

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

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Received: 22 March 2011 / Accepted: 15 September 2011 / Published online: 13 October 2011
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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.

Handling editor: Joseph Dickens.

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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 twenty-first 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 have tested the effect of drought on plant–herbivore interactions (English-Loeb 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; English-Loeb 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 with 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 (English-Loeb 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 with 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. 2007a, b). 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 alters 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: First, we investigated how an

extreme drought, applied under field conditions, alters plant metabolites and thus affects the development of herbivores. And second, 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 and methods

Study organisms

Spodoptera littoralis (Boisduval) (African cotton leaf-worm; 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 moderately 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 greenhouse effects. The intensity of the drought in 2009 was based on the local 1,000-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 1,000-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 × 2 m plots except for the monocultures that were grown in 1 m × 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. 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; 1,000-year recurrent drought since 2008). Monocultures were established in autumn 2006, also from plants pre-grown 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 (ECH₂O, 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:

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

(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, and soluble proteins of 100 mg plant material were extracted using 50 mM sodiumphosphate-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 analysed 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

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)	<i>Holcus lanatus</i> (L.)
2–	One (grass only)	<i>Holcus lanatus</i> (L.) <i>Arrhenatherum elatius</i> (L.)
4–	Two (grass and herb)	<i>Holcus lanatus</i> (L.) <i>Arrhenatherum elatius</i> (L.) <i>Plantago lanceolata</i> (L.) <i>Geranium pratense</i> (L.)
4+	Three (grass, herb, legume)	<i>Holcus lanatus</i> (L.) <i>Arrhenatherum elatius</i> (L.) <i>Plantago lanceolata</i> (L.) <i>Lotus corniculatus</i> (L.)

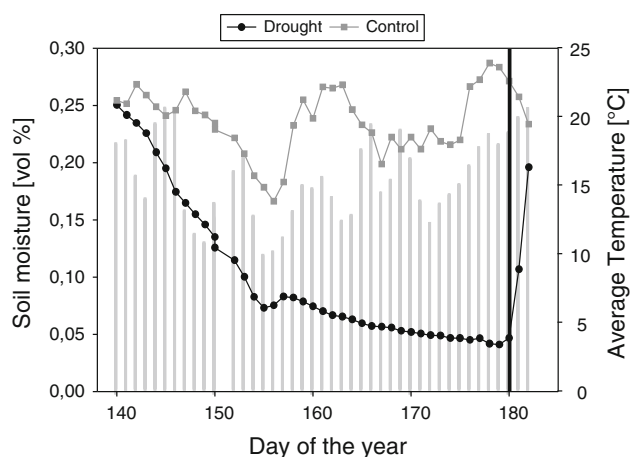


Fig. 1 Course of soil moisture in drought-exposed plots (black circles) and control plots (dark-grey squares), and average daily temperatures, assessed at a height of 1, 20 m (light grey bars). Data are shown from the first day of the drought manipulation (day of the year 140 = May 20th, 2009) until 2 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 was taken here ($n = 5$)

drought period, immediately frozen in liquid nitrogen and lyophilized. 20 mg of leaf material were extracted in 50% methanol. Total soluble carbohydrates were analysed using the anthrone method with glucose as a standard (Kleber et al. 1987). Extinction was measured at 620 nm. Total phenols were analysed using Folin-Ciocalteus reagents and Catechin as a standard and by measuring extinction at 750 nm (Swain and Hillis 1959). Condensed tannins were analysed 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 10 days before the end of the drought period. Thus, only the first 10 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 1 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 analysed 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 analysed 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 analysed 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 1,000 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 with another explanatory variable) of explanatory variables are 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 and Maechler 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 with the second half, but rose quickly again after the drought period was over (Fig. 1).

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).

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 ($P = 0.01$). 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).

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).

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). ($P = 0.007$; $\chi^2 = 12.2$). The drought treatment did not affect larval mortality.

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 the development time until pupation ($P = 0.015$;

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 with control (white bars, C). Asterisks indicate the level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Means ± 1 SE are shown

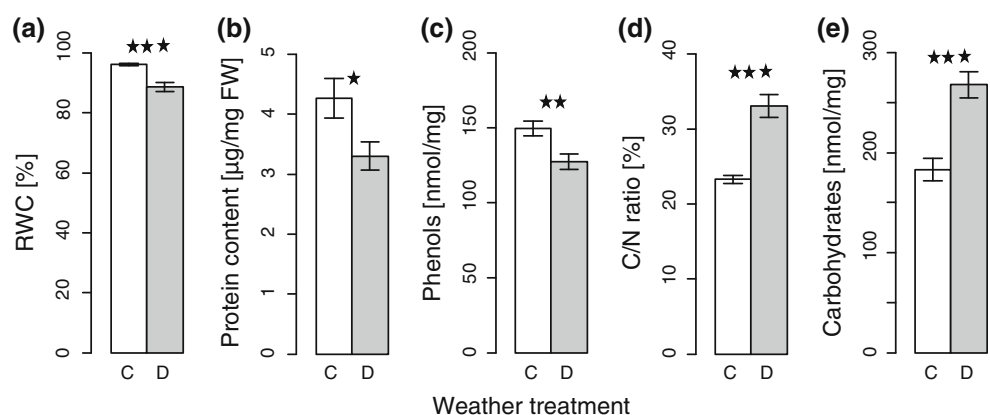


Table 2 ANOVA results of the effects of drought treatment, community composition and, if significant, their interaction on RWC, total soluble protein content, nitrogen concentrations, C/N ratio ($n = 5$, respectively), total soluble carbohydrates, total soluble phenols and condensed tannins ($n = 15$, respectively)

	RWC		Protein		Nitrogen		C/N		Carbohydrates		Phenols		Cond. tannins							
	df	F	df	F	df	MS	F	MS	df	MS	F	MS	F	MS						
Drought	1	0.18	1	28.4***	1	1.02	65.1***	1	1.13	1	76.6***	1	106533	1	5871	11.3**	1	76.4	0.6	
Composition	3	0.01	3	2.26*	3	0.01	0.7	3	0.01	3	0.8	3	3755	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	518		97	124.5		

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: * $P < 0.01$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$

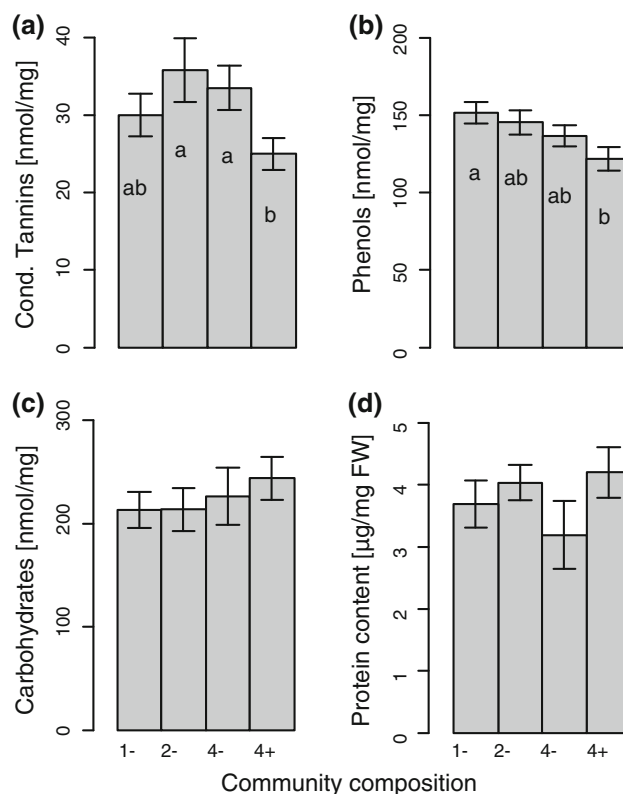


Fig. 3 Effects of plant community composition on **a** the content of condensed tannins ($n = 15$), **b** the total soluble phenol content ($n = 15$), **c** the total soluble carbohydrates ($n = 15$) and **d** the total soluble proteins ($n = 5$) in leaves of *Holcus lanatus* (1-: monoculture, 2-: two 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 ($P < 0.1$)

$F = 3.8$; $df = 3$), as the larvae feeding on *H. lanatus* from the two-species communities reacted with the greatest increase during the larval stage under drought (Table 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 ($P = 0.007$; $\chi^2 = 7.2$) (Fig. 5b), irrespective of community composition.

Hierarchical partitioning showed that protein content had the greatest positive influence on the survival of larvae (Table 5). 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 (Pearson's correlation coefficient: -0.019 ; $P = 0.88$). Pupal survival was positively related to pupal weight ($P = 0.023$; logistic regression).

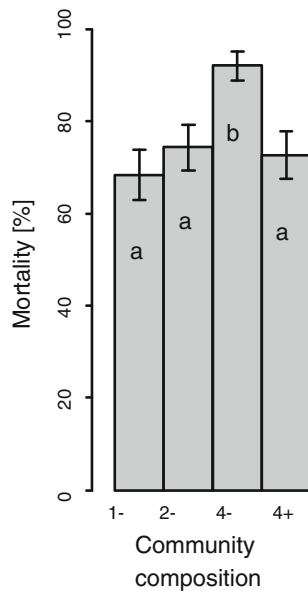


Fig. 4 Effect of the plant community composition, in which *H. lanatus* grows, on the mortality of the 320 *S. littoralis* larvae. Different letters indicate significant differences between the communities ($P < 0.05$) ($n = 20$ /treatment combination)

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, indicates 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

Table 3 Interactive effects of community composition and the drought treatment on the nitrogen concentration in leaves of *H. lanatus* ($n = 5$)

	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

Means ± 1 standard deviation are given. Asterisks behind the community label indicate a significant drought effect in the respective community ($P < 0.05$)

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

	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

Drought significantly increased developmental time with strongest effects in 2– communities ($n = 20$ /treatment combination). Means ± 1 standard deviation are given

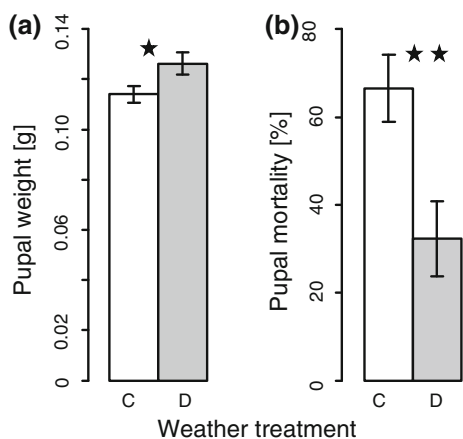


Fig. 5 Differences in **a** the pupal weight and **b** the pupal mortality for *S. littoralis* larvae fed with *H. lanatus* leaves out of the drought (light grey bars, D) and the control (white bars, C) treatments. Asterisks indicate the level of significance: * $P < 0.05$; ** $P < 0.01$ ($n = 20$ treatment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

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 in 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 with four-species communities without legumes supports the idea of an increase in nitrogen

Table 5 Hierarchical partitions of the effects of leaf chemical traits on development duration, pupal weight, larval mortality and pupal mortality of 320 larvae

	RWC	C/N	Nitrogen	Carbon	Protein	Phenols	Tannins	Carbos
<i>Development time</i>								
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
<i>Pupal weight</i>								
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
<i>Larval mortality</i>								
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
<i>Pupal mortality</i>								
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 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 with 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

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 of the same age from the same egg strain grew better when feeding on *Plantago lanceolata* 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 with 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 et al. 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 with other studies (Fischer and Fiedler 2000).

It should be reiterated that the samples for the leaf chemical analysis were taken on the last 2 days of the drought treatment, whereas the larval development lasted from approximately 10 days before to 10 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 (White 1984) 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 (English-Loeb 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 severely 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 (White 1984), does not seem to be widely applicable over all development traits and stages (Fischer and Fiedler 2000). Similar to Fischer and 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 desynchronization 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 it difficult to draw any direct connections between leaf nutrients and accordingly defence compounds and herbivore performance.

Acknowledgments Many thanks to Christian Schemm for his great work in leaf chemical analysis and also to Björn Reineking for advice on statistical problems. This work was kindly supported by the Helmholtz Impulse and Networking Fund through the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE).

References

- Baayen RH (2009) Language R: data sets and functions with “analyzing linguistic data: a practical introduction to statistics” R package version 0.955. <http://CRAN.R-project.org/package=languageR>. Accessed 3 March 2011
- Barrs HD, Weatherley PE (1962) A re-examination of relative turgidity technique for estimating water deficits in leaves. *Austral J Biol Sci* 15(3):413–417
- Bates D, Maechler M (2010) lme4: linear mixed-effects models using S4 classes. R package version 0.999375-33. <http://CRAN.R-project.org/package=lme4>. Accessed 3 March 2011
- Beniston M, Stephenson DB, Christensen OB, Ferro CAT, Frei C, Goyette S, Halsnaes K, Holt T, Jylha K, Koffi B, Palutikof J, Schoell R, Semmler T, Woth K (2007) Future extreme events in European climate: an exploration of regional climate model projections. *Clim Change* 81(Suppl. 1):71–95
- Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecol* 78(4):987–999
- Bradford MM (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. *Analyt Biochem* 72(1–2):248–254
- Broadhurst RB, Jones WT (1978) Analysis of condensed tannins using acidified vanillin. *J Sci Food Agri* 29(9):788–794
- Brown ES, Dewhurst CF (1975) Genus Spodoptera (*Lepidoptera, Noctuidae*) in Africa and near east. *Bull Entomol Res* 65(2):221
- Bruyn L, de Scheirs J, Verhagen R (2002) Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia* 130(4):594–599
- Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecol* 78(7):1958–1965
- Cates RG, Henderson CB, Redak RA (1987) Responses of the western spruce budworm to varying levels of nitrogen and terpenes. *Oecologia* 73:312–316
- Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CP, Osorio ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field. *Photosynth Growth Ann Bot* 89(Sp. Iss. SI):907–916
- Cornelissen T, Fernandes GW, Vasconcellos-Neto J (2008) Size does matter: variation in herbivory between and within plants and the plant vigor hypothesis. *Oikos* 117(8):1121–1130
- Dormann C, Kühn I (2008) *Angewandte statistik für die biologischen wissenschaften*, 2nd edn. <http://www.ufz.de/data/deutschstats/work7649.pdf>
- Dybziński R, Fargione JE, Zak DR, Fornara D, Tilman D (2008) Soil fertility increases with plant species diversity in a long-term biodiversity experiment. *Oecologia* 158(1):85–93
- English-Loeb G, Stout MJ, Duffey SS (1997) Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. *Oikos* 79(3):456–468
- Faraway JJ (2006) Extending the linear model with R—generalized linear, mixed effects and nonparametric regression models. Chapman & Hall/CRC, Boca Raton
- Fischer K, Fiedler K (2000) Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis. *Oecologia* 124(2):235–241
- Goverde M, Erhardt A, Niklaus PA (2002) In situ development of a satyrid butterfly on calcareous grassland exposed to elevated carbon dioxide. *Ecol* 83(5):1399–1411
- Grime JP, Hodgson JG, Hunt R (1988) *Comparative plant ecology. A functional approach to common british species*. Unwin Hyman, London
- Gubsch M, Roscher C, Gleixner G, Habekost M, Lipowsky A, Schmid B, Schulze ED, Steinbeiss S, Buchmann N (2011) Foliar and soil $\delta^{15}\text{N}$ values reveal increased nitrogen partitioning among species in diverse grassland communities. *Plant Cell Environ* 34(6):895–908
- Gumbel EJ (1958) Statistical-theory of extreme values. *Bull Internat Stat Inst* 36(3):12–14
- Heisler-White JL, Blair JM, Kelly EF, Harmoney K, Knapp AK (2009) Contingent productivity responses to more extreme rainfall regimes across a grassland biome. *Glob Change Biol* 15(12):2894–2904
- Herms DA, Mattson WJ (1992) The dilemma of plants- to grow or to defend. *Quart Rev Biol* 67(3):283–335

- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biomet J* 50(3):346–363
- Huberty AF, Denno RF (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecol* 85(5):1383–1398
- Inbar M, Doostdar H, Mayer RT (2001) Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94(2):228–235
- IPCC (2007) Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report for the intergovernmental panel on climate change. Cambridge University Press, Cambridge
- Jentsch A, Kreyling J, Beierkuhnlein C (2007) A new generation of climate-change experiments: events, not trends. *Front Ecol Environ* 5(7):365–374
- Kleber HP, Schlee D, Schöpp W (1987) *Biochemisches praktikum*. Gustav-Fischer, Jena
- Koricheva J, Larsson S, Haukioja E (1998) Insect performance on experimentally stressed woody plants: a meta-analysis. *Ann Rev Entomol* 43:195–216
- Kreyling J, Beierkuhnlein C, Elmer M, Pritsch K, Radovski M, Schloter M, Woellecke J, Jentsch A (2008) Soil biotic processes remain remarkably stable after 100-year extreme weather events in experimental grassland and heath. *Plant Soil* 308(1–2): 175–188
- Larsson S (1989) Stressful times for the plant-stress-insect performance hypothesis. *Oikos* 56(2):277–283
- Li Y, Ye W, Wang M, Yan X (2009) Climate change and drought: a risk assessment of crop-yield impacts. *Clim Res* 39(1):31–46
- Liu Y, Zhang T, Wang J (2008) Photosynthesis and metabolite levels in dehydrating leaves of *Reaumuria soongorica*. *Acta Biol Cracoviensis Ser Bot* 50(1):19–26
- Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* 413(6855):72–76
- Mac Nally R (2002) Multiple regression and inference in ecology and conservation biology: further comments on identifying important predictor variables. *Biodiv Conserv* 11(8):1397–1401
- Morehouse NI, Rutowski RL (2010) Developmental responses to variable diet composition in a butterfly: the role of nitrogen, carbohydrates and genotype. *Oikos* 119(4):636–645
- Myers JH, Post BJ (1981) Plant nitrogen fluctuations of insect populations: a test with the cinnabar moth-tansy ragwort system. *Oecologia* 48:151–156
- Nguyen TT, Michaud D, Cloutier C (2007) Proteomic profiling of aphid *Macrosiphum euphorbiae* responses to host-plant-mediated stress induced by defoliation and water deficit. *J Insect Phys* 53(6):601–611
- Price PW (1991) The plant vigor hypothesis and herbivore attack. *Oikos* 62(2):244–251
- Raubenheimer D, Simpson SJ (1997) Integrative models of nutrient balancing: application to insects and vertebrates. *Nutr Res Rev* 10:151–179
- R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. ISBN 3-900051-07-0. URL <http://www.R-project.org>. Accessed 3 March 2011
- Regier N, Streb S, Coccozza C, Schaub M, Cherubini P, Zeeman SC, Frey B (2009) Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of carbohydrates and oxidative stress defence. *Plant Cell Environ* 32(12):1724–1736
- Schädler M, Jung G, Auge H, Brandl R (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* 103(1):121–132
- Schädler M, Brandl R, Haase J (2007a) Antagonistic interactions between plant competition and insect herbivory. *Ecol* 88(6): 1490–1498
- Schädler M, Roeder M, Brandl R, Matthies D (2007b) Interacting effects of elevated CO₂, nutrient availability and plant species on a generalist herbivore. *Glob Chang Biol* 13:1005–1015
- Scheirs J, de Bruyn L (2005) Plant-mediated effects of drought stress on host preference and performance of a grass miner. *Oikos* 108(2):371–385
- Showler AT, Moran JP (2003) Effects of drought stressed cotton, *Gossypium hirsutum* L., on beet armyworm, *Spodoptera exigua* (Hübner), oviposition, and larval feeding preferences and growth. *J Chem Ecol* 29(9):1997–2011
- Shure DJ, Mooreside PD, Ogle SM (1998) Rainfall effects on plant-herbivore processes in an upland oak forest. *Ecol* 79(2):604–617
- Sinclair TR, Ludlow MM (1985) Who taught plants thermodynamics? The unfulfilled potential of plant water potential. *Aust J Plant Physiol* 33:213–217
- Spehn EM, Scherer-Lorenzen M, Schmid B, Hector A, Caldeira MC, Dimitrakopoulos PG, Finn JA, Jumpponen A, O'Donovan G, Pereira JS, Schulze ED, Troumbis AY, Körner C (2002) The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos* 98(2):205–218
- Swain T, Hillis WE (1959) The phenolic constituents of *Prunus domestica*. *J Sci Food Agric* 10:63–68
- Temperton VM, Mwangi PN, Scherer-Lorenzen M, Schmid B, Buchmann N (2007) Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia* 151(2):190–205
- Trenberth KE, Dai A, Rasmussen RM, Parsons DB (2003) The changing character of precipitation. *Bull Am Met Soc* 84(9): 1205
- Wang H, Fu R, Kumar A, Li W (2010) Intensification of summer rainfall variability in the South Eastern United States during recent decades. *J Hydromet* 11(4):1007–1018
- White TC (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63(1):90–105
- Wurst S, van Beersum S (2009) The impact of soil organism composition and activated carbon on grass-legume competition. *Plant Soil* 314(1–2):1–9