resilience. Therefore, in contrast to the studies on mesic grassland systems of North America (Fay et al., 2003; Knapp et al., 2002), here, in temperate grassland of Central-Europe, overall rainfall amount seemed to influence ANPP stronger than rainfall variability alone. We cannot completely rule out the possibility that the drought effect of 2008 could have enhanced the spring effects of extreme variability in 2009, e.g. by making soil more susceptible to drying. However, as the productivity trend is different from that observed at the end of 2008 (with no differences between the intermediate and low variability treatment in early 2009), we conclude that most of the effect can be attributed to altered spring rainfall variability.

4.2 Effects of increased rainfall variability with constant rainfall amounts

In 2009, which was naturally a wet year, the amount of rainfall that resulted from many small events (as in the low rainfall variability treatment) or from several moderate events (as in the intermediate rainfall variability treatment) did not discriminate soil moisture. The results show that very extreme rainfall events, as in the extreme variability treatment directly after drought, are not efficient in constantly increasing soil moisture, as they also increase water runoff and the length of the dry periods. Furthermore, long dry periods may reduce the water holding capacity of the soil or may even make the rhizosphere hydrophobic (Browning et al., 2007; Carminati et al., 2010), as indicated by the greater responsiveness of soil moisture towards dryness and the lower responsiveness to wet pulses in the extreme variability treatment.

Our results provide evidence of a high short-term sensitivity of grassland after extreme spring rainfall variability that was neutralised until September. Nevertheless, ANPP was affected negatively by the extreme rainfall variability, indicating a possible risk of production losses for agriculture under global climate change. Comparable studies in mesic grassland also show losses in ANPP under increased rainfall variability: Heisler-White et al. (2009) report an 18 % reduction in productivity, although the rainfall variability, which they applied, was greater (up to a 75 % increase in the number of rainfall events) than in our experiment (a 33 % reduction in events between extreme and low variability). Fay et al. (2003) and Knapp et al. (2002) report a 10 % reduction in long-term productivity after subjecting mesic tallgrass prairie to more extreme rainfall patterns.

Root length data in early summer indicate that extreme dryness, as well as regular water availability may decrease root biomass. Although enhanced root growth under drought is viewed as an adaptive feature of many species under drought, other studies also indicate that grassland roots may not respond with enhanced root growth to dryness (Kreyling et al., 2008a). Again, these changes to root length only became apparent in July and were thus relatively short-lived. However, the shoot-root ratio changed consistently as a result of changing rainfall patterns.

The reduction of leaf N under extreme rainfall variability could be due to less microbial activity caused by low soil moisture and long dry periods, or even due to N leaching that is increased after extreme rainfall events (Heisler-White et al., 2008). Surprisingly, the protein content in the target legume *Trifolium pratense* directly after drought was not affected by the extreme variability, but rather showed an increased protein content in the leaves under low rainfall variability, with no differences between intermediate and extreme rainfall variability. This indicates that the leaf quality of different functional groups reacts independently and differently towards rainfall variability. Furthermore, regular water availability might have increased the activity of N fixers in the nodules of *Trifolium pratense.*

4.3 The effects of mowing frequency

The overcompensation in biomass production in more frequently mown communities in 2008 and early 2009 was reversed by a clear negative effect on productivity in September 2009. Most of the previous studies conducted on the effects of defoliation on productivity indicate either negative or neutral effects (Biondini et al., 1999; Green and Detling, 2000; Hejcman et al., 2010; Leriche et al., 2003; Maron and Jeffries, 2001; Milchunas and Lauenroth, 1993), while the effects of overcompensation are reported mostly for very low or intermediate intensities of cutting (Bernhardt-Römermann et al., 2011; Weigelt et al., 2009; Zhao et al., 2008) or for communities without any previous mowing history (Turner et al., 1993) and are often lessened after a history of several mowing events (Loeser et al., 2004). Our study also shows that overcompensation is reversed after one vegetation period of more frequent mowing. Our results therefore indicate that mowing history should be considered and adjusted to optimize productivity.

Our findings of increased leaf quality in terms of N concentration and protein content are consistent with many other studies showing increased N concentration in leaves that were cut more frequently; (Green and Detling, 2000; Maron and Jeffries, 2001; Turner et al., 1993). This might be explained by the generally lower shoot-root ratio in more frequently mown plots, caused by a reduced shoot biomass: This allows for a higher concentration of N in leaf tissue, as root biomass has to allocate resources to less aboveground biomass. Furthermore, increased defoliation intensity accelerates decomposition and N mineralization, thereby increasing the N level in the soil and thus mowing may enhance root N uptake and allocation to the shoots (Green and Detling, 2000; Klumpp et al., 2009; Turner et al., 1993).

Thus, although the effects of mowing on biomass production are ambivalent, the effects of mowing frequency on forage quality are unequivocally positive, as N, which is often a limiting factor for herbivores, increases.

4.4 Interactive effects between rainfall variability and mowing frequency

Mowing frequency and rainfall variability did not interact for most of the assessed parameters. Nevertheless, leaf N concentration and the C/N ratio in early summer were very responsive towards rainfall variability only in the more frequently mown plots, indicating a higher responsiveness of younger leaves with a lower shoot-root ratio towards extreme rainfall variability and drought. Grasses with a lower shoot-root ratio can allocate more N to the leaves, however, water availability is necessary for mineral uptake through the roots. As less frequently mown and thus older leaves have lower leaf N concentrations, they may not depend as largely on temporal water availability. In sum, although frequent mowing increases forage quality it might also increase the fluctuations in forage quality under climate change.

5. Conclusions

Our study shows that increased rainfall variability under climate change may cause losses in temperate grassland productivity and also reduces forage quality. In contrast to other studies, a comparison to the data of the previous year indicates that overall rainfall amount is more important for temperate grassland productivity than rainfall variability. However, changes in variability, that accompany changes in total rainfall amount, surely amplify the effects of differences in rainfall amount. Furthermore, our results indicate that mowing history might be more important for explaining productivity than mowing frequency alone. In sum, positive effects of more frequent mowing on forage quality might be diminished by increased rainfall variability just as increased rainfall variability alone negatively affects forage quality. To conclude, climate change will affect agriculture in Europe by changing meadow usability. Management strategies to buffer adverse effects on forage quality and quantity have yet to be investigated and established, as mowing frequency seems to have a rather small buffering capacity.

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Manuscript 7: Combined effects of multifactor climate change and land-use on decomposition in temperate grassland

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Summary

1. Climate change is likely to alter decomposition rates through direct effects on soil biotic activity and indirect effects on litter quality with possible impacts on the global carbon budget and nutrient cycling. Currently, there is an urgent need to study combined effects of various climatic drivers and of agricultural practise on decomposition.

2. In an *in-situ* litter bag experiment, we studied effects of rainfall variability (including drought plus heavy rain pulses and regular irrigation) interacting with increased winter temperature and precipitation and with changes in cutting frequency, on decomposition in a temperate grassland. Following a realistic scenario, litter bags contained litter out of all different climate and land-use manipulations and were placed within the plots of litter origin. Moreover, to disentangle causes for altered decomposition, we studied decomposition of litter

pre-exposed to the manipulations under ambient standard conditions and decomposition of standard material under differing rainfall variability in additional experimental approaches.

3. Decomposition was reduced when litter bags were exposed to drought for six weeks within an 11 months period. Neither additional winter rain nor winter warming had an effect on decomposition, probably because winter warming reduced snow cover and increased variability of surface temperatures. Climate manipulations did neither change litter quality, nor decomposition under ambient standard conditions. Thus, reduced decomposition under extreme rainfall variability and drought may be mainly caused by a decrease in soil biotic activity, as indicated by reduced decomposition of standard material during drought.

4. More frequent cutting strongly stimulated decomposition, however, this stimulating effect was absent under extreme rainfall variability including drought. The stimulation of decomposition under more frequent cutting was attributed to changes in litter quality, namely a decrease in C/N ratio. Accordingly, litter from more frequently cut communities decomposed faster under ambient standardized conditions.

5. Projected increases in drought frequency under climate change may inhibit decomposition and alter nutrient and carbon cycling along with soil quality. Especially decomposition in frequently cut grassland appears vulnerable towards drought. Under winter warming, a reduction of snow cover leading to more variable surface temperatures may counteract increased carbon loss transiently until the cooling capacity of missing snow cover is exceeded.

Keywords: carbon turnover, climate change, C/N ratio, EVENT experiments, extreme weather event, global warming, litter bag, microbial activity, nutrient cycling

1. Introduction

Litter decomposition plays a major role for the carbon budget as well as for nutrient cycling in terrestrial ecosystems (Aerts 1997; Chapin *et al.* 2002). Decomposition processes are mainly governed by the three factors climate, leaf litter quality and the composition and activity of the decomposer community (Swift *et al.* 1979; Lavelle *et al.* 1993; Aerts 1997). Thus, climate change is likely to alter decomposition processes: Changes in litter decomposition rates might severely affect soil quality along with carbon and nutrient cycling. As grassland biomes store up to 30 % of soil carbon worldwide (Risch *et al*. 2007), effects of climatic change on decomposition in grassland are of major interest, because positive feedback processes may intensify warming due to rising CO_2 levels (Bontti *et al.* 2009). Climate change does not only result in a gradual warming trend, but also increases intra-annual rainfall variability, causing longer dry periods and more intense heavy rain spells (Meehl et al. 2007). Moreover, within Central Europe, warming will be most pronounced during winter, when also the overall precipitation amount is projected to increase (Christensen et al. 2007).

Changing climate is likely to alter decomposition processes through short term changes in soil moisture or temperature which directly affect soil biological processes, including microbial and soil community composition and activity (Hobbie 1996; Aerts 1997). Indirectly, climate change will alter decomposition through chemical changes of litter within single plants as well as through shifts in plant species composition (Hobbie 1996; Aerts 2006; Fortunel *et al.* 2009; Baptist *et al*. 2010; Osanai *et al.* 2012).

Reduced water availability or drought often have a negative effect on litter decomposition or soil respiration (Lensing & Wise 2007; Risch *et al.* 2007; van Meeteren *et al.* 2008; Bontti *et al.* 2009; Joos *et al.* 2010), although these effects may be only short-termed (Kemp *et al.* 2003; O‘Neill *et al.* 2003) or even non-existent (Kreyling *et al.* 2008). Constantly high water availability has also been shown to reduce decomposition (Tiemann & Billings 2011; Lensing & Wise 2007}. Warming has often been found to increase litter decomposition (Hobbie 1996; van Meeteren *et al.* 2008; Kirwan & Blum 2011) due to an increase in microbial and enzymatic activity (Chapin *et al.* 2002; Aerts 2006; Allison & Treseder 2011), although some studies suggest that this effect does not always occur

(Giardina & Ryan 2000; Risch *et al.* 2007). Furthermore, increased winter temperatures are likely to result in colder soil conditions due to snow melting (Kreyling 2010), which may even decrease decomposition. Accordingly, no consensus about the role of global warming on decomposition has emerged yet.

The few existing studies combining multiple climatic factors often found non-additive effects of the different factors, as, for instance, combination of CO_2 enrichment and warming did not react in the same way as both factors alone on microbial biomass carbon (Andresen *et al.* 2010) or as temperature-dependence of decomposition depended on moisture-availability (Butenschoen *et al.* 2011). Thus, acceleration of decomposition caused by warming may be offset under drier conditions (Gavazov 2010; Butenschoen *et al.* 2011).

Therefore, there is an urgent need to further study interactions between different climatic factors according to scenarios of future change, most importantly the simultaneously on-going warming and changed precipitation variability, under natural conditions (Aerts 2006; Butenschoen *et al.* 2011). Moreover, the impact of agricultural practise, such as frequency of cutting on decomposition needs to be addressed, as those may strongly alter decomposition, e.g. by changes in litter quality caused by more frequent cutting (Walter *et al.* 2012).

To study combined effects of increased inter-annual rainfall variability with winter climate change scenarios and agricultural practise on decomposition, we conducted a litter bag experiment in semi-natural grassland under different climate change scenarios and cutting frequencies . Grassland was subjected to summer drought followed by heavy rain pulses (extreme variability), to regular irrigation (low variability) and to ambient rainfall (mid variability) in combination with winter warming, additional winter rain and two cutting frequencies. We wanted to disentangle the causes for possible changes in decomposition, being either leaf chemical alterations or modifications in soil biotic activity, by testing decomposition in an in-situ experiment and under standardized conditions.

Our hypotheses were that

(1.) extreme rainfall variability including drought will reduce decomposition rates

(2.) more frequent cutting will stimulate decomposition independent of summer rainfall variability, caused by more beneficial leaf chemistry, e.g. younger leaves with higher nitrogen content

(3.) winter warming will increase decomposition, except for winter warming leading to actual decreases in temperature on the soil surface due to snow-melt and thus loss of insulation

(4.) additional winter rain will not affect decomposition as winters in Central Europe are already usually wet and decomposition should not be moisture-limited in this time

2. Material and Methods

2.1. Study site and experimental setup

The study was conducted within the EVENT II experiment, which investigates the impact of inter-annual rainfall variability in combination with winter climate change and agricultural practise in temperate grassland. The experiment was established in 2008 in a semi-natural grassland in the Ecological Botanical Garden of the University of Bayreuth, Germany, Central Europe (49°55´19´´N, 11°3455``E, 365 m asl) (Walter *et al.* 2012) and this study was conducted in 2010-2011 when three years of rainfall manipulations were already completed. Communities are dominated by tall grasses, especially *Alopecurus pratensis* L. (meadow foxtail) and *Arrhenatherum elatius* L. (tall oat grass). The regional climate is temperate and moderately continental.

The experimental design for this study consisted of five replications of three rainfall variability regimes applied in the vegetation periods in blocks 6 m x 4 m in size. For the manipulations of rainfall variability the temporal distribution and the magnitude of rainfall per rainfall event in the growing season was altered, but annual rainfall amount was kept constant since 2009 by applying compensation irrigations. The three rainfall variability regimes were:

(1) low variability, with weekly irrigation corresponding to the 30 year average amount of the respective week, ensuring a continuous water supply (low), (2) mid variability, receiving ambient rainfall plus compensation irrigations (4 times per year) to keep the annual rainfall amount constant at quarterly intervals (mid) and (3) extreme variability, subjected to a summer drought treatment, followed by heavy rain pulses (extreme). For the low variability treatment, periods from 1971 to 2000 served as a reference (data: Foken 2003). Missing amounts on natural precipitation were added if the weekly precipitation was less than the long-term average for the same week to ensure continuous water availability. If weekly precipitation exceeded the long-term sum, it was not subtracted for the next irrigation. The irrigated amount of 925 mm from September 1st 2010 until August 31st 2011, applied on all variability treatments, exceeded the 30-year-average sum for this time period by 202 mm, thus simulating a rather wet year. Table 1 lists the irrigated amounts of all compensation irrigations for the mid and extreme variability treatment.

Table 1 Compensation irrigations applied on mid- and extreme variability treatments during the experimental period to ensure an overall identical precipitation sum in all rainfall variability regimes

date	rainfall variability regime	
	mid	extreme
September 27th 2010	17.5	17.5
May 23rd 2011	52.9	52.9
July 4th 2011	26.3	26.3
August 15th 2011	33.9	229.9*

* applied on two consecutive days

For the extreme variability treatment, tunnel-shaped rain-out-shelters excluded natural precipitation from June 22nd until August 3rd in 2010 and from July 5th until August 16th in 2011, resulting in an extreme summer drought of 42 days, followed by two days of extreme irrigation as compensation irrigations. The PE-foil of the rain-out-shelters allowed nearly 90 % of photosynthetic active radiation. Shelters were started off a height of 0.8 m to reduce microclimatic artifacts. Irrigation was applied using portable irrigation systems with a drop size and rainfall intensity comparable to natural rainfall events. A lateral surface flow was reduced by using plastic sheet pilings around all plots reaching down to a depth of 0.2 m – 0.25 m.

In each rainfall variability block four subplots of 1.5 m x 1.5. m were nested, in which differing winter climate change scenarios and cutting frequencies were executed (n=60).

These within-block manipulations mimicked common agricultural practise (two cuts (July and September) versus four cuts (May, July, August, September) and projected winter climate change for Germany, most notably an increase in winter precipitation and temperatures (Jacob 2009; Zebisch *et al.* 2012). Aboveground temperature from October until April was increased by 1.1 ° C on average in the warmed plots at 0.05 m height and by 1.3 ° C in the soil using IR-heating lamps at a height of 1 m. The additional winter rain was applied in four monthly steps from November until February. Table 2 summarizes all rainfall variability regimes and the nested subplot scenarios.

2.2. Soil moisture and Temperature

Soil moisture was logged every hour using FD-sensors in each treatment combination (ECH$_2$O, Decagon devices, Pullman, USA) (n=5/ treatment combination). Each sensor measured the soil moisture between -2 and -7 cm. According to root length data, the majority of root biomass is located within the upper 5 cm of the soil. Figure 1 shows the course of soil moisture over the experimental period with daily averaged values. Temperature was measured at 10-minutes intervals by thermistors (B57863-S302-F40, EPCOS) and logged as hourly average by a data-logger (dl2, Delta) at 0.02 m soil depth and at 0.05 m height for each rainfall variability treatment in warmed and un-warmed plots.

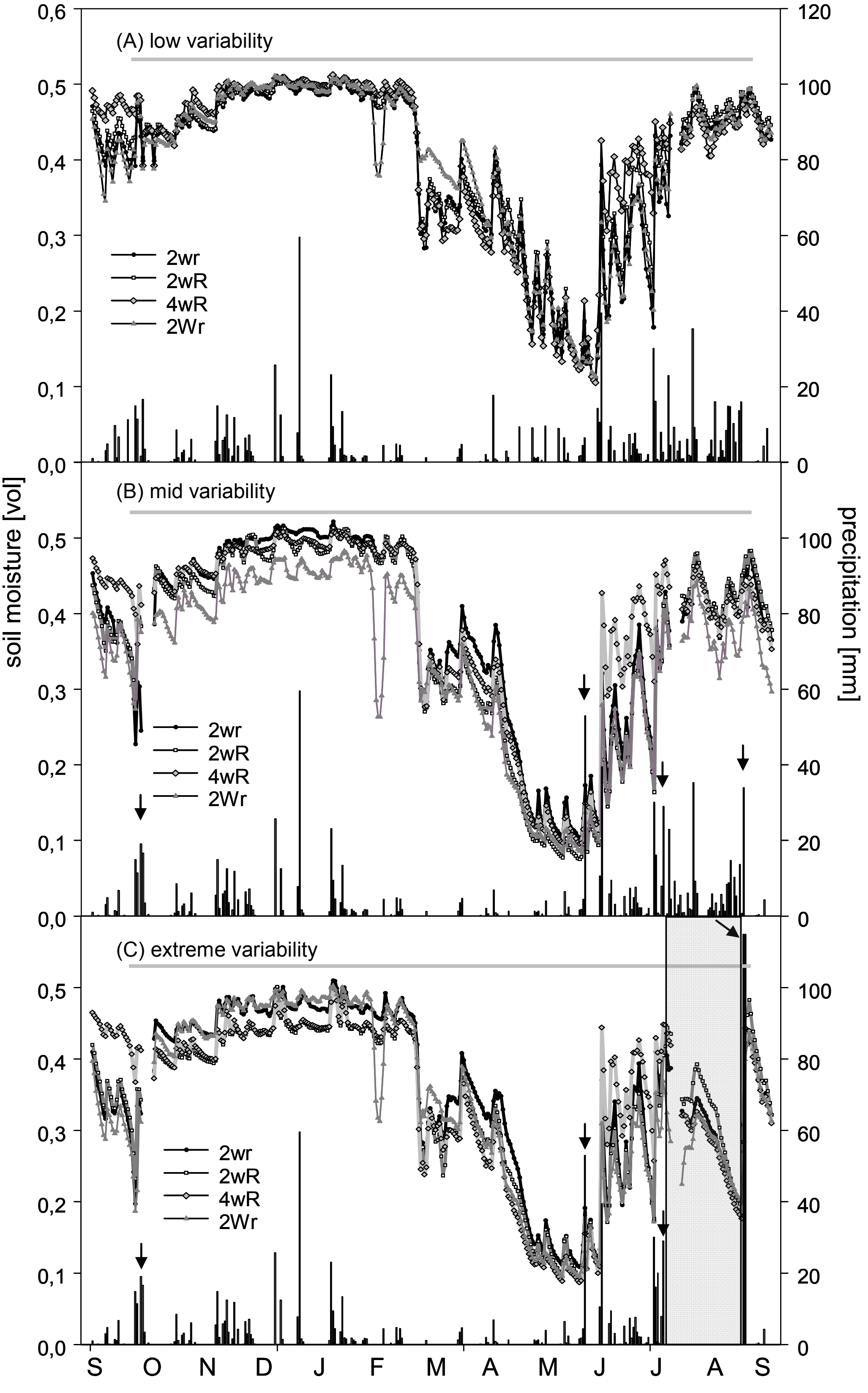

Fig. 1 Course of soil moisture over the experimental period in the winter climate change and cutting frequency manipulations within the low (A), mid (B), and extreme (C) rainfall variability treatments (black circle: cut twice (2wr); white square: cut twice with additional winter rain (2wR); light gray diamond: cut four times with winter rain (4wR); dark gray triangle: cut twice and warmed during winter (2Wr)). The gray area in (C) marks the duration of the extreme drought. Black arrows in B and C mark the compensation irrigations. The gray vertical line shows the exposure time of the litter bags.

Figure 2 shows the course of temperature and snow height during winter for plots warmed and not warmed from October until April.

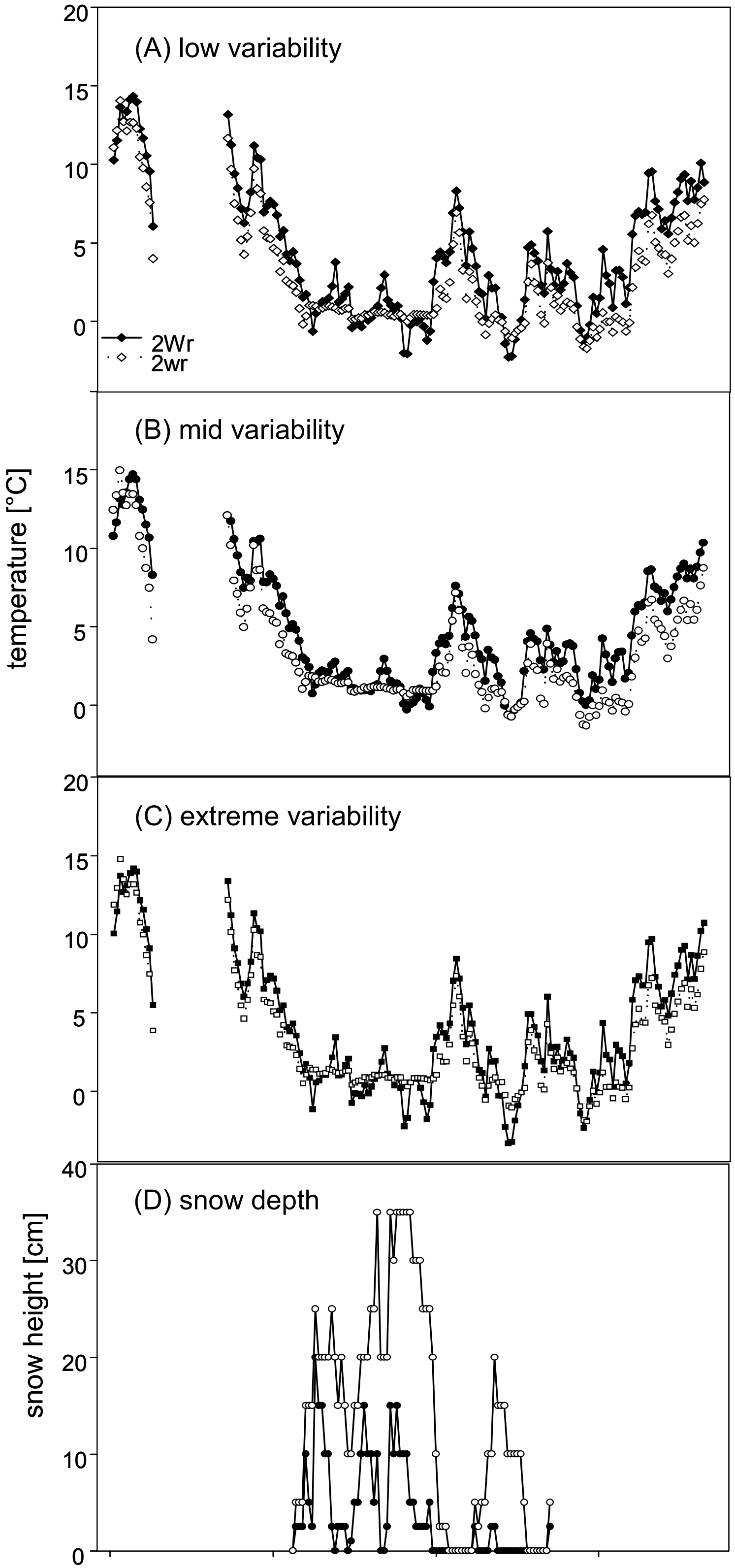

Fig. 2 Course of temperature averaged between -2 cm and +5 cm in the plots warmed (black circles) and not warmed (open circles) during winter within the low (A), mid (B), and extreme (C) rainfall variability treatment and snow depth in warmed (black circles) and un-warmed (open circles) plots. Temperature data between October 16 and November 3 are missing due to technical failure.

2.3. Sampling design for the litter bags and chemical analyses

To investigate the effects of rainfall variability regime in interaction with the different winter climate change and cutting scenarios, biomass sampling for the litter bags was conducted at September 6th and 7th in 2010. We obtained mixed samples by cutting four different circular areas, 0.20 m in diameter in each plot (n=60). To estimate effects of changes in leaf chemicals caused by different cutting regimes and to disentangle intra- from interspecific alterations, we additionally sampled a single grass species, *A. pratensis* out of the subplots 2wR and 4wR. All samples were oven-dried for 72 hours at 40 °C. After drying, 3 g ± 0.03 g were weighed into nylon mesh bags (10 cm x 20 cm) with a mesh size of 1 mm and the exact weight was recorded. This allows fungi, bacteria, microfauna and most of the mesofauna to attack the litter (Chapin *et al.* 2002). Mixed samples and *A.* pratensis out of 2wR and 4wR plots were ground in a ball mill and analysed for carbon (C) and nitrogen (N) with an elemental analyser (Thermo Quest Flash EA 1112).

2.4. Placement of the litter bags

To test decomposition under natural conditions, two mixed litter bags per plot were placed in the respective plots their litter was sampled from in late September. Bags were placed on the vegetation that was cut to the ground and attached to the ground using two plastic coated wires placed diagonally over the litter bag. Litter bags were removed in late August 2011 (after 11 month) and thus received a direct summer drought followed by a rewetting pulse in 2011.

To disentangle the causes for effects of rainfall variability regime and cutting frequency on decomposition, mixed samples out of all rainfall variability regimes and the subplots 2wR and 4wR (n=30) were placed on a standardized, untreated, mulched plot outside the experimental site. To disentangle chemical effects caused by intra-specific (variations of leaf chemicals within single plant species) or inter-specific (variations in leaf chemicals due to changes in plant community composition) alteration the *A. pratensis* samples were also placed on this untreated plot (n=30). Those bags were removed in May 2011, after 8 months of decomposition.

After retrieval, all bags were dried for 72 hours at 40° C and stored in air tight containers with silica gel until they were weighed on a micro-balance. Percentage of dry weight loss was calculated as a proxy for decomposition. Table 2 gives an overview over the sampling design and placement of the litter bags.

Table 2 Descriptions and abbreviations of applied rainfall variability regimes during the vegetation period and of the therein nested winter climate change and cutting frequency scenarios and sampling design for the litter bags

variability	description	mowing frequency/ winter climate	description	mixed litter bags within experiment*	untreated standard plot
low	weekly irrigation with 30-year average	2wr	mown twice/ year, no further manipulation	2 mixed, Sept-August	
		2wR	mown twice, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		4wR	mown four times, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		2Wr	mown twice, winter warming October-April	2 mixed, Sept-August	
mid	ambient rainfall with compensation irrigation	2wr	mown twice/ year, no further manipulation	2 mixed, Sept-August	
		2wR	mown twice, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		4wR	mown four times, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		2Wr	mown twice, winter warming October-April	2 mixed, Sept-August	
extreme	42 days summer drought followed by extreme compensation irrigation pulses	2wr	mown twice/ year, no further manipulation	2 mixed, Sept-August	
		2wR	mown twice, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		4wR	mown four times, 60 mm winter rain added	2 mixed, Sept-August	2 mixed, 2 *A.pratensis*
		2Wr	mown twice, winter warming October-April	2 mixed, Sept-August	

* bags were sampled from and placed on the respective treatment combination

** bags were sampled from the respective treatment combination and placed on untreated control plot to disentangle causes for changes in decomposition rates (litter quality or soil biotic activity)

2.5. Soil biotic activity

A bait-lamina test (Kratz 1998) was performed to measure effects of rainfall variability on soil biotic activity in August 2011, during the drought period in the extreme variability treatment. This approach complements the decomposition trial with plot-specific litter by investigating the treatment effects using a standard material. Each bait-lamina stick (*Terra Protecta*® GmbH, Berlin, Germany) contained 16 baits which consisted of a wet mixture of cellulose, bran flakes and activated coal (70:27:3). Within each rainfall variability regime, only the 2wr, 2wR and 2W subplots were included, as in the preceding year, cutting frequency was shown not to affect soil enzymatic activity (see supplemental information). In each plot, two baited sticks were placed vertically into soil and remained there for 14 days. At the end of the exposure period each stick was carefully removed from soil, placed into plastic bags and stored in a freezer at -30 ° C until analysis. Perforated baits of the cleaned bait sticks were recorded and expressed as percentage of eaten baits per plot.

2.6. Statistical analysis

To test for significant effects of summer rainfall variability in the differing winter climate change and cutting frequency scenarios, two-factorial ANOVA with the fixed factors “rainfall variability regime” and “winter climate change and cutting scenario” were performed. As each rainfall variability block was restricted to occur just once in each row and each column of the design, we included the row and the column of the weather treatment blocks as random factors in our linear mixed effect model. This also implements the nesting of winter climate change/ cutting scenario within the rainfall variability blocks in the model, as one block with its corresponding and unique row and column combination includes four values of the response variable (Faraway 2006). Tukey HSD tests were calculated for post-hoc analysis of differences between rainfall variability treatments. As the subplot scenarios were not all directly comparable with each other we only included the directly comparable data in further mixed models for post-hoc analysis to avoid unnecessary comparisons, if the effect of subplot scenarios or the interaction of subplot scenarios with rainfall variability was significant. Scenarios that are directly comparable as only one factor is varied are 2x +wr with 4x +wr to test for effects of cutting frequency, 2x +wr with 2x to test for effects of winter rain and 2x +ww with 2x to test for effects of winter warming. These data were analysed for effects of winter climate change and cutting frequency and of combined effects of those with rainfall variability.

All statistical analyses were performed using R 2.11.0 (R Development Core Team 2010). For mixed effect models we used the software package lme4 (Bates 2010) and nlme (Pinheiro 2008) to run the Tukey HSD tests. Significance levels in mixed effect models were evaluated by Markov Chain Monte Carlo sampling of 1000 permutations, using the software package language R (Baayen 2009).

3. Results

3.1. Effects of summer rainfall variability regime on decompositionand soil biotic activity

Litter decomposition over 11 months was strongly affected by the extreme variability treatment, as those samples decomposed significantly slower when compared to mid and low rainfall variability (overall effect of rainfall variability: F($_{2,83}$)= 5.5; p=0.006; Fig. 3A).

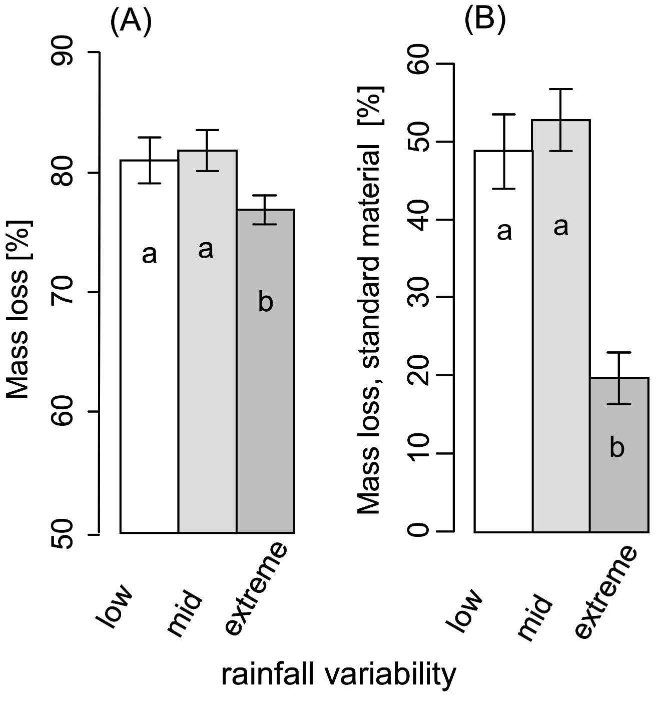

Fig. 3 Effects of rainfall variability on mass loss of mixed litter obtained from and placed within the experiment (white bars: low rainfall variability (weekly irrigation), light gray bars: mid rainfall variability; dark gray bars: extreme rainfall variability (summer drought plus heavy rain)). The bait-lamina sticks (C) were only placed within the experiment for two weeks during the drought period in the extreme variability treatment in 2011. Different letters indicate significant differences (p<0.05).

Extreme rainfall variability significantly reduced soil biotic activity during the drought period as measured by the bait-lamina test when compared to low and mid rainfall variability (overall rainfall variability effect F($_{2,41}$)=11.0, p>0.001; Fig. 3B).

3.2. Effects of cutting frequency and winter climate change on decomposition

Generally, cutting frequency strongly affected the rate of decomposition (F($_{1,41}$)= 34.1; p<0.001), with litter cut four times per year decomposing significantly faster than litter cut only twice (Fig. 4). The stimulating effect of frequent cutting depended on the rainfall variability regime (significant interaction F($_{2,41}$)= 7.9; p=0.001): The effect was not significant in the extreme variability treatment and highly significant under mid and low rainfall variability (p<0.001) (Fig. 4).

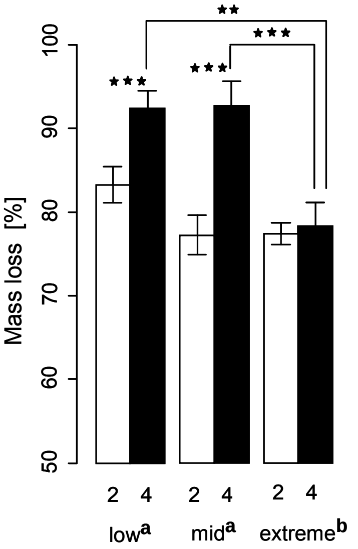

Fig. 4 Interacting effects of cutting frequency and rainfall variability on litter mass loss of mixed litter (black bars: cut four times per year, white bars: cut twice). Asterisks display significant differences between neighbouring bars or the bars connected with braces (*:p<0.001;**:p<0.01;*:p<0.05). Different superscript letters above the rainfall variability manipulations indicate significant differences between the manipulations.**

Thus, litter of plots cut four times per year decomposed faster under mid and low rainfall variability when compared to extreme variability (p<0.001 and p=0.002, respectively).

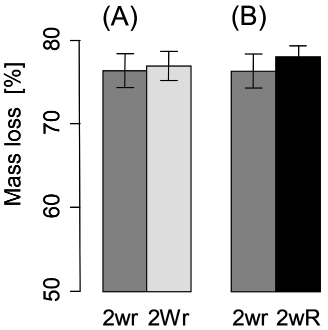

Fig. 5 Effects of winter warming (A) and of winter rain on litter mass loss. Dark gray bars: no winter rain or winter warming; light gray bar: warmed from October until April; black bar: 60 mm additional winter rain.

Neither winter warming nor additional winter rain affected decomposition (Fig. 5 A,B).

3.3. Decomposition under common standard conditions and leaf chemical traits

Pre-exposure of *A. pratensis* and the mixed litter to rainfall variability did not affect their decomposition on the untreated standard plot ($F(_{2,40})$= 0.9; p= 0.43 and $F(_{2,45})$= 2.9; p= 0.06, respectivley (data not shown)). Cutting frequency had a strong effect on decomposition under control conditions, as *A. pratensis* leaves and mixed samples from plots cut four times per year decomposed significantly faster than those from plots cut only twice per year ($F(_{1,40})$= 27.10; p<0.001 and $F(_{1,45})$= 35.9; p<0.001, respectively; Fig. 6A, B).

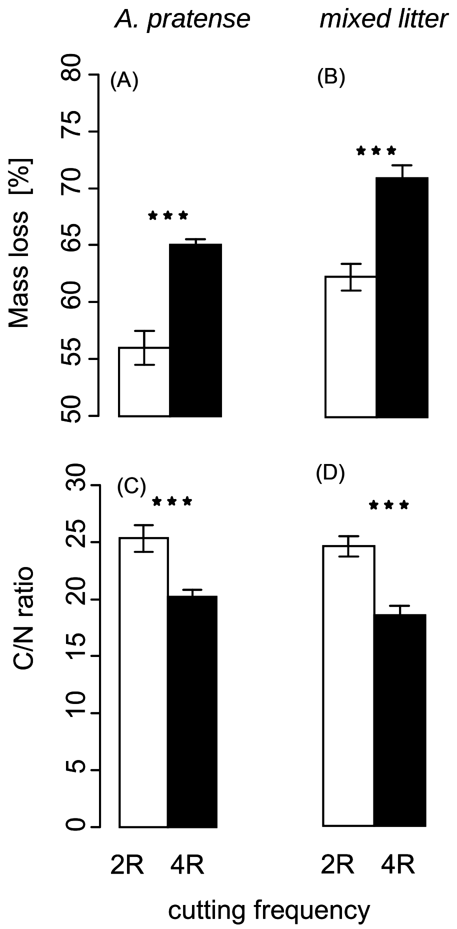

Fig. 6 Effects of cutting frequency on litter mass loss of A. pratensis (A) and of mixed litter (B)under ambient standard conditions, and effects on C/N ratio of A. pratensis (C) and of mixed litter (D). Asterisks indicate significant differences between the bars (*:p<0.001).**

C/N ratio was the best predictor for decomposition of mixed litter (Pearson´s coefficient= -0.51, p=0.007) and of *A. pratensis* litter (Pearson´s coefficient:-0.57, p=0.003). Rainfall variability pre-exposure did not alter C/N ratio of the target grass and of the mixed samples several weeks after the drought in the extreme variability treatment had been applied ($F(_{2,24})$=

1.6; p=0.22 and $F(_{2,24})$= 1.8; p=0.19; data not shown). More frequent cutting decreased C/N ratio of the grass by 24 % ($F(_{1,22})$=13.1; p=0.002) and C/N ratio of the mixed samples by 25 % ($F(_{1,22})$=27.0; p<0.001; Fig. 6C, D).

4. Discussion

4.1. Effects of summer rainfall variability on decomposition

In our study, soil moisture was reduced during the experimental drought, but restored quickly after rewatering. Yet, drought exposure of litter bags for only six weeks within an 11 month period inhibited decomposition. This reduction in decomposition under drought was not caused by changes in leaf litter C or N, but probably rather by a decrease in soil microbial and faunal activity under drought, as indicated by the bait-lamina test. Similar results have been found in other studies (Liao, Hou & Wang 2002; Kemp *et al.* 2003; Emmett *et al.* 2004; Lensing & Wise 2007; Risch *et al.* 2007; van Meeteren *et al.* 2008; Bontti *et al.* 2009; Reed *et al.* 2009; Andresen *et al.* 2010; Joos *et al.* 2010). Contrary to other studies (Sorensen 1974; Xu *et al.* 2004; Miller *et al.* 2005), the rewetting pulses after drought did not stimulate decomposition, at least not sufficiently to compensate for the decrease during drought.

As decomposition should accelerate under warm and moist (but not water-logged) conditions (Chapin *et al.* 2002; Aerts 2006) we expected decomposition to be fastest in communities that were weekly irrigated during summer (the low variability treatment). However, contrary to our expectations, weekly irrigations did not promote decomposition. Accordingly, Lensing et al. (2007) report higher decomposition under ambient rainfall conditions than under irrigation. Tiemann, Billings and Sharon (2011) show decreases in soil respiration under more regular irrigation when compared to soil respiration under higher soil moisture variability. Guenet et al. (2012) find highest soil enzymatic activity under intermediate moisture levels, which might relate to our mid rainfall variability. It was further shown that the microbial and fungal community gets less abundant and diverse under regular watering (Cornejo *et al.* 1994;

Hawkes *et al.* 2011), which might also inhibit a promotion of decomposition under regularly watered conditions.

4.2. Effects of cutting frequency and interactions with summer rainfall variability

As expected, more frequent cutting promoted decomposition. This can be explained by a more beneficial C/N ratio and thus a faster decomposition in community mixtures as well as in the single grass species, which has been shown for decomposition under untreated standard conditions. As leaf chemical changes in the single grass species pointed in the same direction and were of the same magnitude as changes in mixed litter we conclude that the chemical changes of mixed litter are due to intra-specific changes and not to changes in community composition. Thus, in our study the influence on decay processes of intra-specific variation under different environmental conditions was larger than the influence inter-specific variations, which contrasts other findings (Hobbie 1996; Aerts 2006; Wardle *et al.* 2009). Cutting frequency also strongly interacted with summer rainfall variability as decomposition was not stimulated by cutting four times per year when litter was derived out of the extreme rainfall variability regime. Unlike the general accelerating effect of more frequent cutting on decomposition, the reduction of this accelerating effect under drought cannot be explained by changes in leaf chemicals, as it is not mirrored in C/N ratio alterations and did not occur on the untreated standard plot. In the preceding year 2009 it was shown that cutting frequency does not alter soil enzymatic activity (see SI for an example), but we do not have data from our experimental period. Microclimatic conditions might react differently under drought in more frequently cut communities. During summer, soil moisture was often slightly higher in more frequently cut communities, which might have rendered the microbial community more vulnerable towards drought. Further studies should investigate long-term microbial activity and microclimate during and after drought in more and less frequently cut meadows. Our findings imply that decomposition in more frequently cut grassland might be more responsive to drought conditions than less frequently cut grassland. Such an impairment of nutrient turnover may lead to reductions in soil quality and thus also to reductions in productivity and forage quality under more frequent drought events, especially in more intensively managed grassland.

4.3. Effects of winter warming and winter rain on decompostion

A lack of a stimulating effect of warming on decomposition is often due to a concomitant decrease in soil moisture (Aerts 2006; Bontti *et al.* 2009; Gavazov 2010). Our soil moisture data also show a sudden drop under winter warming in February (Fig. 1). However, this drop was not caused by increased evapotranspiration, but probably by a prolonged soil frost in the warmed communities, due to melting of the snow cover in the warmed plots, when compared to un-warmed plots (Fig.2). Our warming treatment increased temperature slightly by 1.1° C on average, but also decreased temperature minima and resulted in a 16 % increase of frost days at 5 cm height, again probably caused by missing insulation due to snow-melt. These findings support evidence that winter warming might well lead to an *increase* in frost stress for many plants (Groffman *et al.* 2001; Gu *et al.* 2008; Kreyling 2010) and might lead to an increase in soil or surface temperature variability during winter, therefore explaining the missing stimulation of decomposition in our winter warming manipulation. In summary, snow cover appears to be the crucial factor controlling decomposition in warmer winters.

Concerns that global warming might lead to a stimulation of decomposition and soil respiration and thus to increases of carbon loss and positive feedback processes on climate, especially under cold conditions (Kirschbaum 1995; Aerts 2006) seem not to be generally justified regarding temperate grassland during winter (Giardina & Ryan 2000). Our results, however, imply that snow cover is critical for this conclusion. With ongoing climate warming, winter conditions in the southern temperate zone reach a point where lack of snow is accompanied by warmer soil conditions (Kreyling & Henry 2011), which is in contrast to more northern regions (Henry 2008) and the situation in our study. Based on this, we conclude that acceleration of decomposition is more likely to take place in southern temperate regions than in northern temperate regions. The lack of a stimulating effect of additional winter rain on decomposition shows that moisture can only stimulate decay processes when it is a limiting factor, which is not the case during Central European winters. With regard to decomposition, the very likely trend towards wetter winters in temperate regions (Christensen et al. 2007) consequently appears unimportant.

5. Conclusions

We show that even a very short drought relative to the exposure period decreases decomposition rate by 5 %.. Especially decomposition in more frequently cut grassland was vulnerable towards drought. Drier climatic conditions under global warming could thus slow down nutrient cycling and alter soil-carbon balance in more intensively managed grassland. Surprisingly, changes in winter climate and especially winter warming had no stimulating effect on decomposition. We attribute this finding to reduced snow cover which caused colder soils due to missing insulation against air temperature variability in the warming treatment during considerable periods of time over winter. We conclude that the interplay between climate warming and decomposition depends on snow cover. Changes in climatic variables directly affected decomposition through changes of soil biotic activity and not through litter quality alterations, as neither C/N ratio nor decomposition under untreated standard conditions was altered by litter pre-exposure to rainfall variability. Contrastingly, the stimulation of decomposition of more frequent cutting can be largely explained by changed litter quality, most notably a decrease in C/N ratio. To conclude, although grassland decomposition and soil biotic activity seemed to be quite resistant towards changes in climatic variables, certain future projections, such as increased drought frequency or continued winter warming beyond the cooling capacity of missing snow cover could necessitate an adaptation of agricultural routines to sustain soil quality and productivity.

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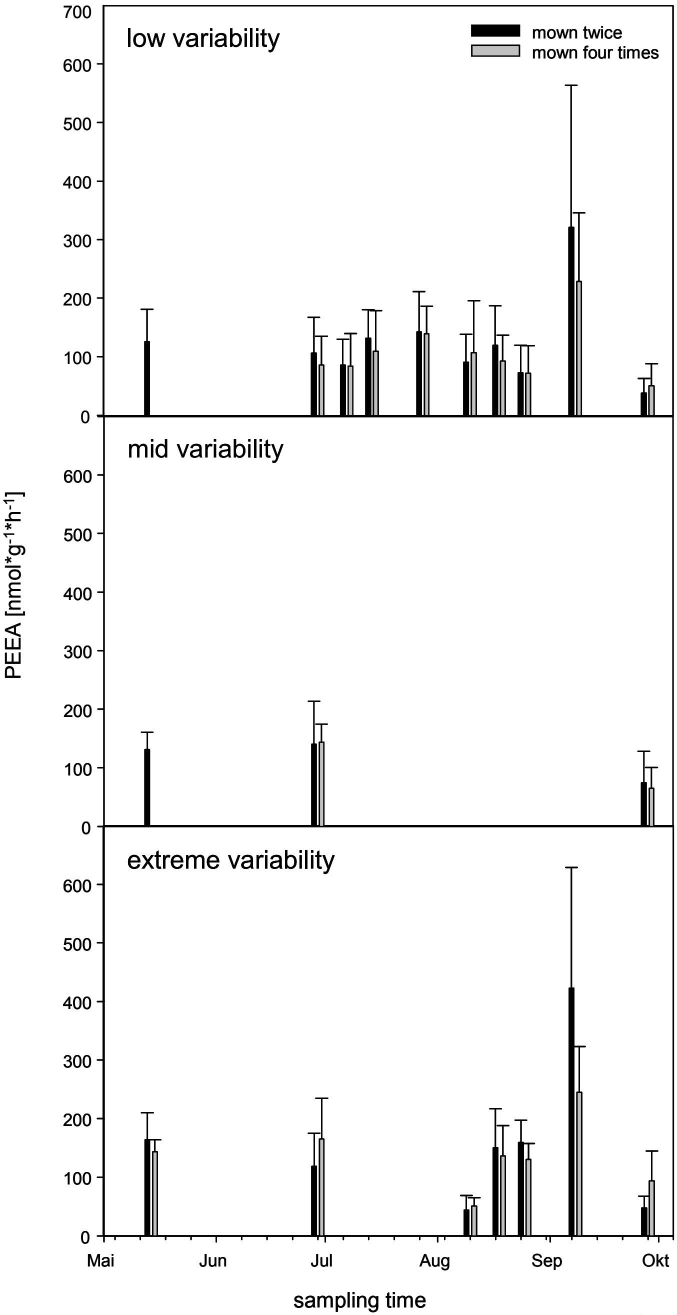
Online Supporting Information



Potential soil enzyme activity (PEEA) in nmol*g^{-1}*h^{-1} of cellobiohydrolase as an example for general soil enzymatic activity out of different measured PEEAs (β-glucosidase, phosphomonoesterase, exochitinase, glucuronidase and xylosidase; data not shown). No significant changes in response to altered mowing frequency occurred.

Method:

For characterisation of soil biological activity, potential extracellular enzyme activity (PEEA) was measured. At each sampling date three soil samples per plot were collected with a small diameter corer (5 - 15 cm depth) and pooled. All samples were stored at 4 °C until further handling within 48 h. The procedure used for sample preparation and fluorescent measurement has been described by Pritsch *et al.* (2005). The enzyme assay based on methylumbelliferone (MUF) labelled substrates was prepared in black microplates. Solutions were prepared as previously described (Pritsch *et al.* 2005; Kreyling *et al.* 2008). Samples were incubated for 120 minutes at a concentration of 400 µM. After incubation, the reaction in the microplates was alkalinised and stopped with a 1 M Tris buffer (pH 10.8) and centrifuged for 5 min at 20 °C and 1120 x g. Fluorescence measurements were performed on a Spectrofluorometer (SpectraMax GEMINI EM, Sannyvale, USA) at excitation/emission wavelengths of 365 / 450 nm. Released amounts of MUF were calculated based on the calibration curves and expressed as PEEA in nmol per gram soil dry weight per hour (nmol g^{-1} h^{-1}).

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Synopsis

More frequent and more extreme weather events, especially drought, will affect temperate grassland in many ways. They will alter biotic interactions and ecosystem processes at multiple levels, thereby also affecting ecosystem services, such as fodder provisison. Some aspects and processes, like forage quality or decomposition seem to be more vulnerable towards drought and extreme rainfall variability in more frequently mown grassland. Further research is necessary to understand mechanisms of grassland resilience and to enable policy makers to take measures for adaptation and mitigation under global climate change and consequently to maintain functionality of temperate grassland, which provides numerous ecosystem services.

Hiermit erkäre ich, dass ich die Arbeit selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ferner erkläre ich, dass ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

Bayreuth, 11.04.2012,