INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–144/2014/16–4–715–722
http://www.fspublishers.org

Full Length Article

Effects of Drought Stress and Sward Botanical Composition on the Nutritive Value of Grassland Herbage

Frank Küchenmeister[1*], Kai Küchenmeister[1], Manfred Kayser[1], Nicole Wrage-Mönnig[2] and Johannes Isselstein[1]
[1]*Institute of Grassland Science, Department of Crop Sciences, Georg-August-University, Göttingen, Germany*
[2]*Faculty of Life Sciences, Rhine-Waal University of Applied Sciences, Kleve, Germany*
*For correspondence: frank.kuechenmeister@agr.uni-goettingen.de

Abstract

The predicted increase of drought incidents even in temperate climates might affect not only yield but the nutritive value of grassland herbage as well. It is not yet clear whether species richness or functional group composition could mitigate a possibly negative reaction of the nutritive value to drought. Here, we report findings of a study investigating the effects of drought stress, species richness (one to five species) and functional group composition (grass, forb and legume) on nutritive value (crude protein, water-soluble carbohydrates, neutral detergent fiber, acid detergent fiber) of herbage under semi-controlled conditions in a vegetation hall. Moderate or strong drought was imposed on plants in one growing season and followed by a recovery period. Drought had no or minor immediate or residual effects on nutritive value, and there was no interaction of species richness or functional group with drought. However, functional group and seasonal variation distinctively influenced the nutritive value of herbage. It was concluded that under conditions of climate change with drought stress events, yield decreases in grassland seem to be by far more important than changes in nutritive value.

Keywords: Crude protein; Water-soluble carbohydrates; NDF; ADF; Functional group composition; Species richness

Introduction

Producing grassland herbage of a good nutritive value is a prerequisite of efficient ruminant livestock production (Gibon, 2005; Hopkins and Wilkins, 2006). Herbage of a high nutritive value is more likely to be taken up in high amounts, is readily digested and facilitates a high performance of ruminants (Hopkins and Wilkins, 2006). The nutritive value of herbage is strongly dependent on factors like grassland management as well as on soil and climatic conditions (Isselstein *et al.*, 2005).

For grassland herbage production an adequate water supply is important. Predicted climate change, with varying precipitation patterns and frequently occurring droughts, may affect herbage production even in temperate climate zones (Alcamo *et al.*, 2007; Hopkins and Del Prado, 2007). It has frequently been shown that drought reduces the yield of crops and forages (Ehlers and Goss, 2002; Jaleel *et al.*, 2009) as well as of temperate grassland (Wrage *et al.*, 2009). However, the effect of drought on the nutritive value of herbage is much less clear. Wang and Frei (2011) reported an increase in crude protein (CP) concentration under drought stress in a wide range of cash and forage crops, e.g. *Arachis hypogea*, *Solanum tuberosum*, *Triticum aestivum* and *Zea mays*. In contrast, Peterson *et al.* (1992) found increased CP concentration for *Lotus corniculatus* and *Trifolium pratense* as well as decreased for *Astragalus cicer*. In opposite, Seguin *et al.* (2002) stated just a minor effect of drought on CP of *Medicago sativa*, *Trifolium ambiguum* and *Trifolium pratense*. Water-soluble carbohydrates (WSC) have been found to increase in graminoids (Bajji *et al.*, 2001; DaCosta and Huang, 2006) and *Glycine max* (Nakayama *et al.*, 2007) under drought conditions, but showed no reaction to drought in forage legumes (Abberton *et al.*, 2002). The reaction of fibre components like neutral detergent fibre (NDF) and acid detergent fibre (ADF) to drought stress is not consistent. Increasing and decreasing concentrations or no reaction of NDF and ADF to drought have been reported for forage legumes, forbs and grasses (Peterson *et al.*, 1992; Seguin *et al*., 2002; Skinner *et al.*, 2004).

Species richness and functional group composition may modify reactions of swards to drought and affect the nutritive value. However, it is not yet clear whether species richness may enhance (Bullock *et al.*, 2007) or decrease nutritive value of grassland herbage (Bruinenberg *et al.*, 2002). Particularly the ratio of grasses, forbs and legumes in swards is known to have a marked effect on the nutritive value (Hopkins and Wilkins, 2006). Previous drought stress incidents might affect plant physiology even during and after a recovery time - more tolerant plants would resume their functioning, while others have undergone severe changes. Mirzaei *et al.* (2008) reported a shift from reproductive to vegetative growth after a period of drought

To cite this paper: Küchenmeister, F., K. Küchenmeister, M. Kayser, N. Wrage-Mönnig, J. Isselstein, 2014. Effects of drought stress and sward botanical composition on the nutritive value of grassland herbage. *Int. J. Agric. Biol.*, 16: 715–722

within a growing season. Van Ruijven and Berendse (2010) and Vogel *et al.* (2012) found inconsistent effects of species richness in a recovery period after drought. So far, it remains unclear whether the nutritive value of a sward during a recovery period after drought would also be modified by species richness.

Here, we report the results of an experiment conducted under semi-controlled conditions in a vegetation hall with two successive drought stress treatments and periods (moderate and strong), each followed by a recovery period. Species richness varied between one, three and five species and we choose three functional groups (grasses, forbs, legumes).

As important parameters for nutritive value of grassland herbages CP, WSC and the fibre components NDF and ADF were analysed. CP is essential for nitrogen supply for ruminants; WSC positively influence fodder intake and are important for efficient utilisation of protein; NDF is an estimation of total cell wall (cellulose, hemicellulose and lignin) and is inversely related to the voluntary fodder intake; ADF includes lignin and cellulose and is an indicator for the digestibility of the cell wall (Hopkins and Wilkins, 2006; Moorby *et al*., 2006). We hypothesize that (i) drought stress of different intensity will have an effect on nutritive value parameters of grassland herbage, during the drought period but also during recovery after drought and that (ii) species richness and functional group will modify the drought response of the nutritive value.

Materials and Methods

Experimental Setup

The experiment was conducted in a vegetation hall at the University of Göttingen, Germany, in mid-July 2009 as a randomized block design with four replicates and two factors (sward and drought stress). Five species were selected for the experiments which are common in a wide range of temperate grassland and they have a high nutritive value and mowing tolerance (Dierschke and Briemle 2002). The species are: *Trifolium repens* L. var. Rivendel (legume), *Dactylis glomerata* L. var. Donata (grass), *Lolium perenne* L. var. Signum (grass), *Plantago lanceolata* L. wild type (forb) and *Taraxacum officinale* F.H. Wigg. specie agg. wild type (forb). Those were either grown in monoculture, in all possible combinations of three-species mixtures, and in one mixture that contained all five species.

Experimental Details

In monocultures, 1000 viable seeds per m^2 for forbs and legume swards and 5000 viable seeds per m^2 for grass swards were sown. For the three and five-species mixtures, sowing density per species was reduced to one third and one fifths of that of the monoculture swards, respectively (replacement design).

A homogeneous mixture of 20 kg sand (air-dried, sieved to pass a mesh of 5 mm; August Oppermann Kiesgewinnung GmbH, Hann. Münden, Germany), 5.5 kg compost (air-dried; Bioenergiezentrum Göttingen GmbH, Göttingen, Germany) and 0.9 kg vermiculite (particle size 8-12 mm; Deutsche Vermiculite GmbH, Sprockhoevel, Germany) was used as growing substrate per container (round plastic container of 33 cm diameter, 42 cm height and a volume of 30 L), and covered with 1.5 kg compost as seed bed. All containers were treated with a rhizobium solution (Radicin, Jost-GmbH Iserlohn, Germany) to enable nodulation of *T. repens* roots. No fertilisation and no extra lighting were provided. The pH of the soil (in $CaCl_2$ suspension) as well as the availability of P, K (extracted with calcium acetate lactate, continuous flow analyser [CFA]) and Mg ($CaCl_2$ extraction, CFA) were measured in summer 2011 (pH, 7.3; 292 mg P kg^{-1}; 430 mg K kg^{-1}; 364 mg kg^{-1} oven-dry soil).

The climatic conditions in the vegetation hall followed a normal seasonal pattern of temperate climates with (mild) frost in winter, lower temperatures in spring and autumn and higher temperatures in summer. The conditions were the same for all species and mixtures. Peak temperatures occurred in June and July with maximal temperatures over 30°C. Temperatures in summer were controlled by ventilation. In winter, a heating system was operating when temperatures fell below 0°C for more than 24 h. Heating was stopped when the temperature reached 5°C. Temperatures were recorded daily at three locations in the vegetation hall.

In an earlier paper (Küchenmeister *et al.*, 2012), the germination of the species used in this experiment, the establishment of the swards, the yields and yield contribution of the functional groups have been studied.

Drought Stress Treatment

In the first full harvest year (2010) swards were subjected to moderate drought stress in spring (mid-April to end of May) and to strong drought stress in summer (early-July to end of August). Water availability was controlled by watering and regular weighing of the containers. Control containers were kept at a water content of 25 Vol. % (-0.03 MPa) and watered once their water content went down to 18Vol. % (-0.3 MPa).

Drought stress was induced by stopping watering of the containers for some time after an initial watering of the containers to a target value of volumetric soil water content of 25 Vol.%. For moderate drought stress, no water was given until three days after the first stress symptoms (wilting of leafs) appeared on the first plant (-1.5 MPa, 10 Vol. %), containers were then watered again (to -0.03 MPa) followed by repetition of the drought phase. To induce strong drought stress, the drought phase was extended to five days after appearance of the first stress symptoms (-1.5 MPa, 10 Vol. %), and was repeated three times with two irrigations in between. Average Vol. % water content of the containers after the end of the moderate drought period was between 11% and 6% and between 10% and 4% after strong drought stress.

Sampling and Measurement

Above ground biomass was harvested two times in 2009 and five times in 2010 (mid-April, end-May, early-July, end-August and mid-October). Shoots were hand-clipped 3-4 cm above the soil surface. Each biomass sample was sorted into species or functional groups (grass species were not separated), dried (60°C for 72 h) and weighed. Chemical analyses were done on bulk samples as biomass of some species was found to be too little for analysis.

Prior to analysis dried samples were ground to 1 mm and analysed by near-infrared reflectance spectroscopy (NIRS). The spectra were analyzed using the large dataset of calibration samples from different kinds of grasslands by VDLUFA Qualitätssicherung NIRS GmbH, Kassel, Germany (Tillmann, 2010). N concentration of the samples was calculated by dividing CP concentration by 6.25. N yield was calculated by multiplying yield and N concentration. We used coefficients of variation (CV) for every sward in control as well as in the drought treatments to assess the variability of nutritive value over the growing season. CV of nutritive value was calculated by dividing standard derivation of the four periods by their mean.

Statistical Analysis

Statistical data analysis was carried out using Genstat 6.1 software package (VSN International, Hemel Hempstead, UK) and STATISTICA 9.1 (StatSoft, Inc., Tulsa, Oklahoma, USA). A two-factorial analysis of variance (ANOVA) was calculated for every period and considered the factors sward and drought stress. Least significant differences (LSD values) were used to compare mean values in case of significant treatment effects ($P < 0.05$). Additionally, we evaluated the relationship between nutritive value parameters and species richness as well as the contribution of functional groups by a linear regression model. The full data set was used for regression calculation, except for CP concentration of forbs: here we excluded mixtures with legume, the strong effect of legume would have obscured the influence of forbs on CP in mixture with grasses.

Results

Influence of Drought Stress on Nutritive Value

The variations in sward composition, from monocultures to three- and five-species mixtures, had a highly significant effect (P<0.001) on all parameters of the nutritive value after both stress and after recovery periods. Moderate or strong drought stress had no significant effect on the nutritive value after stress or after recovery periods, apart from ADF in spring 2010. Independent of stress and sward, contents for CP ranged between 88 g kg^{-1} DM and 273 g kg^{-1} DM (Table 1) and for WSC between 8 g kg^{-1} DM and 227 g kg^{-1} DM (Table 2). The fibre components NDF and ADF ranged between 222 g kg^{-1} DM and 640 g kg^{-1} DM (Table 3) and 175 g kg^{-1} DM and 355 g kg^{-1} DM (Table 4), respectively. There were no significant interactions between sward and drought stress for CP, WSC and ADF, but for NDF. The variability in time for parameters of nutritive value during the growing season, as indicated by the coefficient of variation (CV), was significantly different among swards (P<0.001) and ranged between 0.07 and 0.82. Drought stress, or the interaction of sward and drought stress, showed no significant effect (Table 5).

Influence of Species Richness and Functional Group Composition

Species richness: Nutritive value did not change with species number: values for highest diversity level (five-species mixture, including *T. repens*) did usually not differ from three-species mixtures that contained *T. repens*. However, species and thus functional groups differed significantly in their nutritive value; functional group composition determined the nutritive value of mixed swards. Apart from the influence of sward composition, we found the common seasonal variability in nutritive value. CP concentration (Table 1) and fiber components (Table 3 and 4) increased in summer and WSC (Table 2) was high in spring and autumn.

Functional group composition: CP concentrations were especially high in swards that contained *T. repens* and they varied between 104 g kg^{-1} DM and 273 g kg^{-1} DM (Table 1). Also forb monocultures and mixtures of forbs and grasses produced high CP concentration up to 199 g kg^{-1} DM while grass monocultures had lower CP concentrations between 90 g kg^{-1} DM and 147 g kg^{-1} DM. In contrast to CP, grass monocultures and swards with a larger proportion of grasses had higher WSC values of up to 227 g kg^{-1} DM. Monocultures of dicotyledonous plants and mixed swards with significant contents of dicots were usually low in WSC. For monocultures of dicotyledonous plants WSC concentration varied between 8 g kg^{-1} DM and 128 g kg^{-1} DM (Table 2). Similarly, grass dominated swards were higher in NDF and ADF, while swards with larger contributions of forbs and legumes had lower concentrations of theses fibre components. Grass monocultures showed NDF and ADF concentrations between 481 g kg^{-1} DM and 640 g kg^{-1} DM and 255 g kg^{-1} DM and 355 g kg^{-1} DM (Table 3 and 4).

Table 1: Crude protein concentration (g kg^{-1} DM) of different swards (monocultures and mixtures) with two drought stress treatments each followed by a recovery period from April to October 2010. Means (n=4) with LSD (5%). Results from an ANOVA considering the effects sward and drought stress (Control = not limiting water supply)

Sward[1]	Moderate stress		Recovery period		Strong stress		Recovery period	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Dg	102	101	103	101	96	101	147	138
Lp	92	90	103	111	113	115	112	105
Pl	100	97	97	88	113	111	166	171
To	168	174	164	168	150	161	199	198
Tr	272	264	223	223	240	224	269	273
LpPlDg	93	94	108	100	103	111	112	112
LpToDg	100	104	111	113	109	113	125	122
PlToDg	118	111	121	103	130	117	176	160
LpPlTo	98	93	116	114	119	120	123	119
TrLpDg	110	104	176	193	145	133	166	155
TrLpPl	140	140	213	211	181	157	180	189
TrLpTo	146	161	208	224	165	171	196	196
TrPlDg	152	133	198	188	130	128	191	190
TrToDg	138	160	195	210	166	148	203	221
TrPlTo	206	198	217	210	173	172	226	225
TrPlToDgLp	150	141	208	198	167	144	176	198
LSD value	19.5		20.5		21.7		24.5	
ANOVA Summary	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Sward	94.14	<0.001	96.74	<0.001	39.73	<0.001	52.42	<0.001
Drought stress	0.27	0.607	0.03	0.855	2.91	0.092	0.02	0.899
Sward x Drought stress	1.01	0.448	0.97	0.49	1.08	0.389	0.66	0.816

[1]Dg – *Dactylis glomerata*; Lp – *Lolium perenne*; Pl – *Plantago lanceolata*; To – *Taraxacum officinale*; Tr – *Trifolium repens*

Table 2: Water-soluble carbohydrates concentration (g kg^{-1} DM) of different swards (monocultures and mixtures) with two drought stress treatments each followed by a recovery period from April to October 2010. Means (n=4) with LSD (5%). Results from an ANOVA considering the effects sward and drought stress (Control = not limiting water supply)

Sward[1]	Moderate stress		Recovery period		Strong stress		Recovery period	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Dg	95	105	73	78	79	84	124	116
Lp	218	216	170	147	109	123	214	227
Pl	128	128	88	97	55	62	99	99
To	26	26	18	8	9	15	35	27
Tr	64	74	48	55	71	72	87	88
LpPlDg	195	205	123	152	103	91	198	193
LpToDg	166	180	127	101	90	87	173	174
PlToDg	81	86	46	65	28	42	61	87
LpPlTo	195	206	120	140	77	91	177	195
TrLpDg	191	186	108	80	82	90	146	166
TrLpPl	167	172	68	61	78	86	140	135
TrLpTo	153	142	71	65	64	68	111	122
TrPlDg	103	101	67	63	74	56	99	95
TrToDg	86	80	60	54	53	51	82	75
TrPlTo	56	70	46	54	27	27	75	49
TrPlToDgLp	130	146	61	65	72	75	125	120
LSD value	24.5		28.1		23.7		30.2	
ANOVA Summary	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Sward	87.15	<0.001	28.62	<0.001	19.65	<0.001	48.13	<0.001
Drought stress	2.05	0.156	0.01	0.931	0.91	0.342	0.13	0.715
Sward x Drought stress	0.45	0.96	1.34	0.195	0.55	0.907	0.74	0.735

[1]Dg – *Dactylis glomerata*; Lp – *Lolium perenne*; Pl – *Plantago lanceolata*; To – *Taraxacum officinale*; Tr – *Trifolium repens*

Discussion

The results obtained in the present experiment revealed a considerable variation of data for the different characteristics of the nutritive value, mainly related to the different grassland species and the functional groups. Such range of data has also been found in various other studies, both under field and controlled environment conditions: Buxton (1996), Harris *et al.* (1997) and Seip *et al.* (2011) reported similar values for CP in temperate grasslands,

Table 3: Neutral detergent fibre concentration (g kg^{-1} DM) of different swards (monocultures and mixtures) with two drought stress treatments each followed by a recovery period from April to October 2010. Means (n=4) with LSD (5%). Results from an ANOVA considering the effects sward and drought stress (Control = not limiting water supply)

Sward[1]	Moderate stress		Recovery period		Strong stress		Recovery period	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Dg	611	606	640	635	610	598	524	527
Lp	520	527	551	556	574	590	490	481
Pl	273	257	335	335	327	300	244	222
To	297	282	334	341	310	317	283	302
Tr	340	324	401	394	366	366	334	322
LpPlDg	535	517	577	545	587	574	497	493
LpToDg	529	524	549	573	557	564	473	488
PlToDg	521	569	518	583	499	552	398	462
LpPlTo	505	502	507	514	528	528	453	466
TrLpDg	510	531	475	484	535	547	452	455
TrLpPl	484	468	446	444	476	492	418	408
TrLpTo	485	459	432	421	476	458	391	382
TrPlDg	510	555	467	486	541	577	397	463
TrToDg	536	482	475	439	479	480	411	391
TrPlTo	307	290	375	363	325	314	335	298
TrPlToDgLp	491	487	453	457	483	515	419	407
LSD value	35.8		32.1		39.9		39.6	
ANOVA Summary	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Sward	136.56	<0.001	116.61	<0.001	99.81	<0.001	65.45	<0.001
Drought stress	1.05	0.308	0.25	0.617	1.47	0.229	0.4	0.529
Sward x Drought stress	1.99	0.024	2.04	0.02	1.12	0.354	1.97	0.026

[1]Dg – *Dactylis glomerata*; Lp – *Lolium perenne*; Pl – *Plantago lanceolata*; To – *Taraxacum officinale*; Tr – *Trifolium repens*

Table 4: Acid detergent fibre concentration (g kg^{-1} DM) of different swards (monocultures and mixtures) with two drought stress treatments each followed by a recovery period from April to October 2010. Means (n=4) with LSD (5%). Results from an ANOVA considering the effects sward and drought stress (Control = not limiting water supply)

Sward[1]	Moderate stress		Recovery period		Strong stress		Recovery period	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Dg	350	343	355	354	345	334	274	280
Lp	287	286	302	310	328	331	261	255
Pl	250	246	290	291	287	278	187	175
To	248	241	259	263	273	271	227	235
Tr	257	252	308	302	274	292	247	238
LpPlDg	300	288	325	308	336	334	268	266
LpToDg	302	292	315	329	332	328	264	268
PlToDg	319	339	323	349	324	337	254	264
LpPlTo	289	281	301	296	328	315	259	256
TrLpDg	292	297	297	304	325	328	264	264
TrLpPl	287	278	299	306	311	315	263	259
TrLpTo	288	278	296	291	313	308	261	257
TrPlDg	310	329	305	315	332	339	249	273
TrToDg	324	300	308	295	316	307	269	257
TrPlTo	248	234	293	286	290	276	246	234
TrPlToDgLp	298	289	302	306	311	320	264	256
LSD value	18.5		18.9		19.7		20	
ANOVA Summary	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Sward	42.41	<0.001	19.04	<0.001	19.58	<0.001	19.65	<0.001
Drought stress	4.59	0.035	0.50	0.481	0.08	0.779	0.22	0.639
Sward x Drought stress	1.47	0.134	1.28	0.229	0.92	0.549	0.94	0.521

[1]Dg – *Dactylis glomerata*; Lp – *Lolium perenne*; Pl – *Plantago lanceolata*; To – *Taraxacum officinale*; Tr – *Trifolium repens*

Nakayama *et al.* (2007), DaCosta and Huang (2006) and Abberton *et al.* (2002) showed comparable values for WSC for leguminous plants and temperate grasses; our fiber components were in the range of those described by Buxton (1996), Harris *et al.* (1997) and Seip *et al.* (2011) for temperate grasslands. We therefore assume that our data are relevant also for field conditions.

In the study presented here, a significant effect of the sward was found but no or only an inconsistent effect of drought stress on the nutritive value of herbage harvested immediately after the stress period or after a recovery period. In almost all periods no interaction of sward x drought stress was found. However, yield reduction in the study was on average 12% under moderate stress

Table 5: Coefficient of variation of crude protein (CP), water-soluble carbohydrates (WSC), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in different swards (monocultures and mixtures) with two drought stress treatments each followed by a recovery period from April to October 2010. Means (n=4) with LSD (5%). Results from an ANOVA considering the effects sward and drought stress (Control = not limiting water supply)

	CP		WSC		NDF		ADF	
Sward[1]	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Dg	0.21	0.17	0.27	0.26	0.09	0.08	0.12	0.10
Lp	0.10	0.11	0.31	0.31	0.08	0.09	0.10	0.11
Pl	0.29	0.33	0.38	0.32	0.15	0.18	0.19	0.21
To	0.12	0.10	0.71	0.82	0.10	0.12	0.09	0.09
Tr	0.10	0.11	0.28	0.26	0.09	0.11	0.11	0.12
LpPlDg	0.08	0.09	0.32	0.35	0.08	0.07	0.10	0.10
LpToDg	0.11	0.07	0.31	0.38	0.08	0.07	0.10	0.10
PlToDg	0.20	0.22	0.52	0.36	0.12	0.12	0.11	0.13
LpPlTo	0.11	0.12	0.40	0.36	0.07	0.06	0.10	0.09
TrLpDg	0.19	0.28	0.40	0.45	0.08	0.09	0.09	0.09
TrLpPl	0.19	0.21	0.43	0.46	0.08	0.09	0.08	0.09
TrLpTo	0.17	0.17	0.45	0.40	0.10	0.09	0.08	0.08
TrPlDg	0.21	0.21	0.25	0.33	0.14	0.11	0.12	0.10
TrToDg	0.20	0.21	0.28	0.26	0.11	0.10	0.08	0.08
TrPlTo	0.14	0.12	0.47	0.42	0.11	0.11	0.10	0.11
TrPlToDgLp	0.17	0.20	0.39	0.39	0.08	0.10	0.08	0.10
LSD value	0.060		0.161		0.037		0.031	
ANOVA Summary	F-ratio	P	F-ratio	P	F-ratio	P	F-ratio	P
Sward	17.05	<0.001	9.04	<0.001	6.82	<0.001	13.32	<0.001
Drought stress	1.15	0.286	0.03	0.863	0.04	0.837	0.52	0.471
Sward x Drought stress	1.02	0.442	0.65	0.822	0.80	0.679	0.56	0.895

[1]Dg – *Dactylis glomerata*; Lp – *Lolium perenne*; Pl – *Plantago lanceolata*; To – *Taraxacum officinale*; Tr – *Trifolium repens*

(max. 36%), 22% under strong stress (max. 40%; data not shown). Drought stress had no obvious effect on yields after a recovery period, but there was a tendency for smaller yields in stressed swards even after a longer recovery time. This negative effect of drought stress on biomass production is well known (Farooq *et al.*, 2009).

Drought stress has been found to increase protein concentration in forage plants or to have no consistent effect (Peterson *et al*., 1992; Wang and Frei, 2011). This might be explained by a delayed maturity or a change in the leaf-stem ratio (Peterson *et al.*, 1992; Buxton, 1996). Nakayama *et al.* (2007) reported declining N concentrations under drought due to an impaired N uptake. Seguin *et al.* (2002) reported no influence of drought on the CP concentration. Although we found a reduced N uptake and so a decreased N yield under drought stress, this most likely had no direct effect on CP concentrations as the smaller N uptake can be explained by a reduction in yield.

Although we found no significant effect of drought stress on WSC, there was a small tendency to increased WSC concentrations; however, this tendency might have been obscured by the strong mixture effects. Those effects are due to the varying amount of sward components with either a low or a high WSC concentration when drought is imposed. Also Abberton *et al.* (2002) explained the absence of drought effects on WSC with the strong impact of plant mixtures. On the other hand, significant increases in WSC under drought stress, due to osmotic adjustments of plants, have often been reported in the literature (Bajji *et al.*, 2001; DaCosta and Huang, 2006; Nakayama *et al.*, 2007).

The reaction of NDF and ADF to drought stress was inconsistent in our study with no clear trend. Increased, decreased or unchanged values were found after a stress period. The botanical composition of a sward has greater effect than drought (Skinner *et al.*, 2004). For forage legumes, Seguin *et al*. (2002) observed small effects of drought on NDF but a higher ADF concentration after drought stress. In contrast, Peterson *et al.* (1992) found a reduction in NDF and ADF values of forage legumes. This might be attributed to an increased leaf to stem ratio and a reduced plant maturity at harvest when drought was imposed (Peterson *et al.* (1992). In our experiment there was no visible effect of drought on the plant development so that the variation in the fibre concentration of the mixed sowings is more likely to be related to a variation of the botanical composition.

There was no interaction of drought stress effects and species richness for parameters of nutritive value directly after drought stress or after a period of recovery (Table 1–4). Also species richness, independent of drought, had no obvious effect on nutritive value: we found positive, negative or no reaction to increasing species number. A positive influence of species richness on the nutritive value, e.g. higher CP, might be partially explained by an increased probability of *T. repens* being part of the mixture when the species number increases; the so-called sampling effect (Huston *et al*., 2000). Bullock *et al.* (2007) found increased nutritive values in more species-rich swards as well. This was explained by an improved resource use of stands with an increasing number of species and thus more nitrogen being acquired by the sward. In contrast, White *et al.* (2004) found a decrease in nutritive value with increasing species number and explained this with a dilution effect - more plants with lower nutritive value in the mixture. A lower nutritive value with higher species richness was also reported by Bruinenberg *et al.* (2002), who found a higher variation in plant maturity in species rich swards.

No interaction was found between functional groups and drought stress. However, nutritive value of swards was significantly affected by functional group composition. The nutritive values of the functional groups in our study are in line with values reported in the literature (Ulyatt *et al.*, 1988; Buxton, 1996; Marshall *et al.*, 2004; DaCosta and Huang, 2006; Harrington *et al.*, 2006; Dragomir *et al.*, 2011; Seip *et al.*, 2011; Lukač *et al.*, 2012). Larger proportions of legume and forbs led to increased CP in all harvests (R^2 up to 0.86, $P<0.05$), while the contribution of grass to the mixture was negative correlated to overall CP concentration (R^2 up to 0.63, $P<0.001$). WSC concentrations in our study depended mainly on the yield proportion of the functional group grass (R^2 up to 0.86). The yield proportions of forbs and legume were negatively correlated to WSC concentrations (R^2 up to 0.45, $P<0.05$). NDF and ADF concentrations increased with increasing proportions of grass (R^2 up to 0.96, $P<0.001$). With an increasing contribution of forbs in the mixture, fibre concentrations decreased (R^2 up to 0.65, $P<0.001$). The legume *T. repens* usually had no influence on ADF and NDF, in some cases its presence led to slightly lower fibre concentrations. Sanderson (2010) reported that sward composition could be more important for yield and stability than the species number alone. Our results suggest that functional composition of swards is also more important for nutritive value than species number.

We found no accumulated effect of drought over the growing season. Variability of the nutritive values, measured as CV, was not greater in drought stress exposed swards than in the control. Differences between CV of swards with drought stress and control were not more than 0.16, while CV over the growing season was up to 0.82 (Table 5). This means that seasonal effects on nutritive values were greater than stress caused by drought. Seasonal growth patterns, with a fluctuation in yield of different harvests and changes in CP concentration with varying maturity of grassland plants, are well known (Ulyatt *et al.*, 1988; Suleiman *et al.*, 1999; Skinner *et al.*, 2004; Küchenmeister *et al.*, 2012). Differences in WSC concentrations depending on harvest date were also reported by Conaghan *et al.* (2011). With increasing maturity and under conditions of higher temperatures, as occurred in our experiment in summer, fibre components will increase (Buxton, 1996; Suleiman *et al.*, 1999; Bruinenberg *et al.*, 2002).

In conclusion, drought stress may affect herbage nutritive value from grassland, but the effect was shown to be quite small or inconsistent in our study. It seems that under conditions of predicted climate change, temperate grassland will be more affected by a decrease in yield than by changes in the nutritive value. Furthermore, the common seasonal variation of the nutritive value is considerably higher than influence of drought. The response of swards to drought in our study was not modified by species richness and functional group composition. However, functional group composition, i.e. the percentage of functional groups in the sward, had a strong direct effect on CP, WSC, NDF and ADF. Grass increased WSC and fibre components while it decreased CP. In contrast, legume and forbs increased CP and more or less decreased fibre components. According to our results, it is concluded that for managed temperate grasslands, a balanced sward composition and the time of harvest are largely determining the nutritive value of biomass; this holds true also under conditions of predicted future climate change.

Acknowledgements

The authors thank the Ministry for Science and Culture of Lower Saxony, Hannover, Germany for the financial funding. Furthermore we thank Dr. Peter Tillmann for support with NIRS.

References

Abberton, M.T., A.H. Marshall, T.P.T. Michaelson-Yeates, T.A. Williams and I. Rhodes, 2002. Quality characteristics of backcross hybrids between *Trifolium repens* and *Trifolium ambiguum. Euphytica*, 127: 75–80

Alcamo, J., J.M. Moreno, B. Nováky, M. Bindi, R. Corobov, R.J.N. Devoy, C. Giannakopoulos, E. Martin, J.E. Olesen and A. Shvidenko, 2007. Europe. *In: Climate Change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the Intergovernmental Panel on Climate Change*. pp: 541–580. M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, C.E. Hanson (eds.). Cambridge University Press, UK

Bajji, M., S. Lutts and J.M. Kinet, 2001. Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. *Plant Sci.*, 160: 669–681

Bruinenberg, M.H., H. Valk, H. Korevaar and P.C. Struik, 2002. Factors affecting digestibility of temperate forages from semi natural grasslands: a review. *Grass Forage Sci.*, 57: 292–301

Bullock, J.M., R.F. Pywell and K.J. Walker, 2007. Long-term enhancement of agricultural production by restoration of biodiversity. *J. Appl. Biol.*, 44: 6–12

Buxton, D.R., 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Anim. Feed Sci. Tech.*, 59: 37–49

Conaghan, P., P. O'Kiely and F.P. O'Mara, 2011. Possibilities of increasing the residual water-soluble carbohydrate concentration and aerobic stability of low dry-matter perennial ryegrass silage through additive and cultivar use. *Grass Forage Sci.*, 67: 177–189

DaCosta, M. and B. Huang, 2006. Osmotic adjustment associated with variation in bentgrass tolerance to drought stress. *J. Amer. Soc. Hort. Sci.*, 131: 338–344

Dierschke, H. and G. Briemle, 2002. *Kulturgrasland – Wiesen, Weiden und verwandte Staudenfluren*, 1st edition, Ulmer, Stuttgart, Germany

Dragomir, N., M. Sauer, C. Cristea, C. Dragomir, D. Rechiţean, I. Sauer, S. Toth and D. Văcariu, 2011. Forage quality determined by botanic species' contribution on permanent pastures. *Sci. Papers Anim. Sci. Biotechnol*., 44: 205–207

Ehlers, W. and M. Goss, 2003. *Water dynamics in plant production*, 1st edition, CAB International, Wallingford, UK

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev.*, 29: 185–212

Gibon, A., 2005. Managing grassland for production, the environment and the landscape. Challenges at the farm and the landscape level. *Livest. Prod. Sci.*, 96: 11–31

Harrington, K.C., A. Thatcher and P.D. Kemp, 2006. Mineral composition and nutritive values of some common pasture weeds. *New Zealand Plant Protect*., 59: 261–265

Harris, S.L., D.A. Clark, M.J. Auldist, C.D. Waugh and P.G. Laboyrie, 1997. Optimum white clover content for dairy pastures. *Proc. New Zealand Grassl. Assoc.*, 59: 29–33

Hopkins, A. and A.D. Prado, 2007. Implications of climate change for grassland in Europe: impacts, adaptations and mitigation options: a review. *Grass Forage Sci.*, 62: 118–126

Hopkins, A. and J. Wilkins, 2006. Temperate grassland: key developments in the last century and future perspectives. *J. Agric. Sci. Cambridge*, 144: 503–523

Huston, M.A., L.W. Aarssen, M.P. Austin, B.S. Cade, J.D. Fridley, E. Garnier, J.P. Grime, J. Hodgson, W.K. Lauenroth, K. Thompson, J.H. Vandermeer and D.A. Wardle, 2000. No consistent effect of plant diversity on productivity. *Science*, 289: 1255a

Isselstein, J., B. Jeangros and V. Pavlu, 2005. Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe: a review. *Agron. Res.*, 3: 139–151

Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam, 2009. Drought Stress in Plants: A review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, 11: 100–105

Küchenmeister, F., K. Küchenmeister, N. Wrage, M. Kayser and J. Isselstein, 2012. Yield and yield stability in mixtures of productive grassland species – does species number or functional group composition matter? *Grassl. Sci*., 58: 94–100

Lukač, B., B. Kramberger, V. Meglič and J. Verbič, 2012. Importance of non-leguminous forbs in animal nutrition and their ensiling properties: a review. *Žemdirbystė Agric.*, 99: 3–8

Marshall, A.H., T.A. Williams, M.T. Abberton, T.P.T. Michaelson-Yeates, P. Olyott and H.G. Powel, 2004. Forage quality of white clover (*Trifolium repens* L.) x Caucasian clover (*T. ambiguum* M. Bieb.) hybrids and their grass companion when grown over three harvest years. *Grass Forage Sci*., 59: 91–99

Mirzaei, H., J. Kreyling, M.Z. Hussain, Y. Li, J. Tenhunen, C. Beierkuhnlein and A. Jentsch, 2008. A single drought event of 100-year recurrence enhances subsequent carbon uptake and changes carbon allocation in experimental grassland communities. *J. Plant Nutr. Soil Sci.*, 171: 681–689

Moorby, J.M., R.T. Evans, N.D Scollan, J.C. MacRae and M.K. Theodorou, 2006. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.). Evaluation in dairy cows in early lactation. *Grass Forage Sci.*, 61: 52–59.

Nakayama, N., H. Saneoka, R.E.A. Moghaieb, G.S. Premachandra and K. Fujita, 2007. Response of growth, photosynthetic gas exchange, translocation of ^{13}C-labelled photosynthate and N accumulation in two soybean (*Glycine max* L. Merrill) cultivars to drought stress. *Int. J. Agric. Biol.*, 9: 669–674

Peterson, P.R., C.C. Sheaffer and M.H. Hall, 1992. Drought effects on perennial forage legume yield and quality. *Agron. J.*, 84: 774–779

Sanderson M.A., 2010. Stability of production and plant species diversity in managed grasslands: A retrospective study. *Basic Appl. Ecol.*, 11: 216–224

Seguin, P., A.F. Mustafa and C.C. Sheaffer, 2002. Effects of soil moisture deficit on forage quality, digestibility, and protein fractionation of Kura clover. *J. Agron. Crop Sci*., 188: 260–266

Seip, K., G. Breves, J. Isselstein and H. Abel, 2011. Nitrogen excretion of adult sheep fed silages made of a mixed sward or of pure unfertilised grass alone and in combination with barley. *Arch. Anim. Nutr.*, 56: 278–289

Skinner, R.H., D.L. Gustine and M.A. Sanderson, 2004. Growth, water relations, and nutritive value of pasture species mixtures under moisture stress. *Crop Sci.*, 44: 1361–1369

Suleiman, A., E.K. Okine, L.A. Goonewardene, P.A. Day, B. Yarmico and G. Recinos-Diaz, 1999. Yield and feeding of prairie grasses in east-central Alberta. *J. Range Manage.*, 52: 75–82

Tillmann P., 2010. Anwendung der Nahinfrarotspektroskopie (NIRS) an Grünlandproben. *VDLUFA-Schriftenreihe*, 66: 145–150

Ulyatt, M.J., D.J. Thomson, D.E. Beever, R.T. Evans and M.J. Haines, 1988. The digestion of perennial ryegrass *(Lolium perenne* cv. Melle) and white clover *(Trifolium repens* cv. Blanca) by grazing cattle. *Brit. J. Nutr.*, 60: 137–149

Van Ruijven, J. and F. Berendse, 2010. Diversity enhances community recovery, but not resistance, after drought. *J. Ecol.*, 98: 81–86

Vogel, A., M. Scherer-Lorenzen and A. Weigelt, 2012. Grassland resistance and resilience after drought depends on management intensity and species richness. *PLoS ONE*, 7: e36992

Wang, Y. and M. Frei, 2011. Stressed food – The impact of abiotic environmental stresses on crop quality. *Agr. Ecosyst. Environ.*, 141: 271–286

White, T.A., D.J. Barker and K.J. Moorea, 2004. Vegetation diversity, growth, quality and decomposition in managed grasslands. *Agric. Ecosyst. Environ.*, 11: 73–84

Wrage, N., L. Gauckler, E. Steep, F. Küchenmeister, K. Küchenmeister and J. Isselstein, 2009. Influence of drought stress and fertilisation on carbon isotopes as indicators of water use of grassland differing in diversity. *Grassl. Sci. Eur.*, 15: 860–862

(Received 30 January 2013; Accepted 12 August 2013)