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Influence of drought stress on nutritive value of perennial forage legumes

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Abstract

In the next decades, forage legumes are likely to become more important. However, predicted climate change may increase the risk of droughts and thus influence their agricultural performance. Decreases in yield due to water shortage are well documented, while influences on nutritive values are inconsistent. Therefore, we examined the effects of drought on crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and water-soluble carbohydrates (WSC) of six legumes, birdsfoot trefoil (*Lotus corniculatus* L.); marsh birdsfoot trefoil (*Lotus uliginosus* Schkuhr); black medic (*Medicago lupulina* L.); yellow alfalfa (*Medicago falcata* L.); sainfoin (*Onobrychis viciifolia* Scop.) and white clover (*Trifolium repens* L.) in monoculture and in mixture with perennial ryegrass (*Lolium perenne* L.) in a container experiment in a vegetation hall. Moderate and strong drought stress was applied during three periods in two years. Mean volumetric soil water content at the end of the moderate drought stress period was 11 vol. % and 6 vol. % under strong stress. The effect of drought on nutritive values was considerably less pronounced than on yield. While the impact of moderate stress on nutritive quality was negligible, we found decreases in CP, NDF and ADF and increases in WSC under strong stress. This may indicate that water scarcity could even increase fodder quality and digestibility. However, the choice of legume species and stand (monoculture or mixture) had stronger effects on nutritive values than drought. We conclude that the reaction of temporary drought on nutritive values seems to be less important for the selection of suitable forage legumes species than other agronomic properties under conditions of climate change.

Keywords: Crude protein; NDF; ADF; Water-soluble carbohydrates.

Introduction

Grassland with its potentially high productivity and good fodder quality forms the basis for ruminant nutrition. Legumes are important for grassland productivity and fodder quality, especially under conditions of limited input of nitrogen (N) from mineral fertilisers and/or manures, due to their ability to fix atmospheric N. Increasing prices for energy and N-fertiliser along with higher costs for concentrates, which are expected for the future, will further increase the importance of forage legumes (Watson et al., 2002; Jensen and Hauggaard-Nielsen, 2003; Crews and Peoples, 2005; German Agricultural Research Alliance, 2012).

Forage production from grassland is dependent on adequate water supply (Hopkins and Del Prado, 2007). Under conditions of climate change, water is likely to become more limited in semiarid and in temperate climates as the probability of summer droughts increases (Alcamo et al., 2007; Schindler et al., 2007; Trenberth, 2011). Insufficient water supply can have strong effects on production of forage legumes. A decrease in yield, depending on the strength and duration of drought stress, is common (Foulds, 1978; Farooq et al., 2009; Jalleel et al., 2009). However, knowledge about the influence of drought on important characteristics of the nutritive value of legumes is inconsistent and limited. Under conditions of drought stress, Peterson et al. (1992) found reduced acid detergent fibre (ADF) and neutral detergent fibre (NDF) concentrations in a range of forage legumes, but inconsistent changes in crude protein (CP) concentrations. In contrast, Seguin et al. (2002) described an increased ADF concentration and a minor effect on CP and NDF concentration in cura clover (*Trifolium ambiguum* M.B.), red clover (*T. pratense* L.) and alfalfa (*Medicago sativa* L.). Nakayama et al. (2007) found an increased concentration of water-soluble carbohydrates (WSC) under water shortage in two cultivars of soybean (*Glycine max* L. Merrill). For clover species, Abberton et al. (2002) observed only a small effect of drought on WSC. More research is needed to gain knowledge about the influence of drought stress on the nutritive value of forage legumes.

In this study, we used six perennial forage legumes in monoculture and in mixture with perennial ryegrass (*Lolium perenne*) and examined the effects of drought stress and the interaction of legume species and drought on important parameters of nutritive value, like CP, WSC and the fibre components NDF and ADF. Due to N fixation, legumes are high in CP which is essential for ruminant nutrition. Water-soluble carbohydrates have a positive influence on fodder intake and are important for an efficient utilisation of dietary N. The NDF concentration gives an estimation of the structural part of the plant material (cellulose, hemicellulose and lignin) and is inversely related to the voluntary fodder intake. Acid detergent fibre includes lignin and cellulose and is negative correlated with cell wall digestibility (Sarwar et al., 1999; Hopkins and Wilkins, 2006; Moorby et al., 2006). It is well known that forage legumes differ in drought stress sensitivity (Dierschke and Briemle, 2002). White clover (*Trifolium repens*) is one of the most important legumes in agricultural production, but is also relatively drought-sensitive (Marshall et al., 2001). We selected five promising and better drought-adapted legumes as possible alternatives to white clover (*T. repens*) for future forage production, namely birdsfoot trefoil (*Lotus corniculatus*), marsh birdsfoot trefoil (*L. uliginosus*), black medic (*Medicago lupulina*), yellow alfalfa (*M. falcata*) and sainfoin (*Onobrychis viciifolia*). A possibly better adaptation to drought of these legumes is supposed to stabilize yields and also the nutritive value under conditions of stress. Forage legumes are commonly grown in mixtures with grasses (Hopkins and Wilkins, 2006; Hopkins and Del Prado, 2007). All six legume species were therefore cultivated in monoculture and in mixture with perennial ryegrass (*Lolium perenne*) to examine if legume-typical reactions to drought were also apparent in the mixtures. In the present experiment, we investigated the following hypotheses: (1) the six legumes differ in nutritive value; (2) drought stress can change nutritive value; and (3) legume species differ in their reaction to drought stress, in monoculture as well as in mixtures with grass.

Material and Methods

The study was conducted in 2009 (sowing date: 15th July) in a vegetation hall of the University of Göttingen, Germany, as a three-factorial experiment in a randomized complete blocks design with four replications. The factors were (1) legume species, (2) stand (legumes in monoculture or in mixture with *L. perenne*) and (3) drought stress (regular watering or water shortage). The legumes were birdsfoot trefoil (*Lotus corniculatus* L., var. Bull), marsh birdsfoot trefoil (*Lotus uliginosus* Schkuhr, wild seeds), black medic (*Medicago lupulina* L., var. Ekola), yellow alfalfa (*Medicago falcata* L., wild seeds), sainfoin (*Onobrychis viciifolia* Scop., var. Matra) and white clover

(*Trifolium repens* L., var. Rivendel); perennial ryegrass (*Lolium perenne* L., var. Signum) was used as a companion grass in mixtures. Drought stress was imposed during three periods with varying severity, i.e. a moderate stress in spring 2010 and a strong stress in summer 2010 and spring 2011.

Experimental setup

The growing substrate was a homogeneous mixture composed of 20 kg sand (air-dried, sieved to pass a mesh of 5 mm; August Oppermann Kiesgewinnung GmbH, Hann. Münden, Germany), 0.9 kg vermiculite (particle size 8-12 mm; Deutsche Vermiculite GmbH, Sprockhoevel, Germany) and 5.5 kg compost (air-dried; Bioenergiezentrum Göttingen GmbH, Göttingen, Germany) per container (round plastic containers, diameter 33 cm, height 42 cm, volume 30 l), covered with 1.5 kg compost as seed bed. 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) measured in summer 2011 were 7.3; 292 mg P kg^{-1}; 430 mg K kg^{-1}; 364 mg Mg kg^{-1} (oven-dry soil), respectively.

The six legumes and perennial ryegrass were sown in monoculture with 1000 germinating seeds per m^2 for legumes and 5000 for grass. For the mixtures of each legume with perennial ryegrass, we used 500 germinating seeds per m^2 for legumes and 2500 for grass. This considerably high sowing density was used in order to establish a dense sward immediately after seedling emergence.

We chose a vegetation hall as the conditions there followed a normal seasonal pattern with mild frost in winter and higher temperatures in summer, while drought stress could be fully controlled and recorded. Temperatures were recorded daily at three locations in the vegetation hall. The average temperatures ranged from 8 ^oC (min.) to 27 ^oC (max.) in spring regrowth 2010, from 15 ^oC to 35 ^oC in summer regrowth 2010 and from 9 ^oC to 31 ^oC in spring 2011. Climatic conditions were controlled by ventilation in summer and by a heating system in winter that was switched on when temperatures were below 0 ^oC for longer than 24 hour. Heating in winter was limited to a maximum of 5 ^oC air temperature in the vegetation hall. No extra lighting was provided and no fertilisation applied. In order to ensure nodulation of the legumes, all containers were treated with a rhizobium solution (Radicin, Jost GmbH, Iserlohn, Germany). The Radicin solution contained all rhizobia strains in same proportions for an effective infection of all legumes. Marsh birdsfoot trefoil did not survive the first winter and was therefore re-sown in March 2010. The aboveground biomass was harvested two times in 2009 (2^{th} September and 25^{th} October), five times in 2010 (12^{th} April, 25^{th} May, 5^{th} July, 24^{th} August and 18^{th} October) as well as two times in 2011 (11^{th} April and 30^{th} May).

Drought stress treatment

A moderate stress was induced in spring 2010 (April/May) followed by two periods with strong drought stress in summer 2010 (July/August) and spring 2011 (April/May). In spring, drought stress periods were carried out after the first harvest of the year. After each drought stress period, plants were allowed to recover with regular irrigation and harvests of all containers at the end of the recovery periods. Drought stress was induced by temporarily ceasing the watering of the containers after an initial watering up to a volumetric soil water content of 25 vol. % (-0.03 MPa). For the moderate drought stress, no water was given until three days after the first plants showed signs of drought (e.g. wilt of leaves; ~10 vol. %, -1.5 MPa). Containers were then watered again (~25 vol. %) followed by a second cycle of drying up. In order to induce strong drought, the stress phase was extended to five days after first stress symptoms had appeared and was carried out three times with two waterings in between. The average water content of the containers (except marsh birdsfoot trefoil monoculture) ranged from 15 vol. % to 6 vol. % at the end of the moderate drought stress and from 10 vol. % to 4 vol. % under strong drought stress. All containers were weighed at intervals of one to three days during the stress periods. The control containers (no drought stress) were watered to ~25 vol. % if their water content was below ~18 vol. % (-0.3 MPa).

Sampling and measurements

Aboveground biomass was determined by cutting the plants at a height of 3-4 cm above the soil surface. The cut herbage was separated into species immediately after harvest. Dry weight was determined after drying of the herbage samples at 60 ^{o}C for 72 hours in a drying oven (ULM 800, Memmert GmbH und Co KG, Schwabach, Germany).

For analysis of CP, NDF, ADF and WSC, dried samples were ground to 1 mm and analysed by near-infrared reflectance spectroscopy (NIRS). The spectra were analysed using the large dataset of calibration samples from different kinds of grasslands by the Institute VDLUFA Qualitätssicherung NIRS GmbH, Kassel, Germany (Tillmann, 2010). Mixtures were separated into grasses and legumes for yield assessment, but the nutritive value was only analysed on the bulk sample.

Statistical analysis of data

Statistical data analysis was carried out using the Genstat 6.1 (VSN International, Hemel Hempstead, UK) software package. We did a three-factorial analysis of variance (ANOVA) for CP, NDF, ADF and WSC concentrations of all species in monoculture and in mixture with perennial ryegrass of the harvest following each stress period (Payne, 2002). The three factors were legume species (L), stand (S) and drought stress (DS). In case of significant treatment effects (α <0.05), least significant differences (LSD values) were used to compare mean values. Relationships between selected variables were examined with a linear regression model.

Results

The effect of the main factors legume species (L) and stand (S) as well as that of the interaction L×S on all parameters of nutritive value was in most cases significant ($P<0.05$) in all three drought periods. Drought stress (DS) led to significant effects in spring 2011. Interactions between L×DS and S×DS, as well as the three-way interaction, were not significant, with the exception of some cases after strong drought stress; the pattern, however, was inconsistent. Generally, the effects of drought stress on the nutritive value were considerably smaller than on yield. It was only during the last strong drought stress period in spring 2011 that effects became apparent and statistically significant.

Crude protein concentration

Crude protein concentrations in legume monocultures and mixtures were hardly affected by drought stress, but there was a tendency for reduced concentrations in monoculture under strong stress (Table 2). Among the legume species, particularly yellow alfalfa, but also white clover, black medic and birdsfoot trefoil had high CP concentrations in monocultures in all stress periods. For these legumes, CP values ranged from 225 g kg^{-1} DM for birdsfoot trefoil to 274 g kg^{-1} DM for yellow alfalfa with no drought stress, and from 212 g kg^{-1} DM for birdsfoot trefoil to 278 g kg^{-1} DM for yellow alfalfa under water shortage. In contrast, CP values for sainfoin and marsh birdsfoot trefoil in monoculture were usually rather small. Generally, the grass-legume mixtures had a smaller CP concentration than corresponding monocultures. Mixtures with white clover had highest CP concentrations, followed in most cases by the mixture containing black medic. When the drought stress treatment started in spring 2010, marsh birdsfoot trefoil and sainfoin had already been outcompeted by perennial ryegrass in the mixed sowings and did not produce any biomass.

Neutral detergent fibre concentration

Neutral detergent fibre concentrations varied with the severity of drought stress. Moderate drought stress had no or a relatively small effect on NDF, while strong stress, particularly in spring 2011, decreased NDF concentrations (Table 3). When legumes were grown in monoculture, highest NDF values were found in yellow alfalfa in both control and stress treatment (439 g kg^{-1} DM; 425 g kg^{-1} DM), while for white clover NDF concentrations were always the lowest in both treatments (315 g kg^{-1} DM; 293 g kg^{-1} DM). Mixtures had considerably higher NDF concentrations than monocultures in both, control and stress treatments. Concentrations of NDF in perennial ryegrass were comparatively high. Grass-legume mixtures with yellow alfalfa, especially, had high NDF concentrations of up to 599 g kg^{-1} DM, while white clover mixtures always showed lowest concentrations ranging from 405 to 554 g kg^{-1} DM.

Acid detergent fibre concentration

The ADF concentrations differed relatively little between control and drought treatments in monocultures and in mixtures, but decreased under strong stress, especially in spring 2011 (Table 4). Values for ADF concentrations in legume monocultures ranged from 242 g kg^{-1} DM for marsh birdsfoot trefoil to 328 g kg^{-1} DM for sainfoin with sufficient water supply (control) and from 236 g kg^{-1} DM for marsh birdsfoot trefoil to 304 g kg^{-1} DM for yellow alfalfa under drought stress. Grass-legume mixtures usually showed slightly higher ADF concentrations than the corresponding monoculture in both, control and stress treatments.

Water-soluble carbohydrates concentration

The influence of drought stress on WSC concentrations was generally small, but there was a trend to higher concentrations under strong drought (Table 5). Sainfoin and marsh birdsfoot trefoil monocultures had high concentrations of WSC of up to 129 g kg^{-1} DM, while the WSC concentrations of yellow alfalfa and birdsfoot trefoil were comparatively low, ranging from 15 g kg^{-1} DM to 59 g kg^{-1} DM. WSC concentrations were in most cases higher in mixtures than in the corresponding legume monoculture.

Discussion

Temporary drought influenced biomass yield depending on strength and duration of stress. Moderate and strong drought stress reduced yields up to 36% and 57%, respectively (Table 1). Moderate stress had no effect on nutritive value, while strong stress had a significant effect (spring, 2011). Our hypothesis (2) that drought stress can change the nutritive value of legumes could thus be rejected for moderate stress while for strong stress it could not. The six forage legumes in our study differed in their nutritive value under conditions of sufficient water supply, which confirmed our first hypothesis. Also Peterson et al. (1992) and Fulkerson et al. (2007) found differences in nutritive value among legume species.

Interactions between legume species (L) and drought stress (DS) as well as between stand (S) (monoculture or mixture) and DS were not significant under moderate stress. There were significant interactions L×DS and S×DS under strong drought stress, but they were usually weak and inconsistent among the different parameters. Therefore, our hypothesis (3) that legume species react differently to drought needs to be rejected for moderate stress, and can only partially be confirmed for strong stress.

Irrespective of the water supply treatment, the legumes showed a nutritive value comparable to values found in the literature (Peterson et al., 1992; Fulkerson et al., 2007); they would be ranked as having a moderate to high quality (Buxton, 1996; Schwarz, 2008).

Table 1. Yield reduction (%) of legume species (plus *L. perenne*) in monoculture (Mono) and in mixture (Mix) with *L. perenne* under different levels of drought stress in spring 2010 (moderate stress), summer 2010 (strong stress) and spring 2011 (strong stress); means (n=4).

Forage plant species	Yield reduction [%]					
	Spring 2010 moderate		Summer 2010 strong		Spring 2011 strong	
	Mono	Mix	Mono	Mix	Mono	Mix
Birdsfoot trefoil[1]	1	15	40	23	47	30
Marsh birdsfoot trefoil[2]	27	6[#]	29	17[#]	57	18[#]
Black medic[3]	13	4	5	19	52	18
Yellow alfalfa[4]	5	-2	28	21	42	19
Sainfoin[5]	1	18[#]	29	18[#]	49	33[#]
White clover[6]	36	18	36	44	56	53
Perennial ryegrass[7]	6		10		18	

Scientific names: [1] *Lotus corniculatus*; [2] *L. uliginosus*; [3] *Medicago lupulina*; [4] *M. falcata*; [5] *Onobrychis viciifolia*; [6] *Trifolium repens*; [7] *Lolium perenne*.
[#] The legume partner did not produce any biomass in these periods.

Crude Protein concentration

CP concentrations differed among the legume species. Strong drought stress showed a tendency to decrease CP concentrations in monoculture. The CP concentration of legumes is generally depending on the amount of available N. For legumes, especially under N limited conditions as was the case in our experiment, N fixation is very important for N nutrition (Zahran, 1999; Watson et al., 2002) and it differs among legume species. We used the difference method (Gierus et al., 2012) to investigate the N fixation performance (g N per container). According to the results, legumes could be divided in two groups. The group containing yellow alfalfa, white clover, black medic and birdsfoot trefoil had 10 to 30% higher Ndfa (mean N derived from atmosphere in %) than marsh birdsfoot trefoil and sainfoin, also under drought stress. This resulted in an at least 40% higher N fixation performance (g N per container) in the high fixing group and led to differences in CP concentration among the legumes. Marked differences in CP concentration among a variety of temperate clover species were also found by Ates (2011).

Nitrogen fixation determines the availability of N, but the N concentration in the plant is also depending on the amount of biomass production. A specific CP concentration is then the result of N uptake and the development of biomass production in time which is greatly determined by water availability. Nakayama et al. (2007) found an impaired N uptake in soybean under drought and Pimratch et al. (2013) measured a decreased N fixation under drought stress in peanuts (*Arachis hypogaea* L.). In our study, strong drought stress resulted in a reduction of N fixation performance that was on average 15% larger than the decrease in yield. This explains why the CP concentration in the stress treatments was smaller than the corresponding concentrations in the control treatment.

As CP concentrations were considerably higher in legumes than in perennial ryegrass, CP concentrations of mixtures strongly depended on the yield contribution of the legume component of mixture (R^2 up to 0.95; $P<0.001$). When the legume partner was no longer present, as was the case with marsh birdsfoot trefoil and sainfoin, mixtures produced similar CP concentrations as perennial ryegrass in monoculture (Table 2). It seemed that under strong drought stress, the competitive ability of the legume against the grass partner decreased. This effect was enhanced by the fact that perennial ryegrass was much less affected by drought. Particularly white clover suffered from drought stress (decrease in yield contribution in mixture of up to 73%) and lost strongly in competitive ability against perennial ryegrass. Therefore, total mixture yield decreased and CP concentration in the mixture was reduced as well.

Neutral detergent fibre and acid detergent fibre concentrations

We observed a tendency for lower NDF and, to a lesser extent, ADF concentrations under strong drought stress. Legume species generally differed in fibre concentration.

Fibre concentration is generally influenced by many interacting factors among which are the stage of plant development, leaf-stem ratio, environmental conditions (drought, temperature, photoperiod etc.) or availability of nutrients (Peterson et al., 1992; Buxton, 1996; Fulkerson et al., 2007).

The reduction of NDF and ADF concentration under strong stress supports the findings of Peterson et al. (1992) and Buxton (1996) that a delayed maturity under drought is associated with lower NDF and ADF concentrations.

Drought effects on NDF (including cellulose, hemicellulose and lignin) were stronger than for ADF (including cellulose and lignin). This might be explained by the fact that the hemicellulose concentration, as a part of NDF, is more affected by drought than cellulose and lignin. However, results on the effects of drought on hemicellulose concentrations are inconsistent in the literature: some authors have reported decreased hemicellulose concentrations under drought (Jiang et al., 2012), while other reported increases (Al-Hakimi, 2006).

The cell walls of monocots and dicots differ in their composition. The lignification of cell walls in dicots is stronger, but the concentration of hemicellulose is smaller (Buxton and Mertens, 1995; Ebringerová et al., 2005) resulting in higher NDF of grasses and grass-legume mixtures than that of legumes (Buxton, 1996; Table 4). Additionally, the ADF concentration in most legumes is approximately 100 g kg^{-1} lower than that of NDF, while this difference is usually about 200 g kg^{-1} for most grasses (Buxton, 1996). Similar results were found in our experiment (Tables 3 and 4). A lower fibre concentration may lead to a higher herbage intake and to an increase in digestibility of forage (Buxton, 1996).

Water-soluble carbohydrates concentration

Under strong drought stress in summer 2010 and spring 2011 WSC concentrations mostly increased. Positive effects of drought stress on WSC have been reported elsewhere (Da Costa and Huang, 2006; Nakayama et al., 2007). An increase in the WSC concentration in plants will change the osmotic potential, which maintains the uptake of soil water under drought stress conditions (Morgan, 1984; Nakayama et al., 2007). This osmotic adjustment is a physiological mechanism in response to drought (Da Costa and Huang, 2006). Apart from stress, the WSC concentrations varied among legume species and differed between legume monoculture and legume-grass mixture (Table 5). WSC concentrations were highest in marsh birdsfoot trefoil and sainfoin. It seems that in legume monocultures higher WSC concentrations are related to lower CP concentrations (Sanada et al., 2007). Water-soluble carbohydrate concentrations in perennial ryegrass are usually higher than in legumes (Ulyatt et al., 1988; Dewhurst et al., 2003; Marshall et al., 2004). There were significant ($P<0.05$) negative linear correlations between legume contents in the mixtures and WSC concentrations of the mixture in all three periods and in both, control and stress treatments.

Digestibility of legumes may even increase under strong drought stress due to a tendency to higher WSC and lower fibre concentrations. Moreover, higher WSC concentrations, associated with a lower ratio of CP to WSC, which is an indicator for N utilisation, could enhance N use and reduce N excretion in urine of ruminants (Moorby et al., 2006).

Table 2. Crude protein (CP) values of legume species (plus *L. perenne*) in monoculture and in mixture with *L. perenne* under different levels of drought stress in spring 2010 (moderate stress), summer 2010 (strong stress) and spring 2011 (strong stress); means (n=4).

Forage plant species	CP [g kg^{-1} DM]											
	Spring 2010 moderate				Summer 2010 strong				Spring 2011 strong			
	Monoculture		Mixture		Monoculture		Mixture		Monoculture		Mixture	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Birdsfoot trefoil[1]	270	259	95	100	226	212	144	146	227	229	162	149
Marsh birdsfoot trefoil[2]	205	220	92[#]	91[#]	179	149	113[#]	115[#]	251	188	77[#]	81[#]
Black medic[3]	253	249	114	112	261	230	144	149	239	222	152	167
Yellow alfalfa[4]	274	278	96	93	248	239	122	128	261	252	156	141
Sainfoin[5]	135	132	93[#]	97[#]	186	203	113[#]	119[#]	162	158	81[#]	86[#]
White clover[6]	272	264	175	150	240	224	188	150	254	234	205	169
Perennial ryegrass[7]	93	90			113	115			87	79		
LSD value	20.4				28.9				17.6			
ANOVA Summary	*F*-ratio		*P*		*F*-ratio		*P*		*F*-ratio		*P*	
Legume (L)	91.79		< 0.001		22.24		< 0.001		133.09		< 0.001	
Stand (S)	1807.98		< 0.001		371.02		< 0.001		1190.42		< 0.001	
Drought Stress (DS)	0.62		0.432		3.92		0.052		24.55		< 0.001	
L×S	49.48		< 0.001		5.87		< 0.001		24.65		< 0.001	
L×DS	1.25		0.296		1.55		0.187		4.45		0.001	
S×DS	0.18		0.676		1.70		0.196		5.32		0.024	
L×S×DS	0.82		0.537		1.26		0.291		6.72		< 0.001	

Results from an Analysis of variance (ANOVA) considering the effects of legume, stand (monoculture or mixture) and drought stress. *L. perenne* was not included in the analysis.

Scientific names: [1] *Lotus corniculatus*; [2] *L. uliginosus*; [3] *Medicago lupulina*; [4] *M. falcata*; [5] *Onobrychis viciifolia*; [6] *Trifolium repens*; [7] *Lolium perenne*.

[#] The legume partner did not produce any biomass in these periods.

Table 3. Neutral detergent fibre (NDF) values of legume species (plus *L. perenne*) in monoculture and in mixture with *L. perenne* under different levels of drought stress in spring 2010 (moderate stress), summer 2010 (strong stress) and spring 2011 (strong stress); means (n=4).

Forage plant species	NDF [g kg^{-1} DM]											
	Spring 2010 moderate				Summer 2010 strong				Spring 2011 strong			
	Monoculture		Mixture		Monoculture		Mixture		Monoculture		Mixture	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Birdsfoot trefoil[1]	345	346	513	523	390	383	580	583	384	320	475	452
Marsh birdsfoot trefoil[2]	337	324	560#	530#	373	354	618#	606#	364	352	504#	504#
Black medic[3]	398	376	528	531	383	389	576	578	391	334	480	443
Yellow alfalfa[4]	378	379	535	530	439	425	598	599	395	362	477	483
Sainfoin[5]	358	361	522#	530#	354	333	631#	599#	414	351	510#	509#
White clover[6]	340	324	469	487	366	366	490	554	315	293	411	405
Perennial ryegrass[7]	520	527			574	590			478	468		
LSD value	31.6				35.0				26.0			
ANOVA Summary	*F*-ratio		*P*		*F*-ratio		*P*		*F*-ratio		*P*	
Legume (L)	12.01		< 0.001		13.46		< 0.001		46.47		< 0.001	
Stand (S)	1317.22		< 0.001		1634.38		< 0.001		927.73		< 0.001	
Drought Stress (DS)	0.58		0.449		0.24		0.627		47.69		< 0.001	
L×S	5.55		< 0.001		13.77		< 0.001		3.87		0.004	
L×DS	0.87		0.503		2.61		0.032		3.46		0.008	
S×DS	0.84		0.364		1.71		0.195		17.52		< 0.001	
L×S×DS	0.67		0.650		1.16		0.337		1.03		0.409	

Results from an Analysis of variance (ANOVA) considering the effects of legume, stand (monoculture or mixture) and drought stress. *L. perenne* was not included in the analysis.

Scientific names: [1] *Lotus corniculatus*; [2] *L. uliginosus*; [3] *Medicago lupulina*; [4] *M. falcata*; [5] *Onobrychis viciifolia*; [6] *Trifolium repens*; [7] *Lolium perenne*.

The legume partner did not produce any biomass in these periods.

Table 4. Acid detergent fibre (ADF) values of legume species (plus *L. perenne*) in monoculture and in mixture with *L. perenne* under different levels of drought stress in spring 2010 (moderate stress), summer 2010 (strong stress) and spring 2011 (strong stress); means (n=4).

Forage plant species	ADF [g kg^{-1} DM]											
	Spring 2010 moderate				Summer 2010 strong				Spring 2011 strong			
	Monoculture		Mixture		Monoculture		Mixture		Monoculture		Mixture	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Birdsfoot trefoil[1]	248	254	284	289	270	279	339	330	289	258	292	280
Marsh birdsfoot trefoil[2]	242	236	314[#]	285[#]	286	280	355[#]	337[#]	278	290	276[#]	269[#]
Black medic[3]	277	267	301	301	254	265	335	334	280	262	298	284
Yellow alfalfa[4]	261	264	298	293	302	304	343	340	286	272	288	291
Sainfoin[5]	294	300	285[#]	293[#]	296	279	362[#]	334[#]	328	292	282[#]	277[#]
White clover[6]	257	252	287	284	274	292	320	337	263	256	291	266
Perennial ryegrass[7]	288	286			328	331			263	251		
LSD value	18.9				21.2				18.8			
ANOVA Summary	*F*-ratio		*P*		*F*-ratio		*P*		*F*-ratio		*P*	
Legume (L)	9.36		< 0.001		6.31		< 0.001		6.46		< 0.001	
Stand (S)	120.93		< 0.001		348.43		< 0.001		1.63		0.206	
Drought Stress (DS)	0.98		0.326		0.35		0.554		22.90		< 0.001	
L×S	10.78		< 0.001		3.04		0.015		9.16		< 0.001	
L×DS	1.83		0.118		3.43		0.008		1.98		0.093	
S×DS	0.24		0.625		2.49		0.119		1.24		0.270	
L×S×DS	0.70		0.623		0.15		0.980		2.32		0.052	

Results from an Analysis of variance (ANOVA) considering the effects of legume, stand (monoculture or mixture) and drought stress. *L. perenne* was not included in the analysis.

Scientific names: [1] *Lotus corniculatus*; [2] *L. uliginosus*; [3] *Medicago lupulina*; [4] *M. falcata*; [5] *Onobrychis viciifolia*; [6] *Trifolium repens*; [7] *Lolium perenne*.

[#] The legume partner did not produce any biomass in these periods.

Table 5. Water-soluble carbohydrates (WSC) values of legume species (plus *L. perenne*) in monoculture and in mixture with *L. perenne* under different levels of drought stress in spring 2010 (moderate stress), summer 2010 (strong stress) and spring 2011 (strong stress); means (n=4).

Forage plant species	WSC [g kg^{-1} DM]											
	Spring 2010 moderate				Summer 2010 strong				Spring 2011 strong			
	Monoculture		Mixture		Monoculture		Mixture		Monoculture		Mixture	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Birdsfoot trefoil[1]	30	40	222	207	47	59	66	71	25	51	110	129
Marsh birdsfoot trefoil[2]	113	109	198[#]	227[#]	103	129	87[#]	100[#]	53	95	240[#]	242[#]
Black medic[3]	56	68	178	179	63	78	85	75	55	78	133	126
Yellow alfalfa[4]	37	33	195	211	20	26	90	85	15	25	135	147
Sainfoin[5]	129	121	224[#]	210[#]	86	92	78[#]	97[#]	74	101	230[#]	229[#]
White clover[6]	64	74	132	162	71	72	69	72	67	83	87	142
Perennial ryegrass[7]	218	217			109	123			235	260		
LSD value	22.9				24.2				27.2			
ANOVA Summary	*F*-ratio		*P*		*F*-ratio		*P*		*F*-ratio		*P*	
Legume (L)	40.29		< 0.001		18.01		< 0.001		58.55		< 0.001	
Stand (S)	1365.26		< 0.001		9.51		0.003		677.44		< 0.001	
Drought Stress (DS)	2.52		0.117		4.56		0.036		22.35		< 0.001	
L×S	26.58		< 0.001		11.74		< 0.001		25.75		< 0.001	
L×DS	1.90		0.106		0.78		0.567		1.08		0.377	
S×DS	0.62		0.434		0.95		0.332		1.96		0.166	
L×S×DS	1.99		0.091		0.55		0.734		2.31		0.053	

Results from an Analysis of variance (ANOVA) considering the effects of legume, stand (monoculture or mixture) and drought stress. *L. perenne* was not included in the analysis.

Scientific names: [1] *Lotus corniculatus*; [2] *L. uliginosus*; [3] *Medicago lupulina*; [4] *M. falcata*; [5] *Onobrychis viciifolia*; [6] *Trifolium repens*; [7] *Lolium perenne*.

[#] The legume partner did not produce any biomass in these periods.

Conclusion

The effect of drought stress on the nutritive value of six different legume species was considerably less pronounced than the influence on yield. The impact of drought was more visible under strong drought stress than under moderate stress. Strong drought led to increased WSC concentrations and decreased fibre concentrations which may increase the digestibility of the herbage. Also the ratio of CP to WSC, an indicator for N utilisation, was smaller under drought and which could thus enhance the ruminal N retention and decrease the N surplus in ruminates. However, in most cases legume species and stand (monoculture or mixture) influenced quality parameters stronger than drought stress. We conclude that the effect of temporary drought on the nutritive value of legumes seems to be less important than other properties for the selection of suitable forage legumes for agronomic productions under conditions of predicted climate change.

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