Sustainable grassland herbage production under drought stress - the role of plant species number and functional group composition

Dissertation

Zur Erlangung des Doktorgrades

der Fakultät für Agrarwissenschaften

der Georg-August-Universität Göttingen

vorgelegt von

Frank Küchenmeister

Geboren am 07.06.1983 in Bad Salzungen

Göttingen, März 2013

D 7

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Tag der mündlichen Prüfung: 07. Mai 2013

Habe nun, ach! Philosophie,
Juristerei und Medizin,
Und leider auch Theologie
Durchaus studiert, mit heißem Bemühen.
Da steh ich nun, ich armer Tor!
Und bin so klug als wie zuvor;
Heiße Magister, heiße Doktor gar
[…]
Daß ich erkenne, was die Welt
Im Innersten zusammenhält,
[…]
O sähst du, voller Mondenschein,
Zum letzen mal auf meine Pein,
Den ich so manche Mitternacht
An diesem Pult herangewacht:
Dann über Büchern und Papier,
Trübsel'ger Freund, erschienst du mir!
Ach! könnt ich doch auf Bergeshöhen
In deinem lieben Lichte gehen,
Um Bergeshöhle mit Geistern schweben,
Auf Wiesen in deinem Dämmer weben,
Von allem Wissensqualm entladen,
In deinem Tau gesund mich baden!
[…]
Ha! welche Wonne fließt in diesem Blick
Auf einmal mir durch alle meine Sinnen!
Ich fühle junges, heil'ges Lebensglück
Neuglühend mir durch Nerv' und Adern rinnen.
War es ein Gott, der diese Zeichen schrieb,
Die mir das innre Toben stillen,
Das arme Herz mit Freude füllen,
Und mit geheimnisvollem Trieb
Die Kräfte der Natur rings um mich her enthüllen?

Faust, der Tragödie erster Teil   Johann Wolfgang von Goethe

Contents

General Introduction

Permanent grassland covers approximately 70% of the agriculturally used area worldwide (Panunzi 2008) and more than one third in Europe (Smit et al. 2008). Grassland is an important agricultural resource (White et al. 2000; Isselstein et al. 2005) and forms the basis for ruminant nutrition and livestock production (White et al. 2000; Hopkins and Wilkins 2006). The productivity of ruminant husbandry relies on quantity and quality of herbage (Gibon 2005; Hopkins and Wilkins 2006). Grassland production strongly depends on factors like soil, climatic conditions and the grassland management. Productive grasslands in temperate climate zones require a considerably high water supply over the growing season (Dierschke and Briemle 2002; Hopkins and Del Prado 2007). Climate change is expected to vary the future pattern of rainfall and to increase the frequency of extreme weather events like drought (Alcamo et al. 2007; Trnka et al. 2011). While herbage production and yield is known to be generally adversely affected by drought, less information is available if about the role of sward botanical composition and how it may modify drought effects (Grime et al. 2000; Ehlers and Gross 2003; Hopkins and Del Prado 2007).

Generally, increasing plant biodiversity has been proposed as a means to obtain higher herbage yields from grassland (e.g. Marquard et al. 2009; Tilman et al. 2012), more stable yields (Tilman et al. 2006; Biondini 2007; Isbell et al. 2009) and an enhanced nutritive value (Bullock et al. 2007). Other authors found no impact of species richness on agricultural features like productivity (Wrage et al. 2011; Seither et al. 2012) and water utilization (Rose et al. 2012) and no consistent (Seither et al. 2012), or even a negative influence on nutritive value (Bruinenberg et al. 2002). Isbell et al. (2011) concluded from a review of the literature and Lanta et al. (2012) showed with their experiments that species richness has a positive influence on grassland functions like productivity even under stress conditions while Kahmen et al. (2005) found neither increasing nor decreasing effects under stress conditions. A suppressed productivity of diverse swards with stress was reported by de Boeck et al. (2008) while van Peer et al. (2004) found an increasing water use in more diverse swards under stress which did not result in larger yields.

A number of investigations have stressed species identity and composition of functional groups as important factors for productivity and stability in grassland (Mokany et al. 2008; Grman et al. 2010; McLaren and Turkington 2010; Sasaki and Lauenroth 2011). Sanderson (2010) suggested species identity and composition to have more influence on yield stability than the mere number of species. That there is an influence of sward composition on

nutritive value has been known for a long time (Hopkins and Wilkins 2006) but it is not clear how swards of different composition react to drought. Under drought the nutritive value may increase (DaCosta and Huang 2006; Wang and Frei 2011), decrease (Peterson et al. 1992; Skinner et al. 2004) or show inconsistent effect (Abberton et al. 2002; Seguin et al. 2002). In short, the role of sward diversity and functional group composition for sustainable herbage production and how grassland reacts under drought stress is still under discussion.

Our major aims were to:

1) Analyze the overall effects of species richness and functional composition on yield and yield stability of temperate grasslands (Chapter I)

2) Determine if species richness and/or functional composition have a positive influence on yield and water utilization under drought stress (Chapter II)

3) Investigate the effect of drought stress on nutritive value of grassland herbage and evaluate if species richness and functional group composition modify quality under drought conditions and if the degree of diversity interacts with drought stress (Chapter III)

4) Test if diverse grasslands would suffer less from drought stress in terms of biomass production and $\delta^{13}C$ than less diverse grassland, with or without nitrogen fertilization (Chapter IV)

We, therefore, conducted a drought stress experiment in a vegetation hall over three growing seasons (2009-2011; Chapter I–III) and a drought stress field experiment (2009; Chapter IV). Different drought stress conditions in the vegetation hall were performed in two growing seasons where the conditions followed normal seasonal pattern with frost in winter and higher temperatures in summer. Drought stress was induced by leaving the containers unwatered for a defined period of time, during which soil water availability could be controlled. We chose productive agricultural species of temperate grasslands. Plants were sown in monoculture and three- and five-species mixtures. This range of species number has been shown to affect productivity in biodiversity-productivity experiments (Roy 2001; Tracy and Sanderson 2004). To support our results from the vegetation hall, a field experiment was carried out. On established grassland swards we installed rainout-shelters and created a gradient of species diversity by removing dicots from some plots with herbicides.

The investigation was part of the research co-operation "KLIFF Klimafolgenforschung in Niedersachsen" (Climate impact and adaptation research in Lower Saxony). Our study was part of the research area "Animal production".

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Chapter I

Yield and yield stability in mixtures of productive grassland species: Does species number or functional group composition matter?

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Abstract

In productive agricultural grasslands the relative importance of species identity, species richness and functional group composition for production and yield stability is not clear. We, therefore, tested diversity effects in mixtures of five productive species common in temperate agricultural grasslands in a greenhouse study: *Trifolium repens* (legume), *Lolium perenne*, *Dactylis glomerata* (grasses) and *Plantago lanceolata*, *Taraxacum officinale* agg. (forbs). Diversity levels were (i) monoculture; (ii) all possible three-species mixtures; and (iii) five species mixture. Biomass production increased with greater species richness, an effect that was interpreted as a functional response to a higher proportion of legumes in the mixtures. Species identity and functional composition influenced yield and yield stability in different ways. Larger contents of the legume in mixtures increased yield but decreased yield stability while grasses showed the opposite effect in mixtures. The biomass production of forbs was mostly small in mixtures and yield stability decreased with increasing presence of forbs. In productive agricultural grassland, functional group composition, especially the presence of legumes and grasses, seems to be more important for productivity and yield stability than diversity.

Keywords: Diversity; Forbs; Grasses; Legume; Species identity

Introduction

In experimental grasslands, plant species richness has been found to be positively correlated to biomass production (Hector et al. 1999; Isbell et al. 2009; Marquard et al. 2009a, b) as well as to more stable yields (Schläpfer et al. 2002; Tilman et al. 2006; Biondini 2007; Isbell et al. 2009). Other investigations have stressed species identity and composition of functional groups as important factors for productivity (Mokany et al. 2008; McLaren and Turkington 2010). In conclusion, Sanderson (2010) suggested species identity and composition to have more influence on yield stability than the mere number of species. Dominant species have also been found to affect community stability and, therefore, yield stability in a positive way (Grman et al. 2010; Sasaki and Lauernroth 2011).

So far, the relative importance of species identity, species richness and functional group composition for production and yield stability in agricultural grasslands is not clear. Few studies have been carried out under agricultural conditions (e.g. Bullock et al. 2007;

Nyfeler et al. 2009). In most biodiversity experiments, mixtures of a wide range of very low or low to high productive species were sown, weeded intensively and harvested only once or twice per year to understand the basic relation between biodiversity and ∕or functional groups and production (e.g. Spehn et al. 2000; Tilman et al. 2006; Marquard et al. 2009b; McLaren and Turkington 2010; Mommer et al. 2010).

In the greenhouse study presented here, we therefore examined the effects of species richness and functional composition on yield and yield stability of highly productive agricultural species of temperate grasslands, i.e. two forbs (*Plantago lanceolata* and *Taraxacum officinale* agg.), two grasses (*Lolium perenne* and *Dactylis glomerata*) and one legume (*Trifolium repens*). Plants were sown in monoculture and three- and five-species mixtures. This range of species number has been shown to affect productivity in biodiversity-productivity experiments (Roy 2001; Tracy and Sanderson 2004). With five harvests in the main production year, we simulated a high but common cutting regime in frequently used grassland (Dierschke and Briemle 2002).

Materials and Methods

The experiment was set up in a randomized block design with four replicates in a greenhouse in mid-July 2009. Five species had been selected for the experiment (Table 1) and were either grown as monocultures, all possible three species mixtures and as a five species mixture (for details see Table 2). All plant species are common in productive agricultural grassland and have a high nutritive value and mowing tolerance (Table 1).

As a growing substrate, a homogeneous mixture of 20 kg sand (air-dried, sieved to pass a mesh of 5 mm; August Oppermann Kiesgewinnung GmbH, Hann. Muenden, Germany), 0.9 kg vermiculite (particle size 8–12 mm; Deutsche Vermiculite GmbH, Sprockhoevel, Germany) and 5.5 kg compost (air-dried; Bioenergiezentrum Goettingen GmbH, Goettingen, Germany) was used per container (round plastic pots, diameter 33 cm, height 42 cm), and covered with 1.5 kg compost as seed bed. In monocultures, 1000 germinable seeds per m^2 for dicots and 5000 germinable seeds per m^2 for monocots were sown. For the three-species mixtures, we used 333 germinable seeds per m^2 for dicots and 1666 for monocots and in the five-species mixture 200 seeds for dicots and 1000 for monocots – this is one-third or one-fifth, respectively, of the amounts sown in monoculture. Prior to sowing, the germination was tested in Petri dishes over 14 days in June 2009. Night temperatures ranged from 17 to 19°C and day temperatures ranged from 20 to 33°C.

Germination rates were 96% for *T. officinale*, 91% for *L. perenne*, 86% for *D. glomerata*, 85% for *T. repens* and 34% for *P. lanceolata*. All pots were watered with tap water to ensure that water was not a limiting factor and soil water tension should not exceed a pF-value of 3.2 throughout the experiment.

Table 1 The plant species and cultivars used in the greenhouse experiment, mowing tolerance, nutritive value and plant association

Plant species	Cultivar	Mowing tolerance	Nutritive value	Plant association
Legume				
Trifolium repens	Rivendel	8	9	*Molinio-Arrhenatheretea*
Grass				
Lolium perenne	Signum	8	9	*Cynosurion cristati*
Dactylis glomerata	Donata	8	8	*Arrhenatheretalia*
Forb				
Plantago lanceolata	Wild	7	7	*Molinio-Arrhenatheretea*
Taraxacum officinale agg.	Wild	8	7	*Molinio-Arrhenatheretea*

Mowing tolerance, nutritive value and plant association according to Dierschke and Briemle (2002). Values range from 1 (low) to 9 (high).

In order to enable nodulation of white clover roots, all pots were treated with a rhizobium solution (0.015 mL Radicin dissolved in 250 mL water per square meter and application, Radicin; Jost-GmbH, Iserlohn, Germany). No fertilization was conducted. Temperatures were recorded daily at three locations in the greenhouse (Figure 1).
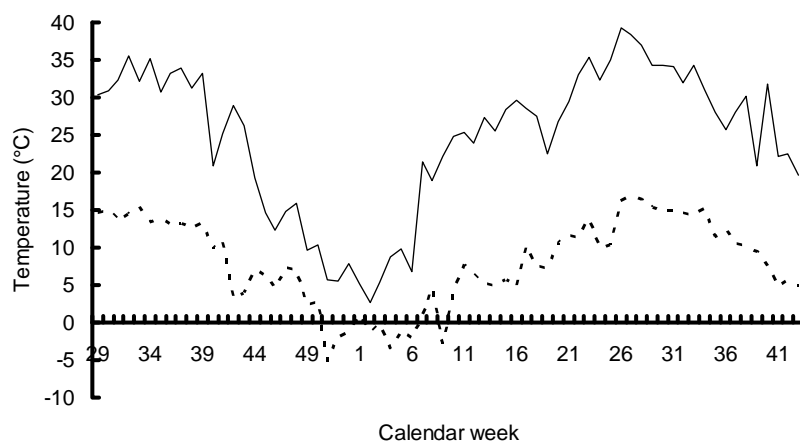
Figure 1 Weekly air temperature in the greenhouse during the experimental period form July 2009 to October 2010. The solid and broken lines represent the mean maximum and minimum temperatures, respectively.

Climatic conditions were controlled by forced venting in summer and by a heating system in winter that was switched on when temperatures fell below 0°C for longer than 24 h. Heating should not exceed 5°C and forced venting was open almost all summer. No extra lighting was provided. Harvests took place twice in 2009 (50 and 104 days after sowing, sowing year) and five times in 2010 (272, 315, 356, 407 and 462 days after sowing, main production year). Shoots were hand-clipped 3–4 cm above ground. Each sample was sorted into species or functional groups (for grasses, where the biomass was not separated), dried (60°C for 72 h) and weighed.

Statistical data analysis was carried out using statistica 9.1 (StatSoft, Inc., Tulsa, Oklahoma, USA). Analysis of variance (ANOVA) considered the factor diversity. The data were tested for normal distribution and homogeneity of variances. Where significant treatment effects ($P < 0.05$) were found, least significant differences (Tukey honestly significant difference [HSD] test) were used to compare means. Regressions were calculated with a linear regression model. The relationships between dry matter and species number or yield proportion as well as the relationship between coefficient of variation and yield proportion were tested.

Results

The data presented here refer to two time periods of the experiment: two harvests in the sowing year and then, after a winter period, five harvests in the first main production year.

The productivity of the first two harvests in the sowing year was used to characterize the establishment of the species in the mixtures. None of the species failed to emerge after sowing and to produce herbage, neither in the pure stands nor in the mixtures. Among the monocultures, total yields ranged from 43.7 g pot^{-1} for the legume and 53.9 g pot^{-1} for *L. perenne*, with forbs being intermediate (Table 2). In the three- and five-species mixtures, there were differences in yield contribution across the functional groups, with the legume having smaller contributions to the total yield than the grasses, and forbs being intermediate. Three-species mixtures containing *T. repens*, *L. perenne* and one of the forbs, along with the five-species mixture, showed the largest yields being significantly higher than the yield of the *T. repens* pure stand.

Table 2 Total dry matter yields of monocultures and mixtures in the establishing year and yield contribution of functional groups (legume, grasses and forbs) to the mixtures

Sward	Yield (g pot^{-1})			
	Legume	Grass	Forb	Total
Monoculture				
T. repens (Tr)	–	–	–	43.7[b]
D. glomerata (Dg)	–	–	–	49.6[ab]
L. perenne (Lp)	–	–	–	53.9[ab]
P. lanceolata (Pl)	–	–	–	49.4[ab]
T. officinale agg. (To)	–	–	–	51.6[ab]
Three-species mixture				
TrDgPl	3.2	32.9	13.9	50.0[ab]
TrDgTo	1.9	36.4	15.1	53.4[ab]
TrLpPl	2.8	44.6	13.1	60.5[a]
TrLpTo	2.2	42.5	13.1	57.8[a]
TrDgLp	1.0	46.0	–	47.0[ab]
TrPlTo	4.3	–	45.1	49.4[ab]
DgLpPl	–	45.2	7.4	52.6[ab]
DgLpTo	–	48.2	7.9	56.1[ab]
DgPlTo	–	29.4	19.6	49.0[ab]
LpPlTo	–	37.3	17.4	54.7[ab]
Five-species mixture				
TrDgLpPlTo	1.8	39.1	17.0	57.9[a]

Yields are sum of two harvests ($n = 4$).

Total yields with different letters differ ($P < 0.05$).

For the main production year (five harvests), there was a significant positive correlation between the mean dry matter production per pot and the number of species in the mixture ($R^2 = 0.56$, $P = 0.005$; Figure 2). However, yield stability measured as the coefficient of variation of yield over successive harvests was not significantly affected by species number ($P = 0.681$; data not shown).
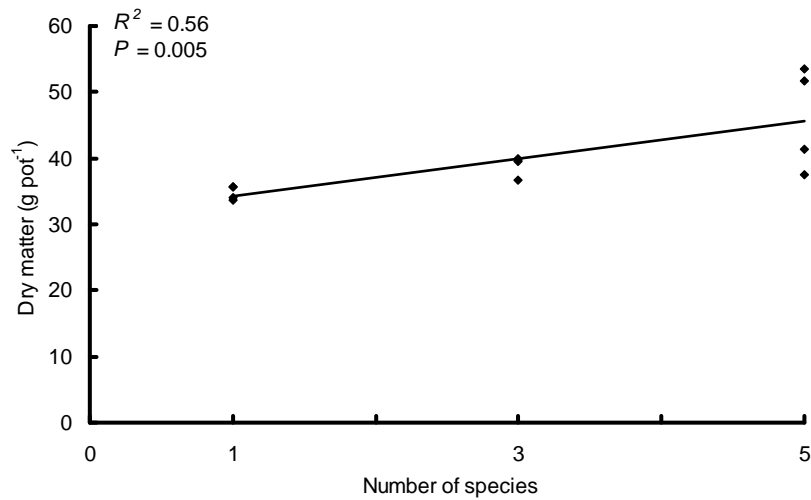
Figure 2 The relationship between the mean annual dry matter yield and the number of species for the main production year (five harvests). Results for different pure stands and three-species mixtures, respectively, were averaged while there was only one five-species mixture. The results for every replicate ($n = 4$) are shown.

Among the monocultures total annual yields in the main production year ranged from 123.9 g pot^{-1} year^{-1} for *T. officinale* to 325.6 g pot^{-1} year^{-1} for *T. repens* (Table 3). The pure stand of *T. repens* showed the highest yield of all swards. Among the three-species mixtures, the combination containing all three functional groups had the largest yields, while the mixtures without *T. repens* produced significantly lower yields. The yield of the five-species mixture was similar to those three-species mixtures that contained *T. repens*. The coefficient of variation was highest in monocultures of the two forbs and the legume and in the mixtures containing *T. repens*.

17

Table 3 Total dry matter yields and coefficient of variation (CV) of monocultures and mixtures in the main production year and yield contribution of functional groups (legume, grasses and forbs) to the mixtures

Sward	Yield (g pot^{-1})				CV
	Legume	Grass	Forb	Total	
Monoculture					
T. repens (Tr)	–	–	–	325.6[a]	0.45
D. glomerata (Dg)	–	–	–	136.4[de]	0.28
L. perenne (Lp)	–	–	–	151.3[de]	0.18
P. lanceolata (Pl)	–	–	–	132.4[ef]	0.40
T. officinale agg. (To)	–	–	–	123.9[ef]	0.45
Three-species mixture					
TrDgPl	71.2	134.5	10.7	216.4[bc]	0.34
TrDgTo	79.7	115.8	30.7	226.2[bc]	0.33
TrLpPl	116.1	143.0	5.5	264.6[b]	0.38
TrLpTo	101.0	120.6	28.1	249.7[bc]	0.34
TrDgLp	47.5	145.0	–	192.5[cd]	0.24
TrPlTo	112.8	–	116.3	229.1[bc]	0.45
DgLpPl	–	136.9	2.3	139.2[df]	0.17
DgLpTo	–	135.3	15.5	150.8[df]	0.19
DgPlTo	–	85.5	45.2	130.7[ef]	0.27
LpPlTo	–	121.9	28.4	150.3[df]	0.20
Five-species mixture					
TrDgLpPlTo	87.2	124.1	18.8	230.1[bc]	0.35

Yields are sum of five harvests ($n = 4$).

Total yields with different letters differ ($P < 0.05$).

With an increasing yield proportion of white clover, the dry matter production increased ($R^2 = 0.86$, $P < 0.001$), but the coefficient of variation also increased ($R^2 = 0.25$, $P = 0.041$), indicating a larger yield variability (or smaller yield stability; Figure 3a, b). The contrary was true for the grasses (Figure 3c, d), which showed decreased dry matter production with increased proportion ($R^2 = 0.11$, $P = 0.007$), but increased yield stability ($R^2 = 0.64$, $P < 0.001$). With increasing proportions of forbs (Figure 3e, f), both dry matter yield and yield stability decreased ($R^2 = 0.14$, $P = 0.002$ and $R^2 = 0.23$, $P < 0.001$).
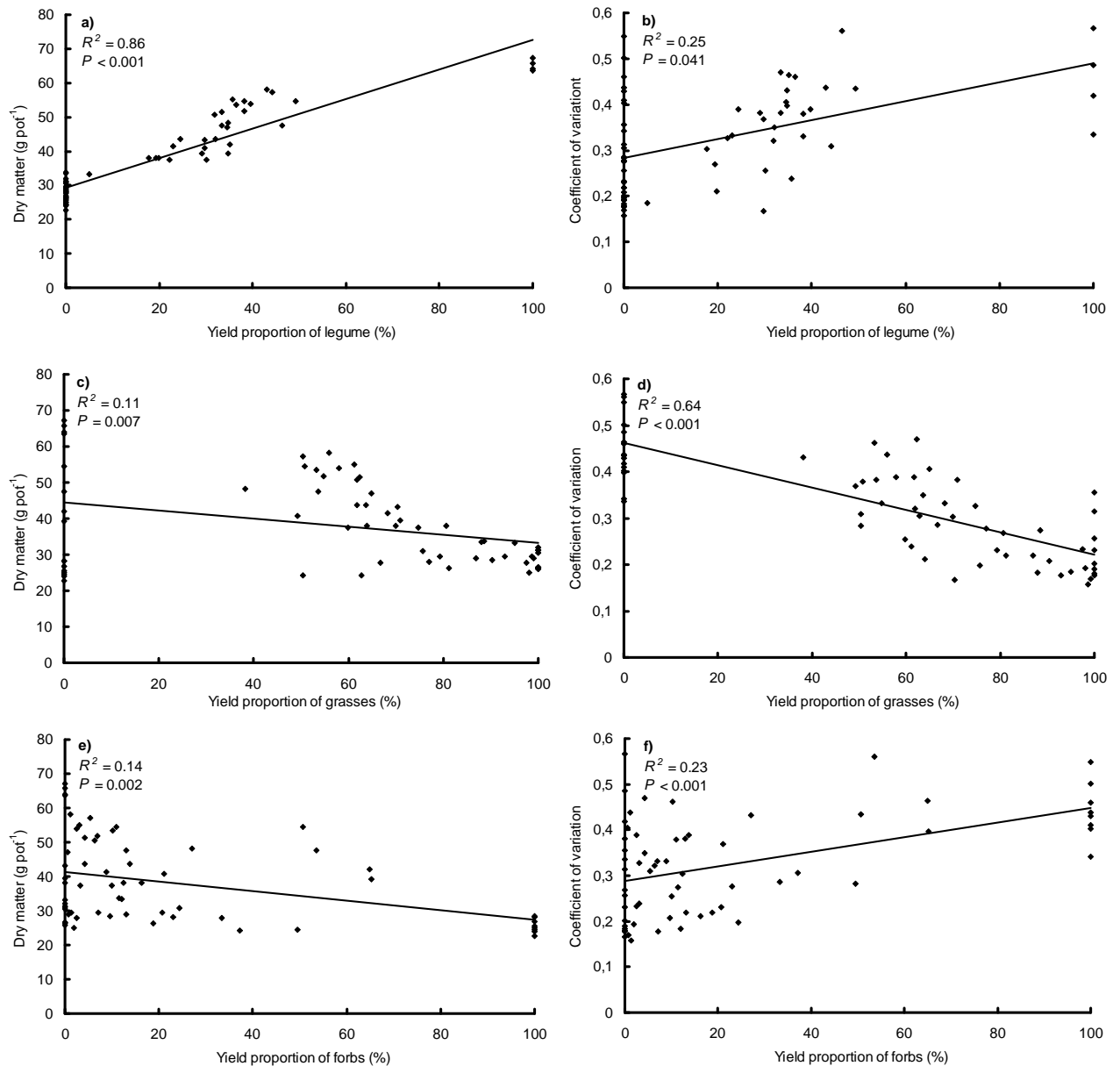
Figure 3 Annual dry matter yield and coefficient of variation of dry matter yield over time plotted against yield proportion of legume (a, b), grasses (c, d) and forbs (e, f). For dry matter, one data point represents the annual dry matter yield in the main production year of one replicate averaged over five harvests ($n = 4$). The coefficients of variation were calculated similarly and thus show the variability over time.

Figure 4 shows the seasonal yield contribution of the functional groups legume, grasses and forbs to the annual yield as means over all three-species mixtures in the main production year. The contribution of the legume strongly varied with time and showed a distinctive peak around calendar week 27 (July).
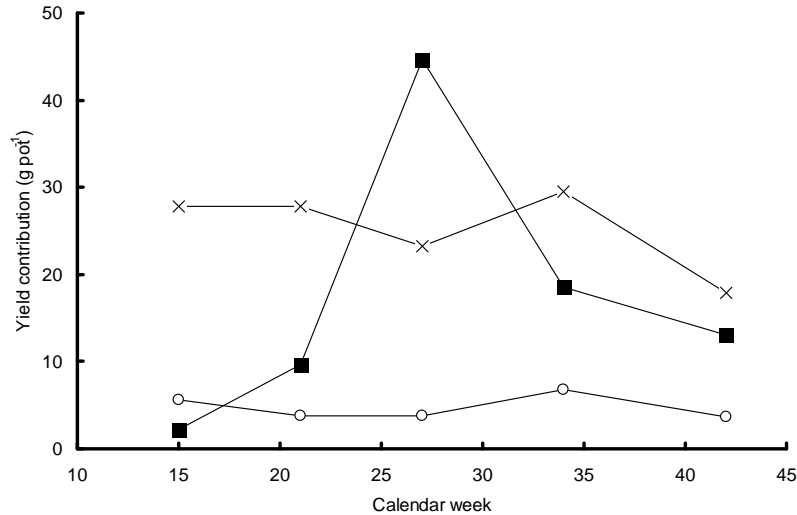
Figure 4 Seasonal contribution of functional groups (legume (■), grasses (×) and forbs (○)) to the annual yield of the three-species mixtures in the main production year.

Discussion

In this study, we examined the effects of species number and functional composition on dry matter yield and yield stability. In the establishing year, neither species number nor functional group showed an effect on dry matter (Table 2). Due to their general yield potential, all species developed well in monoculture and in mixtures. However, *T. repens* was the lowest yielding monoculture and performed equally weakly in mixtures for the first two harvests. Annicchiarico and Proietti (2010) found similarly low yields of *T. repens* in the first year when grown in mixtures with competitive grasses. Furthermore, temperatures in the greenhouse were optimal for grasses, but less so for the development of *T. repens* (Figure 1).

We found that an increased biomass production in the main production year could be related to increased species richness (Figure 2) and functional group (legume) (Figure 3a). For yield stability, no significant relationship with species richness was found but an increased proportion of grass in the species mixture appears to have had a stabilizing effect on dry matter yields (Figure 3d). The observed larger biomass production with increasing species richness can also be interpreted as a functional response. *T. repens* was the highest-yielding monoculture in the main production year (Table 3) and mixtures containing this legume had a higher dry matter production than other mixtures and monocultures. However, the mixture containing the two grasses and *T. repens* had lower dry matter yields and a minor yield contribution of *T. repens* than mixtures of *T. repens* with one grass and

20

one forb. This effect is in accordance with Sanderson et al. (2005) who state that dominant grasses can suppress the growth of *T. repens*. The positive effect of increasing proportions of *T. repens* was probably due to its nitrogen-fixing ability (Frame et al. 1998; Huston et al. 2000). *T. repens* was present in one of five monocultures, in six of 10 three-species mixtures and in the five-species mixture. Thus, the increasing effect of the presence of *T. repens* with increasing species richness can be interpreted as a sampling effect (Huston 1997; Huston et al. 2000) or the validity of the ''mass ratio hypothesis'' (Grime 1998). However, increasing proportions of *T. repens* did not have a similarly positive effect on yield stability (Figure 3b). This is in line with reports on fluctuating seasonal yields, with lower yields in spring and higher yields in summer (Archer and Robinson 1989; Schils et al. 1999; Elgersma et al. 2000; see also Figure 4). *T. repens* has a high temperature demand of 25°C for optimal growth (Frame et al. 1998) ad largest yields occurred during summer when temperatures in the greenhouse exceeded that value (Figure 1). Increasing proportions of grasses coincided with slightly decreasing yields, corresponding with smaller proportions of the highest-yielding species *T. repens*; an effect that has also been reported by Turkington and Jolliffe (1996). However, this led to improved yield stability. The grasses used in this study have a wide temperature optimum, ranging from 15 to 25°C (Davidson and Milthorpe 1965; Eagles 1967; Wilson and Ford 1971), which was reached earlier and continued longer than that of the legume (Figure 1). A relatively stable biomass production by grasses over the vegetation season has been observed before (Waldron et al. 2002; Suter et al. 2008; see also Figures 3d and 4 and Tables 2 and 3). The forbs sown in our study are generally known as having a good yield potential and comparatively high competitive strength (Miller and Werner 1987; Hofmann and Isselstein 2005; Dierschke and Briemle 2002; Assaf and Isselstein 2009; but also see Neal et al. 2009 for *P. lanceolata*). However, in the present experiment *T. officinale* agg. and *P. lanceolata* contributed relatively little to the total yield of most mixtures (Table 3). With increasing yield contribution of the two forbs in mixtures, there was a tendency to slightly smaller total dry matter yields (Figure 3e). Yield stability also slightly decreased with increasing presence of the forbs. Considerable seasonal yield variations of *T. officinale* agg. and *P. lanceolata*, with a peak in spring and autumn, have been observed before (Mølgaard 977; Cavers et al. 1980; Berendse 1983; Vavrek et al. 1997). The dry matter productions of *P. lanceolata* used in this study has previously been shown to be sensitive to cuttting (Sagar and Harper 1964; Berendse 1983). The harvesting in the sowing year and the cutting frequency of five times in the main production year in this experiment represent a rather

21

intensive production. A frequent cutting has been shown to improve the establishment of forb seedlings (Sagar and Harper 1964; Mølgaard 1977; Hofmann and Isselstein 2005). In the first main production year especially, frequent cutting is not beneficial for the development of *T. officinale* agg. (Mølgaard 1977; Isselstein and Hofmann 1996) and *P. lanceolata* (Sagar and Harper 1964). Under conditions of frequent cutting, good water supply and competition from grasses, the yield of *P. lanceolata* is likely to decline over time (Sagar and Harper 1964; Cavers et al. 1980; Neal et al. 2009). For *T. officinale* agg., on the other hand, the competitive ability and yield will increase over time, once the plant is established (Isselstein and Hofmann 1996; Assaf and Isselstein 2009). Yield stability also slightly decreased with increasing presence of the forbs. Seasonal growth patterns of *T. officinale* agg. and *P. lanceolata* with a peak in spring and autumn were observed before by different authors (Mølgaard 1977; Cavers et al. 1980; Berendse 1983; Vavrek et al. 1997). In conclusion, the present study indicated a stronger effect of species identity and functional composition than of species richness on both, yield and stability of yield. The grasses, forbs and legume functional groups had different effects on yield and yield stability that were consistent within functional groups. Whether these are indeed inherent characteristics of functional groups in agricultural grassland remains to be tested with research being extended to the field scale. However, this could hold important information on development of grassland seed mixtures and community composition.

Acknowledgments

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Chapter II

Do phytodiversity and plant functional composition of grass swards alleviate productivity losses under drought stress?

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Abstract

Water is a main production factor in grassland husbandry. Because of climate change, alterations in precipitation patterns and an increase of the frequency of drought periods are expected for the future also in the temperate climate zone. Enhancing the species richness or functional group composition of swards has been proposed of ensuring herbage production and water utilization under drought stress. We tested this hypothesis in an experiment under controlled conditions in a vegetation hall from 2009 to 2011. Monocultures, mixtures of ten different combinations of three species, and a five-species mixture of five productive grassland plant species representing three functional groups, *Trifolium repens* (legume), *Lolium perenne*, *Dactylis glomerata* (grasses), *Plantago lanceolata*, and *Taraxacum officinale* agg. (forbs), were established in containers and subjected to one moderate and two strong drought stress periods in 2010 and 2011; control treatments were not subjected to water limitation. Drought stress decreased yield depending on strength of stress while only strong drought stress decreased agronomic water use efficiency in all swards. The species number had no effect on yield and water use efficiency under drought stress, whereas under well watered conditions a slight positive effect was found. A positive response could be explained by the sampling effect, i.e. an increased probability of the productive *T. repens* being part of the mixture with an increasing species number. As *T. repens* was susceptible to drought no positive effect of species number was found under drought stress. The grasses and the other forbs had a lower yield potential and water use efficiency compared to *T. repens*. However, the response to drought was also lower. Investigated nitrogen yield and concentration were suitable indicators for agronomic water use efficiency while δ^{13}C, as measurement of intrinsic water use efficiency, was less appropriate. Agronomic water use efficiency was increased by nitrogen.

Keywords: Forb; Grass; Legume; Species richness; Water use efficiency; Yield

Introduction

Due to climate change it is expected that in the future precipitation patterns will vary and the frequency of extreme weather events like droughts will increase (Alcamo 2007; Trnka 2011). Yield and yield stability of productive grassland is dependent on a sufficient and regular water supply during the growing season (Hopkins and Del Prado 2007; Dierschke

and Briemle 2002). Adaptation strategies to more frequent droughts are necessary to ensure a future sustainable herbage production from grassland (Alcamo et al. 2007).

Increasing plant biodiversity has been proposed as a way to higher yields in grassland (e.g. Marquard et al. 2009; Tilman et al. 2012). Other investigations have stressed species identity and composition of functional groups as important factors for productivity (e.g. McLaren and Turkington 2010). There is conflicting evidence as to how diverse swards respond to drought stress and how productivity is affected. In a review, Isbell et al. (2011) conclude that species richness has a positive influence on grassland functions like productivity even under stress conditions. According to the "insurance-hypothesis" species richness can stabilize ecosystem functioning against environmental changes (e.g. Hector et al. 2010). In contrast, Kahmen et al. (2005) found neither increasing nor decreasing effects on production under drought. In addition, Rose et al. (2012) did not found any effect of species richness on the agronomic water use efficiency. With drought stress, $\delta^{13}C$ signatures, as a measurement for intrinsic water use efficiency, were also not influenced by species richness (Kahmen et al., 2005). De Boeck et al. (2008) report on an experiment with sown grasslands, with three diversity levels, that highest diverse swards had a detrimental effect on productivity when temperature and drought were increased at the same time. So, the role of sward diversity and/or functional group composition and how grassland swards react under drought stress is still discussed.

We hypothesised that species richness and/or functional composition have a positive influence on yield and water utilization under drought stress.

A drought stress experiment was conducted in a vegetation hall where the conditions followed the normal seasonal pattern with frost in winter and higher temperatures in summer. Different drought stress conditions were generated over three periods in two growing seasons. Drought stress was induced by leaving the containers un-watered for a defined period of time, while soil water availability was measured. We chose productive agricultural species of temperate grasslands. Plants were sown in monoculture and three- and five-species mixtures. This range of species number has been shown to affect productivity in biodiversity-productivity experiments (Roy 2001). We examined the effects of species richness and functional composition on yield and water utilization. The agronomic water use efficiency (WUE) was used to characterize the relation of yield and water consumption. The intrinsic water use efficiency was assessed by measuring the ^{13}C signatures of the plants. Due to a varying stomatal conductance with changing water availability $^{13}CO_2$ is more or less discriminated by the photosynthesis of the plants

(Farquhar et al. 1989). As nitrogen increases carbon utilization, the efficiency of various enzymes, plant canopy and decreases unproductive water loss via leaf over night (Brueck 2008) nitrogen concentration and nitrogen yield of the harvested plants were determined. This comprised measuring the nitrogen fixation of the swards that contained *T. repens* as it does also affects the efficiency of water use (Farooq et al. 2009; Carlsson and Huss-Danell 2003; Ehlers and Goss 2003).

Materials and Methods

Experimental Setup

The experiment was set up in a randomized block design with four replicates and two factors (sward and drought stress) in a vegetation hall in mid-July 2009. Five species had been selected for the experiment and were either grown as monocultures, as mixtures of all possible combinations of three species, and as one five species mixture. The plant species are common in productive agricultural grassland and have moderate to high nutritive value and mowing tolerance (Dierschke and Briemle 2002). The plant species were *Trifolium repens* L. var. Rivendel, *Dactylis glomerata* L. var. Donata, *Lolium perenne* L. var. Signum, *Plantago lanceolata* L. wild type and *Taraxacum officinale* F.H. Wigg. agg. wild type.

As a growing substrate, a homogeneous mixture 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) was used per container (round plastic container, diameter 33 cm, height 42 cm), and covered with 1.5 kg compost as seed bed. The relation between volumetric soil water content and soil water tension was determined with a soil-water retention curve using a pressure plate extractor (Or and Wraith 2002).

In monocultures, 1000 germinable seeds per m^2 for dicots and 5000 germinable seeds per m^2 for monocots 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 monocultures, respectively (replacement design).

All containers were treated with a rhizobium solution (Radicin, Jost-GmbH Iserlohn, Germany, 0.015 ml Radicin dissolved in 250 ml water per square metre and application) to enable nodulation of white clover roots. No fertilisation was conducted. The pH of the soil

(in $CaCl_2$ suspension) as well as the plant available P and K concentration (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 Mg kg^{-1} oven-dry soil). Temperatures were recorded daily at three locations in the vegetation hall (Figure 1). Climatic conditions were controlled by forced venting in summer and heating on frost days in winter (temperatures should not fall below 0°C for longer than 24 h). Heating in winter was limited to a maximum of 5°C air temperature in the vegetation hall. Generally, the climatic conditions in the vegetation hall followed closely outside conditions with (mild) frost in winter and higher temperatures in summer. No extra lighting was provided. The above-ground biomass was harvested two times in 2009, five times in 2010 (calendar week 15, 21, 27, 34 and 42) and two times in 2011 (calendar week 15 and 22).

Drought Stress Treatment

Drought stress was imposed on three periods with a varying severity, i.e. a moderate stress in spring 2010 (harvest week 21), and a strong stress in summer 2010 (harvested week 34) and spring 2011 (harvested in week 22). Intermittent periods were fully watered (no drought stress) and plants were allowed to recover from drought. Drought stress was induced by temporarily ceasing the watering of the containers after an initial watering to approximately -0.03 MPa (25 vol.%). For the moderate drought stress, no water was given until three days after the first plants showed signs of drought (e. g. wilting; soil water tension around -1.5 MPa, i.e. 10 vol.%). Containers were then watered again (to -0.03 MPa) followed by a second drought cycle. In order to induce strong drought, the stress phase was extended to five days after first stress symptoms had appeared and was repeated three times with two irrigations in between. All containers were weighted regularly during stress periods. The number of days without irrigation until a soil water tension of -0.3 MPa was reached, is shown in Tab. 1. This was an indicator for rapidity of water use of the different plant species and mixtures. Means of the soil water contents (vol.%) at the end of drying cycles (Tab. 1) indicated the individual severity of drought for every plant species and mixture.

Table 1: Number of days without irrigation in the drought stress treatment until a water tension of -0.3 MPa was reached in first drought cycle and final volumetric water content (%) as mean of the drought cycles; each drought stress period consisted of two (spring 2010) or three (summer 2010, spring 2011) successive drought cycles; n=4; 10 vol.% correspond to -1.5 MPa.

Sward	Moderate stress spring 2010		Strong stress summer 2010		Strong stress spring 2011	
	Days to reach -0.3 MPa	vol.% water	Days to reach -0.3 MPa	Vol.% water	Days to reach -0.3 MPa	Vol.% water
Monocultures						
T. repens (Tr)	10	6	8	7	11	8
D. glomerata (Dg)	11	9	10	5	12	4
L. perenne (Lp)	9	11	11	8	14	8
P. lanceolata (Pl)	11	8	10	7	14	5
T. officinale agg. (To)	12	9	12	6	15	6
Three-species mixtures						
TrDgPl	10	11	9	6	12	4
TrDgTo	12	6	9	4	11	5
TrLpPl	11	8	10	5	11	5
TrLpTo	10	10	8	5	10	4
TrDgLp	11	8	10	5	11	3
TrPlTo	9	10	9	5	11	4
DgLpPl	11	10	11	6	14	10
DgLpTo	11	10	11	6	14	9
DgPlTo	11	10	12	9	14	9
LpPlTo	11	10	11	9	14	8
Five-species mixture						
TrDgLpPlTo	8	7	8	4	9	4

Sampling and Measurement

Shoots were hand-clipped 3-4 cm above the soil surface. Each biomass sample was sorted into species or functional group (for grasses, where the biomass was not separated into the grass species), dried (60°C for 72 h) and weighed. To determine the agronomic water use efficiency we divided the yield of a single drought period by the total water use (evaporation plus transpiration) in the same period (Gregory et al. 2000).

To assess the influence of drought on the intrinsic WUE the $\delta^{13}C$ signatures of the shoot biomass was measured. The intrinsic WUE is the ratio of assimilated CO_2 and stomatal conductance. When stomata are open, i.e. when sufficient water is available, CO_2 can exchange freely between ambient air and internal stomata space. This effect causes a $^{13}CO_2$ discrimination (meaning $\delta^{13}C$ depletion) in photosynthesis and plant tissue. Under drought stress the stomata close, leading to increased $\delta^{13}C$ signatures in plants (Farquhar et

al. 1989). We selected the strong drought stress period in summer 2010 for these measurements as we expected large discrimination effects here. For the $\delta^{13}C$ analysis, subsamples of the aboveground biomass were ground to 0.2 mm. The isotopic analyses were carried out with an isotope ratio mass spectrometer Finnigan MAT 251 (IRMS; Finnigan, Bremen, Germany), linked via a Conflo II-Interface (Thermo-Finnigan, Bremen, Germany) to an elemental analyser NA1500 (Carlo Erba Instruments, Milano, Italy). The standard was V-PDB, with acetanilide as internal standard. The internal reproducibility of the 13C measurements was better than ± 0.2‰.

The crude protein content was obtained by near infrared spectroscopy (NIRS). Therefore, samples were ground to 1 mm. The spectra were analyzed using the large dataset of calibration samples from different kinds of grasslands by the Institute VDLUFA Qualitätssicherung NIRS GmbH Kassel, Germany (Tillmann, 2010). For calculating the nitrogen concentration (mg g^{-1}), the crude protein was divided by 6.25. Nitrogen concentration and yield were multiplied. Nitrogen fixation was calculated with the help of a difference method (Gierus et al. 2012). Fixation of nitrogen was considered from the nitrogen content in legume-containing swards minus that of non-fixing control swards. As a control sward, we used the mean of all swards not containing *T. repens*.

Statistical Analyses

Statistical data analysis was carried out using the Genstat 6.1 software package and STATISTICA 9.1. Analysis of variance (ANOVA) considered the factors sward and drought stress. Residuals were used to check the validity of the models. Least significant differences (LSD values) were used to compare mean values in case of significant treatment effects (α<0.05). Relationships between selected variables were examined with a linear regression model.
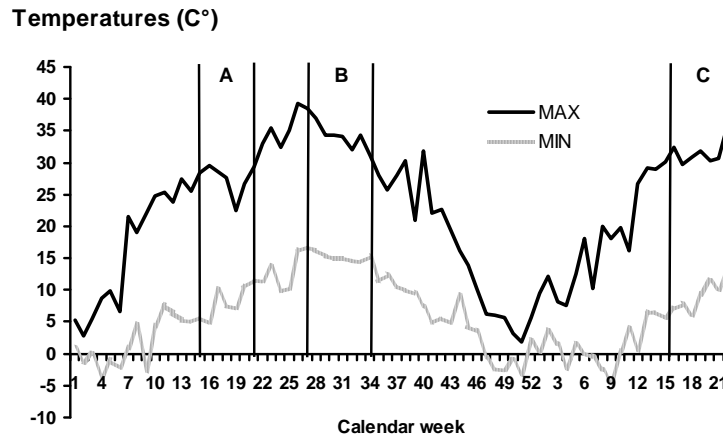
Temperatures (C°)

Figure 1: Weekly air temperature in the vegetation hall from January 2010 to May 2011. The dotted and solid lines represent the mean minimal and maximal temperatures, respectively. Section A – moderate drought stress spring 2010; Section B – strong drought stress summer 2010; Section C – strong drought stress spring 2011.

Results and Discussion

General Drought Stress Effects

Drought stress as well as the factor sward had highly significant effects on the dry matter yield and the agronomic water use efficiency in all drought periods (Table 2 and 3). Production and water use are closely related (Ehlers and Goss 2003) which was also true for our data with R^2 of 0.67 ($P<0.001$) for the whole data set. We also found highly significant interactions for sward and drought on the yield. For agronomic WUE, there was a significant interaction only in spring 2011. On average of the different swards drought stress reduced the dry matter yield by 12% under moderate stress and 27% under strong stress (Table 2). The agronomic WUE was either constant or slightly increased under moderate drought stress but was reduced und strong drought (Table 3). An increase of agronomic WUE with moderate stress could be due to lower transpirational losses and lower stomatal conductance and yet not decreased photosynthesis (Farquhar et al. 1989). The reduction of the agronomic WUE with strong drought stress might have several reasons. The evaporation, i.e. the unproductive water loss, could have been different between the drought stress treatments. We measured evaporation via the water loss of containers that were free of vegetation. Water losses amounted to max. 0.4 kg day^{-1} container^{-1} on the first two days after watering to field capacity. Later on, evaporation decreased rapidly and within two weeks was almost zero. In containers with vegetation, evaporation should be considerably less. Therefore, evaporation is probably not a key

factor explaining decreasing agronomic WUE with increasing drought. Obviously, other reasons have to be considered.

We observed higher mean maximum temperatures under strong stress in summer 2010 and spring 2011, 31°C and 35°C, respectively than under moderate stress. The mean maximum temperature of moderate stress was just 27°C (Figure 1). Such heat stress is likely to have a stronger adverse effect on production when water is limited compared to the regularly watered treatment (Farooq et al. 2009).

The intrinsic WUE was assessed by measuring the $\delta^{13}C$ signature of the harvested biomass. The ANOVA results revealed significant effects of the sward ($F=35.1$, $P<0.001$), the drought stress ($F=65.18$, $P<0.001$), as well as their interaction ($F=2.01$, $P=0.022$). $\delta^{13}C$ values increased under drought (Figure 2) in summer 2010. This obviously is a direct stress response as it displays a change in carbon assimilation and/or stomatal conductance. Increased values of $\delta^{13}C$ (i.e. less negative $\delta^{13}C$) mean increased intrinsic WUE (Farquhar et al. 1989). Although the data from summer 2010 suggest an increased intrinsic WUE with drought stress (Figure 2) there was no such result for the agronomic WUE. In addition, the correlation between $\delta^{13}C$ and agronomic WUE including the data from the drought stress and the control treatments was, albeit significant, low ($R^2=0.13$, $P<0.001$). As Farquhar et al. (1989) point out the intrinsic WUE is only part of the agronomic WUE and there might be other factors having a stronger effect on the agronomic WUE than the intrinsic WUE.

Nitrogen is another important factor for the agronomic WUE. Increasing the nitrogen supply and concentration of plants has positive effects on carbon assimilation, increases leaf area ratio and plant canopy and contributes to reductions of unproductive water losses via leaf over night (Brueck 2008; Brueck and Senbayram 2009). Using the drought stress and control treatment data from summer 2010 a positive relationship between agronomic WUE and nitrogen yield (g N container^{-1}) as well as N concentration (mg g^{-1}) was found ($R^2=0.75$, $P<0.001$ and of $R^2=0.43$, $P<0.001$, respectively) confirming the beneficial role of nitrogen for an efficient water use. Yet, we observed a mean decrease of the nitrogen yield of 21% under drought.

Apart from those effects mentioned above more factors may be responsible for the reduction of the agronomic WUE. Rising severity of drought could increase oxidative stress, as well as membrane and cell damages, change genetic pathways and tissue die-back (Chaves and Oliveira 2004; Farooq et al. 2009) In our experiments we observed increased wilting, tissue die-back and leave losses under strong stress.

Table 2: Dry matter yield of swards at different levels of species richness in three drought stress periods (moderate drought stress in spring 2010 and strong drought stress in summer 2010 and spring 2011); means (n=4) with LSD values. Results from an ANOVA considering the effects sward and drought stress (Control = non-limited water supply)

| Sward | Dry matter yield (g container^{-1}) | | | | | |
| | Spring 2010 moderate | | Summer 2010 strong | | Spring 2011 strong | |
	Control	Stress	Control	Stress	Control	Stress
Monocultures						
Trifolium repens (Tr)	70.5	44.9	60.9	39.1	128.9	57.0
Dactylis glomerata (Dg)	26.4	27.1	37.6	34.4	33.4	32.1
Lolium perenne (Lp)	29.1	27.3	36.1	32.5	38.2	31.4
Plantago lanceolata (Pl)	37.0	36.7	34.9	29.5	32.5	32.0
Taraxacum officinale agg. (To)	19.8	17.1	29.4	27.3	18.5	18.9
Three-species mixtures						
TrDgPl	41.4	34.1	47.9	34.2	121.0	52.4
TrDgTo	35.9	30.5	57.1	34.2	89.7	45.3
TrLpPl	45.4	33.9	62.1	37.9	118.8	52.3
TrLpTo	41.3	37.1	52.7	40.3	118.2	52.7
TrDgLp	34.0	32.4	46.9	40.0	95.2	44.0
TrPlTo	39.3	29.1	51.6	33.1	95.5	37.9
DgLpPl	29.3	27.3	34.0	32.1	31.9	28.7
DgLpTo	29.9	24.7	37.3	30.3	31.6	26.1
DgPlTo	25.4	28.5	33.9	28.5	31.6	27.7
LpPlTo	32.5	30.7	34.9	28.5	29.1	29.3
Five-species mixture						
TrDgLpPlTo	40.7	34.1	52.5	34.8	107.1	47.5
LSD values	5.29		8.14		15.55	
ANOVA	*F*-ratio	*P*	*F*-ratio	*P*	*F*-ratio	*P*
Sward	41.96	<0.001	12.17	<0.001	46.28	<0.001
Drought stress	58.45	<0.001	111.65	<0.001	261.13	<0.001
Sward x Drought stress	6.34	<0.001	3.73	<0.001	15.39	<0.001

Species Richness and Drought Stress Response

Species Richness and Yield

In our study species richness did not show a consistent effect on the productivity of the swards. When water was not-limiting no effect was found in spring period 2010. However, a beneficial effect was found in summer 2010 (R^2=0.45, P<0.05, slope of 3.2) and spring 2011 (R^2=0.84, P<0.001, slope of 14.2). Increasing production (Tilmann et al. 2012) and also no effect (Kahmen et al. 2005) with higher species richness were also mentioned in literature. Positive effects of species richness on productivity can be explained by the so called sampling effect, i.e. an increasing probability of the occurrence of a productive species in the sward with increasing species diversity. The most productive species in the

present experiment was *T. repens*. A positive relationship between the yield contribution of *T. repens* and the dry matter yield of the sward existed of up to R^2=0.94 (*P*<0.001) over the three periods. A rising yield with an increased yield contribution of forage legumes was also found by Carlsson and Huss-Danell (2003). In contrast to the conditions of unlimited water supply, under drought stress a positive species richness and production relation was only found in spring 2011 and not in the other periods. Also the determination of the correlation was lower (R^2=0.71, *P*<0.001, slope of 3.3). An alleviation of the productivity response to drought by an increased phytodiversity was also found by Lanta et al. (2012). On the other hand, Kahmen et al. (2005) did not find any diversity effect on the productivity under drought in a study on permanent grasslands. De Boeck et al. (2008) even report a negative effect of species richness on production under drought stress and they found this to be related to interspecific competition. The observed lower influence of species richness under drought in our experiment could be explained by the marked susceptibility of *T. repens* to water shortage. The monoculture of *T. repens* and the mixtures containing *T. repens* showed reductions in yield of up to 60% (Table 2). This is underpinned by the observation that the relationship between the yield contribution of *T. repens* and the dry matter yield for all three periods decreased to max. R^2=0.73 (*P*<0.001) compared to the control treatment without drought stress. A strong negative stress response of *T. repens* to drought has often been shown (Belaygue et al. 1996; Frame et al. 1998).
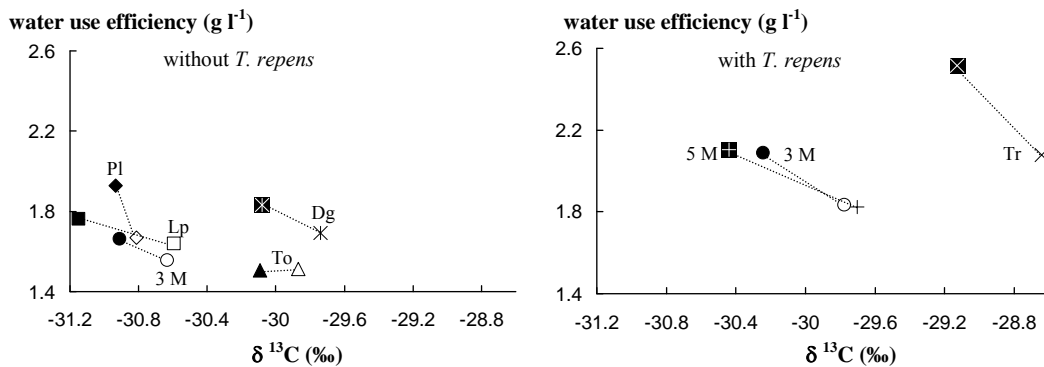
Figure 2: Water use efficiency and δ^{13}C signatures (as indicator for intrinsic WUE) of swards at different levels of diversity; left: monocultures and three-species mixtures without *T. repens* and right: *T. repens*, three-species mixtures with *T. repens* and the five-species mixture under strong drought stress (summer 2010); means (n=4); without *T. repens*: Tr = *T. repens*; 3 M = mean of three-species mixtures with *T. repens*; 5 M = five-species mixture; with *T. repens*: Dg = *D. glomerata*; Lp = *L. perenne*; Pl = *P. lanceolata*; To = *T. officinale* agg.; 3 M mean of three-species mixtures without *T. repens*. Filled symbols show control and open symbols show stress treatment.

Species Richness and Water Utilization

The present experiment revealed a positive relationship between species richness and agronomic WUE under the control treatment in spring 2010, summer 2010 and spring 2011 (R^2=0.68, P<0.01, slope 0.17; R^2=0.34, P<0.05, slope 0.05; R^2=0.83, P<0.001, slope 0.33). Under drought stress the effect of species richness on agronomic WUE was always lower (R^2=0.36, P<0.05, slope 0.07; not significant; R^2=0.7, P<0.001, slope 0.15). As has been demonstrated for the effect of species richness on the production response to drought this finding is obviously related to the presence of *T. repens* and the sampling effect. With an increasing yield contribution of *T. repens* to the swards, N yield (R^2=0.91; P<0.001), N concentration (R^2=0.85; P<0.001) and agronomic WUE increased (R^2 up to 0.87; P<0.001) under control conditions. The influence of *T. repens* to the above named parameters decreased under drought. δ^{13}C values were also affected by the presents of *T. repens* (Figure 2). However, species richness had no effect on agronomic WUE and δ^{13}C. The five-species mixture was comparable to the three-species mixtures containing *T. repens*. Only the time needed to exploit the soil water until a water potential of -0.3 MPa was reached was on average two days faster in the five-species mixture compared to the three-species mixtures containing *T. repens* (Table 1). This, however, did not affect the water use efficiency. But, total water consumption was not different between the five-species mixture and the three-species mixtures containing *T. repens*, irrespective of the water treatment.

Comparable to our results, Rose et al. (2012), in a field experiment, found no relationship between species richness and agronomic WUE as well as water use. In contrast, de Boeck et al. (2006) observed an increased agronomic WUE with higher species richness when temperature and drought were increased at the same time and they explained this with a complementary effect. Different to the results of Rose et al. 2012 and de Boeck et al. 2006, van Peer et al. (2004) reported a proportionally more increased water use than yield in more species rich mixtures under drought, leading perhaps to a decreased agronomic WUE. Our finding that the most species rich mixture exploited the soil water resources earlier than the other swards suggests that the timing of using a limited water resource may also play a role when analyzing the relationship between production and water consumption.

Table 3: Agronomic water use efficiency of swards at different levels of species richness in three drought stress periods (moderate drought stress in spring 2010 and strong drought stress in summer 2010 and spring 2011); means (n=4) with LSD values. Results from an ANOVA considering the effects sward and drought stress (Control = non-limited water supply)

	Water use efficiency (g l^{-1})					
	Spring 2010		Summer 2010		Spring 2011	
Sward	Control	Stress	Control	Stress	Control	Stress
Monocultures						
Trifolium repens (Tr)	3.5	3.5	2.5	2.1	3.6	2.7
Dactylis glomerata (Dg)	2.1	2.3	1.8	1.7	1.7	1.7
Lolium perenne (Lp)	2.4	2.6	1.8	1.6	2.1	1.8
Plantago lanceolata (Pl)	2.4	2.9	1.9	1.7	1.7	1.6
Taraxacum officinale agg. (To)	1.6	1.7	1.5	1.5	1.2	1.1
Three-species mixtures						
TrDgPl	2.8	2.7	2.0	1.7	3.7	2.4
TrDgTo	2.5	2.5	2.1	1.7	2.9	2.2
TrLpPl	3.0	3.2	2.3	1.9	3.5	2.6
TrLpTo	2.9	3.0	2.1	2.0	3.6	2.6
TrDgLp	2.5	2.8	2.0	1.9	3.1	2.2
TrPlTo	2.5	2.4	2.1	1.8	3.2	1.9
DgLpPl	2.1	2.5	1.6	1.6	1.7	1.6
DgLpTo	2.3	2.4	1.7	1.6	1.8	1.5
DgPlTo	2.0	2.4	1.6	1.5	1.7	1.4
LpPlTo	2.3	2.8	1.7	1.5	1.7	1.7
Five-species mixture						
TrDgLpPlTo	2.7	2.9	2.1	1.8	3.4	2.4
LSD values	0.45		0.26		0.40	
ANOVA Summary	*F*-ratio	*P*	*F*-ratio	*P*	*F*-ratio	*P*
Sward	14.03	<0.001	10.79	<0.001	45.77	<0.001
Drought stress	9.58	0.003	36.06	<0.001	126.32	<0.001
Sward x Drought stress	0.55	0.906	1.39	0.169	5.55	<0.001

Drought Stress and Functional Groups

In the present experiment, the dry matter yield and the agronomic WUE of monocultures and mixtures of grasses and forbs was lower compared to the swards that contained *T. repens* (Table 2 and 3). A main reason for this is that no nitrogen fertilizer was applied and *T. repens* contributed nitrogen through nitrogen fixation. An increasing yield contribution of grasses and forbs in the control treatment of all periods had either no or a lightly negative effect on yield and agronomic WUE (R^2 up to 0.27). In general, this result was to be expected (Dierschke and Briemle, 2002; Küchenmeister et al. 2012). However, a similar contribution of grasses and forbs was also found under drought stress. Production and agronomic WUE of swards that consisted only of grasses or forbs or both, was more stable, i.e. was less susceptible to drought. Compared to the control treatment reductions in yield

and agronomic WUE of the drought stress treatment never exceeded 23% and were in most cases not significant. The lower and more stable yield of grass and forb containing swards compared to *T. repens* containing swards could be explained with $\delta^{13}C$ and nitrogen. In general grasses and forbs showed lower intrinsic WUE compared to *T. repens* (Table 2). Also the nitrogen yield and the nitrogen concentration were lower. The decrease of the nitrogen yield due to drought was 12% for swards that consisted only of grasses and forbs whereas losses were 29% for swards that had *T. repens* as a constituent. The larger yield losses of swards containing *T. repens* were due to a decline in nitrogen fixation performance (g N container^{-1}) of 42%. The drought sensitivity of nitrogen fixation is known (Frame et al. 1998). The difference of grasses and forbs on the one hand and the legume on the other is also evident in the overall water consumption and the speed with which the water resources of the soils are exploited. Grasses and forbs had a general lower water use and needed more time to reduce the soil water content to a low level compared to *T. repens* (Table 1). The lower susceptibility of grasses and forbs to drought is also confirmed by the $\delta^{13}C$ data which were much less different between the control and the drought stress treatment as compared to the respective data of *T. repens* (Figure 2). As a result, grasses and forbs in the present experiment seemed to better cope with drought than the swards that contained *T. repens*. In general, programmed moderate water use, also reached by less yields or low agronomic WUE, is an adaptation to drought (Blum 2005; Ehlers and Goss 2003).

In this study we investigated the relationship of drought and species richness as well as functional groups. The hypotheses that species richness would have a clear beneficial effect on the dry matter yield and agronomic WUE under drought could not be confirmed. There was a slight diversity effect when water was unlimited, however, this effect diminished under drought stress. Functional composition, however, had a positive influence on yield and water utilization. The content of *T. repens* strongly affected both, the productivity and the agronomic WUE. Due to a marked susceptibility to drought, this beneficial effect of *T. repens* to the performance of the swards decreased under drought stress. Grasses and forbs had minor effects on yield and agronomic WUE and had a higher tolerance to drought.

Conclusion

To summarize, functional group legume is generally important for productivity of swards. *T. repens* increased yield and agronomic WUE under not limited water supply, but agronomic WUE and production strongly decreased under strong drought because of its drought sensitivity. Also the use of the limited resource water was high and not drought adapted. A more drought tolerant legume may be advantageous for production under predicted climate change. The stable yields and the lower water use of grasses and forbs could be suitable for lower intense production. To forecast the agronomic WUE of different sward, also under drought, nitrogen yield and concentration seem to be good indicators.

Acknowledgment

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Chapter III

Effects of drought stress and sward botanical composition on the nutritive value of grassland herbage

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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, legume) on nutritive value (crude protein, water-soluble carbohydrates, neutral detergent fibre, acid detergent fibre) 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) report increased crude protein (CP) concentrations

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 concentrations for *Lotus corniculatus* and *Trifolium pratense* as well as decreased for *Astragalus cicer*. In opposite, Seguin *et al.* (2002) state 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 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.

Material and Methods

Experimental Details and Treatments

Experimental setup: The experiment was set up 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 had been selected for the experiment. These species 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. agg. wild type (forb). They 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 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 hours. Heating was stopped when the temperature had reached 5°C. Temperatures were recorded daily at three locations in the vegetation hall.

In an earlier paper we examined the germination of the species used in this experiment, the establishment of the swards, the yields and yield contribution of the functional groups. For more detailed information to that topics see Küchenmeister *et al.* (2012).

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 18 Vol. % (-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 ($\alpha < 0.05$). 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 effect (Table 5).

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*

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 fibre

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 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*

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)

Sward[1]	CP Control	CP Stress	WSC Control	WSC Stress	NDF Control	NDF Stress	ADF Control	ADF 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*

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, 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 fibre 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, we found a significant effect of the sward 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 (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. However, our results are in line with those of Seguin *et al.* (2002) who also found 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. Similar

results for NDF were reported by Skinner *et al.* (2004) who found that the botanical composition of a sward had a greater effect than drought. 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 finding of Peterson *et al.* (1992) was attributed to an increased leaf to stem ratio and a reduced plant maturity at harvest when drought was imposed. 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.

We found no interactions 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

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Chapter IV

Influence of drought stress and fertilisation on carbon isotopes as indicators of water use of grassland differing in diversity

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Abstract

Plant diversity in grassland may influence the water use of a sward due to complementary use of the rooting zone. An indicator of the water use of C_3 plants is their natural abundance of the carbon isotope ^{13}C. Without water stress, plants may fractionate more against ^{13}C than with water stress, leading to a stronger depletion of ^{13}C in plant material. We have used this principle to investigate the water use of grassland differing in diversity. In old grassland, diversity was altered three years before this experiment by applying a herbicide against dicots in half of the plots. On sub-plots, different combinations of rainout shelters (yes-no) and N fertilisation (0 or 90 kg ha^{-1}) were established. Differences between drought treatments were clearly reflected in ^{13}C values. N fertilisation led to a further enrichment in ^{13}C, especially in unsheltered conditions. This could be explained by increased biomass production, which might have led to higher water use, and enhanced CO_2 fixation capacity. Unexpectedly, plant diversity did not have a significant influence on the isotopic composition. This was perhaps due to the occurrence of the deep-rooted *Taraxacum* sp. on all plots and the similar species numbers in herbicide-treated and -untreated plots.

Introduction

Climate change will likely increase the occurrence of droughts (IPCC, 2007). To enable sufficiently large production, agricultural management needs to become efficient not only in terms of nutrients and energy, but also in water use. Plant diversity has been suggested to influence the water use of a sward. Different rooting depths of coexisting species increased the water use of more diverse grassland systems (Caldeira *et al.*, 2001). So far, experiments testing for the relationship between phytodiversity and water stress have been conducted on experimental grassland under conditions not readily comparable to agricultural situations. Here, we carried out an experiment on an old grassland sward where diversity was altered by herbicide application. As one indicator of water use, carbon isotopes were measured in plant material. When sufficient water is available and the stomata are wide-open, CO_2 can exchange rapidly between ambient air and the leaf internal spaces. For a given photosynthetic capacity, this effect causes a strong $^{13}CO_2$

discrimination (meaning ^{13}C depletion) in photosynthesis. The reverse occurs under drought, leading to a relative enrichment of ^{13}C in plant biomass.

We hypothesised that diverse grassland would suffer less from drought stress, leading to larger biomass production and depleted ^{13}C values compared to less diverse grassland. N fertilisation should aggravate drought conditions, thus increasing the observed effects.

Material and Methods

The experiment was carried out in 2009 at the experimental farm of the University of Goettingen at Relliehausen on grassland that was at least 14 years old. In September 2006, main plots differing in diversity were established by treating half of them with herbicides against dicots (n = 4). We combined sub-plots with or without rainout shelters and with or without N fertilisation on each main plot, giving four different sub-plots per main plot. Rainout-shelters were installed for five weeks from the beginning of May. N fertilisation was with 90 kg ha^{-1} (calcium ammonium nitrate) one week before installation of the shelters.

As a measure of phytodiversity, the yield percentage of main species was estimated three weeks after establishment of the shelters. Aboveground biomass was harvested two weeks later, dried (60 °C), weighed, milled, and samples measured for 13C on an isotope ratio mass spectrometer. We analysed bulk samples, as earlier measurements gave no significant differences in δ^{13}C between functional groups on these plots. Isotopic values are given as δ^{13}C values:

δ^{13}C [‰] = (^{13}C/^{12}C$_{sa}$ - ^{13}C/^{12}C$_{std}$)/(^{13}C/^{12}C$_{std}$) x 1000, where sa = sample and std = standard (V-PDB).

At the time of harvest, soil samples were taken of the top 10 cm to measure gravimetric water content. Differences between treatments were analysed with ANOVA according to a hierarchical design with diversity on the main plot and drought stress and fertilisation on sub-plots (R, α = 0.05). Correlations were analysed with Pearson's correlation coefficient (Statistica 9.0).

Results

The yield percentage of main dicots was larger on diverse than on herbicide-treated plots (average 21% vs. 3%, Table 1). Irrespective of the herbicide treatment, the dicot biomass

was mainly composed of *Taraxacum sp*. The most abundant species were *Phleum pratense*, *Dactylis glomerata* and *Festuca arundinacea*. Species numbers were similar on both diversity treatments (Table 1).

The rainout shelters led to a significant decrease of the water content in the top 10 cm of soil at the end of the sheltering period (Table 1; 11% vs. 18% and 10% vs. 20% in unfertilised diverse and non-diverse plots, respectively). Fertilisation only significantly reduced the water content on fertilised, unsheltered plots compared to unfertilised, unsheltered plots (15% vs. 18% and 17% vs. 20% for diverse and non diverse plots, respectively). Diversity did not have a significant influence on the water content in the top 10 cm, although there was a trend to lower water content on diverse, unsheltered plots compared to herbicide-treated, unsheltered plots (Table 1).

Table 1: Main results of the experiment. Diversity was manipulated by herbicide treatment against dicots. Drought stress was applied by installation of rainout shelters for five weeks. N fertilisation was carried out on subplots with or without 90 kg ha-1. Shown are means (n = 4). The first two parameters are shown for species with a percentage cover larger than 2%

	diverse				non diverse			
	unsheltered		sheltered		unsheltered		sheltered	
	0 N	90 N	0 N	90 N	0 N	90 N	0 N	90 N
Number main species	8.0	8.0	7.0	7.3	6.3	7.0	6.0	6.8
Yield percentage main dicots [%]	24.5	17.8	31.3	8.8	1.5	4.5	2.3	2.3
Water content 0-10 cm [%]	17.8	15.0	10.7	10.2	19.8	17.0	9.8	9.4
Biomass [g/m²]	451.4	581.5	288.4	458.3	437.4	565.1	360.5	434.7
$\delta^{13}C$ [‰]	-28.1	-27.7	-27.4	-26.2	-28.2	-27.8	-27.4	-26.6

The biomass production was not significantly influenced by plant diversity (Table 1). It was, however, significantly larger on unsheltered, fertilised relative to unfertilised plots (573 vs. 444 g m^{-2}) and smaller on sheltered, unfertilised plots (325 g m^{-2}). The biomass production on sheltered, fertilised plots did not differ significantly from that on the other treatments (447 g m^{-2}).

Plant diversity did also not affect ^{13}C values significantly (Table 1). Biomass on sheltered plots was significantly less depleted in ^{13}C than that on unsheltered plots. N fertilisation led to a significant further enrichment of ^{13}C.

There was a significant correlation between soil water content in the top 10 cm and the $\delta^{13}C$ values of the vegetation ($P < 0.05$; $r = -0.612$). However, there was no significant

correlation between biomass production and soil water content, while percentage cover and soil water content ($P < 0.05$, $r = 0.475$) as well as percentage cover and biomass production ($P < 0.05$, $r = 0.644$) revealed significant correlations.

Discussion

Unexpectedly, phytodiversity had no significant effect on water content in the top 10 cm of soil, biomass production, or the ^{13}C value as an indicator of water use. This could have been due to the occurrence of *Taraxacum sp.* on all plots. With its deep roots, this species could have allowed a complementary use of the soil resources, including water, also on the herbicide-treated plots. However, as its yield percentage was much lower on herbicide treated than -untreated plots, the missing effect of phytodiversity could also be due to the comparable number of species in both diversity treatments (Table 1).

N fertilisation increased biomass production as expected on rainfed plots. At the same time, soil water content decreased and $\delta^{13}C$ increased. Probably, the increased biomass led to a larger use of water (indicated by the correlation between percentage cover and soil water content), in turn increasing water stress for the plants, leading to the observed ^{13}C values. Furthermore, an increase in Rubisco and thus CO_2 fixation capacity following N fertilisation could have caused the increased $\delta^{13}C$. A similar effect of fertilisation on biomass production and soil water was missing under rain-sheltered conditions. Here, the reduced water content, which was not affected further by fertilisation, probably limited a significant increase in biomass production. However, there was a trend towards a larger biomass production, influencing ^{13}C values.

Conclusions

We did not find indications for increased productivity or improved water use with higher phytodiversity in this old grassland system, perhaps due to the similar species numbers and the occurrence of deep-rooted *Taraxacum sp.* on all plots. Therefore, the experiment should be repeated in grassland showing larger differences in plant composition before conclusions are drawn concerning the importance of biodiversity in old grassland with respect to water stress. N fertilisation was shown to increase water stress of grassland vegetation, probably due to enhanced biomass production and CO_2 fixation capacity.

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General Discussion

In the context of prognosticated climate change, water for agricultural production is predicted to become limiting especially due to temporal water shortage during the vegetation period (IPCC 2007). Particularly grassland will be affected by drought (Hopkins and Del Prado 2007). Therefore, adaptation strategies to secure future herbage production from grassland are necessary. Species richness and functional group composition are often proposed to increase production, stabilize yield and providing numerous ecosystem services (Isbell et al. 2009; McLaren and Turkington 2010; Isbell et al. 2011). But the effect of drought on production, water utilization and nutritive value as well as the interactions with species richness and functional group composition are still being discussed and yet not clear.

So, this study was conducted to provide information about drought stress, species richness and functional group composition and their influence on production, stability, water utilization and nutritive value. We wanted to answer the questions: 1) Do species richness and/or functional group composition have a general influence on yield and yield stability? 2) Do species richness and/or functional composition have a positive influence on yield and water utilization even under drought stress? 3) Does drought stress have an effect on nutritive value of grassland herbage and will species richness and functional group composition interact and modify quality under drought conditions? 4) Does diverse grassland suffer less from drought stress under field conditions than less diverse grassland? Generally, drought stress influenced most agricultural features negatively and the impact was dependent on the strength of stress (Jaleel et al., 2009; Farooq et al., 2009). Moderate drought stress in the vegetation hall decreased yield about 12 % (max. 36 %) on average and the reduction under both strong stresses was 27 % (max. 60 %) on average. Significant yield reductions were also found in the field experiment. There were only low positive or no effects of moderate drought stress on agronomic water use efficiency (WUE; relation of yield and water use) while there were significant decreases under strong stress (up to 41 %). Farooq et al. (2009) summarized that drought stress could influence WUE either positively or negatively. Increase in agronomic WUE under moderate stress in the vegetation hall could be an adaptation (Blum 2005) because in our study water consumption decreased more than yield. Reduced transpiration does not necessarily lead to a lower photosynthesis capacity (Farquhar et al. 1989). In contrast, strong drought stress decreased yield more than water consumption.

Generally, nitrogen is a good indicator for WUE (Brueck 2008; Brueck and Senbayram 2009). Nitrogen yield (g N container^{-1}) and nitrogen concentration (mg g^{-1}) were suitable indicators for agronomic WUE in our study. Increasing concentrations of nitrogen yield ($R^2 = 0.75$; $P < 0.001$) and nitrogen concentration ($R^2 = 0.43$ $P < 0.001$) led to increased agronomic WUE. However, we measured a decrease in nitrogen yield of 21 % under drought in summer 2010. In contrast, signatures of $\delta^{13}C$, as indicators for intrinsic WUE, which means the ratio of assimilated CO_2 and stomatal conductance (Farquhar et al. 1989), were less suitable for explaining agronomic WUE in our study.

Of course, there are more impacts on yield and WUE under drought (Farooq et al. 2009). Any additional stress, like e.g. high temperatures measured in both strong drought stress periods, might enhance negative drought effects (de Boeck et al. 2008; Farooq et al. 2009). So, the impact of drought stress on yield and WUE already started under moderate stress conditions, while even under stronger stress no or minor immediate or residual effects on nutritive value were obvious. As expected (e. g. Buxton 1996; Seip et al. 2011), swards differed in nutritive value due to their botanical composition. Mixture effects may, therefore, obscure any drought effect. Those effects are due to the varying amount of sward components with either a low or a high nutritive value. Drought stress did not influence the variability of the nutritive value over growing season either. Impact of drought was less imposed than seasonal effects. Seasonal growth patterns with fluctuations in yield contributions and changes in nutritive value with varying maturity within the growing period of grassland plants are well known (Buxton 1996; Suleiman et al., 1999; Skinner et al., 2004).

Species richness did not influence nutritive value or yield stability over the growing season. The positive influence of species richness on yield and agronomic WUE could be explained by a sampling effect. The yield contribution of well performing *T. repens* increased with species richness. An increasing *T. repens* contribution led to high nitrogen yield and concentration and thus to an increased yield (R^2 of up to 0.94; $P < 0.001$) and WUE (R^2 up to 0.87; $P < 0.001$). Because *T. repens* is drought sensitive (Frame et al. 1998), the influence of the legume and, thus, of species richness decreased under drought in our study. The performance of the species rich sward was comparable to less diverse, but legume containing swards. However, a higher species richness tended to increases water use rapidity (days to reach -0.3 MPa) in the vegetation hall and showed tendency to decease soil water content on diverse, unsheltered plots in the field experiment. The "insurance hypothesis" (Hector et al. 2010) could not be confirmed. Insurance means that

species richness can stabilize ecosystem functioning against environmental changes. There are a lot of studies which underline the positive impact of species richness even under stress conditions (Isbell et al. 2009; Tilman et al. 2012; Vogel et al. 2012). Species richness should maintain ecosystem services e.g. positive species interactions also during different environmental change scenarios (Isbell et al. 2011), complementary resource use (Spehn et al. 2000; Loreau and Hector 2001; Eisenhauer 2012), different plant functional traits (Gubsch et al. 2011) and a high forage quality (Bullock et al., 2007). But our results rather support the investigations of Kahmen et al. (2005), Wrage et al. (2011), Rose et al. (2012) and Seither et al. (2012) which did not found consistent effect of species richness on yield, nutritive value and water utilization, neither in mown nor in grazed grassland. We did not detect any no negative influence of species richness on production like de Boeck et al. 2008 or decreased resistance to stress like Allison 2004. But here were hints in our studies and in literature (van Peer et al. 2004) that water use could be increased and faster in more species rich swards without enhancing production in an equal relation.

The functional group composition influenced the agronomic performance of the swards. A positive impact of the functional group legume was especially visible in yield, WUE and crude protein concentration because of their ability to fix nitrogen (Frame 1998). But increasing legume content in the sward increased yield variability over the growing season and water use. Under field conditions, nitrogen fertilization reduced the soil water content. Grass and forb seemed to have a wider tolerance to soil moisture and stabilized yield and water use also under drought, but had lower yield and WUE. Crude protein was increased by forb whereas water-soluble carbohydrates and fibre components were increased by grass, both under drought and non-limited water supply. In general, our results support the findings of McLaren and Turkington (2010) and Sanderson (2010) who summarized that sward composition could be more important for yield and stability than species richness. Our results suggest that the functional composition of swards is also more important for nutritive value than species number. Furthermore, management, like in our case nitrogen application in the field, had a more pronounced effect on yield than number of species.

To sum up, drought stress could lead to substantial decreases in yield and WUE dependent on the strength of stress, while nutritive value is hardly affected. For future sustainable herbage production, species richness is less important than an adapted functional composition and management. Legume increased production and water utilization but stability the swards decreased. In contrast, grass and forbs stabilize yield and water utilization but on a lower level. The drought sensitive *T. repens* may perhaps be replaced

by more drought resistant legumes (Küchenmeister 2013). Our experiments provide valuable information about the influence of species richness and functional groups on yield, yield stability, water utilization and nutritive value under well watered and drought stress conditions.

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Summary

Producing grassland herbage with high yield and good nutritive value is a prerequisite of an efficient ruminant livestock production. Due to climate change, precipitation patterns are expected to vary and the frequency of extreme weather events like droughts are supposed to increase. Productive grassland requires a particularly sufficient and regular water supply during the growing season. Thus, grassland production, yield stability and nutritive value will be affected by drought. Because of this, adaptation strategies are necessary to ensure a sustainable future herbage production from grassland.

Increasing plant biodiversity has been proposed as a way to improve ecosystem functions like productivity and nutritive value in grassland. There is an ongoing discussion on species richness' reactions to stress, especially drought stress, and on how productivity, nutritive value and water utilization are affected. Other investigations have stressed species identity and composition of functional groups as important factors for productivity and nutritive value.

We, therefore, conducted a drought stress experiment in a vegetation hall from July 2009 to June 2011. The climatic conditions followed the normal seasonal pattern with frost in winter and higher temperatures in summer. Different drought stress conditions were performed over three periods in two growing seasons. Drought stress was induced by temporarily ceasing the watering of the containers after initial watering while soil water availability could be controlled. We chose productive agricultural species of temperate grasslands. Plants were sown in monoculture and three- and five-species mixtures and included the three functional groups legume (*Trifolium repens* L.), grass (*Lolium perenne* L., *Dactylis glomerata* L.) and forb (*Plantago lanceolata* L., *Taraxacum officinale* F.H. Wigg. agg.). This range of species number has been shown to affect productivity in biodiversity experiments. Effects of species richness and functional composition on yield, yield stability, water utilization and nutritive value (crude protein, water-soluble carbohydrates, neutral detergent fibre and acid detergent fibre) were examined. As indicators for the performance of yield and particularly agronomic water use efficiency (relation of yield and water use), we used nitrogen yield and concentration of swards as well as signatures of $\delta^{13}C$ under not limited water supply and drought. Furthermore, in 2009, we conducted a short term field experiment at the experimental farm of the University of Goettingen at Relliehausen on old grassland. Here, influence of drought and species richness on yield and water utilization were also investigated.

Our data show that drought stress lowered production and influenced water utilization. Both increased with the severity of the stress. Agronomic water use efficiency was unchanged or slightly increased under moderate stress but decreased under strong drought stress. Nitrogen yield and concentration were suitable indicators for agronomic water use efficiency while $\delta^{13}C$ was less appropriate. Agronomic water use efficiency was increased by nitrogen. There was no or minor immediate impact of drought on nutritive value. Seasonal effects on nutritive value were more distinct. Generally, yield decrease of grassland herbage seems to be more important than changes in nutritive value.

Species richness did not influence nutritive value or yield stability over the growing season. The occasional positive influence of species richness on yield and agronomic WUE and decrease of the influence under drought could be explained by a sampling effect. Well performing but drought sensitive legume increased with species richness. There was a hint that species richness also increased the rapidity of water use. Results of the field experiment support these data concerning drought effects, yield and water utilization. The "insurance hypothesis" that species richness can stabilize ecosystem functioning against environmental changes could not be confirmed.

But functional groups composition of swards was an important determinant of performance in both unlimited water supply and drought stress. Especially legume had a positive influence on yield, agronomic water use efficiency and crude protein concentration but increased water use and seasonal variability. Grass stabilized yield and water use and increased water-soluble carbohydrates and fibre components while yield and agronomic water use efficiency decreased under the nitrogen limited conditions of our experiment. The functional group forb showed quite similar results regarding yield and water utilization but increased crude protein.

Our results indicate that the predicted increase of droughts will reduce production and lead to a change in water utilization in productive grassland. Alteration in nutritive values will not be as important as decrease in yield. The investigated species richness seems to be less important than functional composition of grassland swards for production, water utilization and nutritive value. Thus, to cope with future climate change, an adapted sward composition might be one possibility to ensure ruminant livestock production from grassland.

Zusammenfassung

Grünlandfutter mit einem hohen Ertrag und gutem Futterwert ist eine Grundvoraussetzung für die effiziente Produktion von wiederkäuenden Nutztieren. Im Zuge des prognostizierten Klimawandels werden sich die Niederschlagsmuster ändern und das Auftreten von Extremwetterereignissen, wie temporärer Trockenheit, wird sich erhöhen. Besonders produktives Grünland benötigt aber eine ausreichende und regelmäßige Wasserversorgung während der Wachstumsperiode. Deshalb werden die Futterproduktion von Grünland, die Ertragsstabilität und der Futterwert von temporärer Trockenheit beeinflusst werden. Aus diesem Grund sind Anpassungsstrategien nötig, um eine zukünftige und nachhaltige Grünlandfuttererzeugung zu sichern.

Erhöhte pflanzliche Biodiversität wird oft als Möglichkeit angesehen, Funktionen von Ökosystemen, wie Produktivität und Futterwert, im Grünland zu verbessern. Es gibt eine fortlaufende Diskussion wie eine erhöhte Artenzahl auf Stress, besonders Trockenstress, reagiert und wie dabei Produktivität, Futterwert und Wassernutzung beeinflusst werden. Andere Untersuchungen zeigten, dass Artidentität und die Zusammensetzung der funktionellen Gruppen wichtige Faktoren für Produktivität und Futterwert sind.

Auf Grund dessen haben wir von Juli 2009 bis Juni 2011 ein Trockenstressexperiment in einer Vegetationshalle durchgeführt. Verschiedene temporäre Trockenstressereignisse wurden in drei Aufwüchsen in zwei Vegetationsperioden durchgeführt. Die klimatischen Verhältnisse in der Vegetationshalle folgten normalen saisonalen Verläufen mit Frost im Winter und höheren Temperaturen im Sommer. Trockenstress wurde induziert, indem, nach einer anfänglichen Bewässerung, die Wasserversorgung für einen bestimmten Zeitraum eingestellt wurde. Die Wasserverfügbarkeit des Bodens konnte dabei immer kontrolliert werden. Für das Experiment wählten wir ertragsstarke und landwirtschaftlich nutzbare Arten des Grünlands der gemäßigten Zonen aus. Die Arten wurden in Monokultur und Drei- sowie Fünfartenmischungen gesät und enthielten die funktionellen Gruppen Leguminose (*Trifolium repens* L.), Gras (*Lolium perenne* L., *Dactylis glomerata* L.) und Kraut (*Plantago lanceolata* L., *Taraxacum officinale* F.H. Wigg. agg.). Der von uns gewählt Umfang der Artenzahl zeigte schon in anderen Biodiversitätsexperimenten einen Einfluss auf die Produktion. Untersucht wurden die Effekte von Artenzahl und funktionellen Gruppen auf Ertrag, Ertragsstabilität, Wassernutzung und Futterwert (Rohprotein, wasserlösliche Kohlenhydrate, neutrale und saure Detergenzienfasern). Als Indikatoren für die Ertragsentwicklung und die agronomische Wassernutzungseffizienz (Verhältnis von Ertrag zu Wasserverbrauch) dienten der Stickstoffertrag und die

Stickstoffkonzentration der Bestände sowie $\delta^{13}C$ Signaturen, sowohl mit unlimitierter Wasserversorgung als auch mit Trockenstress. Überdies führten wir 2009 ein Kurzzeitfeldexperiment auf einem alten Grünlandbestand auf dem Versuchsgut der Universität Göttingen in Reliehausen durch. In diesem Versuch wurde ebenso der Einfluss von Trockenstress und Artenzahl auf den Ertrag und die Wassernutzung untersucht.

Unsere Daten zeigten, dass Trockenstress die Produktivität verringert und die Wassernutzung beeinflusst, beides abhängig von der Stärke des Stresses. Bei moderatem Stress war die agronomische Wassernutzungseffizienz unverändert oder stieg leicht an, bei starkem Stress verringerte sie sich jedoch. Der Stickstoffertrag und die Stickstoffkonzentration waren brauchbare Indikatoren für die agronomische Wassernutzungseffizienz, wohingegen $\delta^{13}C$ weniger geeignet war. Die agronomische Wassernutzungseffizienz wurde von Stickstoff erhöht. Es gab keinen oder nur einen sehr geringen Einfluss von Trockenstress auf den Futterwert. Saisonale Effekte hatten mehr Einfluss auf den Futterwert. Allgemein scheint der Ertragsrückgang wichtiger als die Veränderungen des Futterwerts zu sein.

Die Artenzahl beeinflusste den Futterwert und die Ertragsstabilität über die Vegetationsperiode nicht. Mit Hilfe des "sampling effect" (Probennahmeeffekt) können der manchmal positive Einfluss der Artenzahl auf den Ertrag und die agronomische Wassernutzungseffizienz und der Rückgang dieses Einflusses unter Trockenheit erklärt werden. Mit erhöter Artenzahl stieg der Anteil der leistungsfähigen, aber trockenheitssensitiven Leguminose. Weiterhin gab es einen Hinweis, dass die Artenzahl die Geschwindigkeit des Wasserverbrauchs erhöht. Die Ergebnisse des Feldexperiments bekräftigten die Befunde bezüglich der Effekte des Trockenstresses, des Ertrages und der Wassernutzung. Aus diesen Gründen kann die „insurance hypothesis" (Versicherungshypothese), die besagt, dass eine erhöhte Artenzahl Ökosystemfunktionen gegenüber Umweltveränderungen stabilisieren kann, nicht bestätigt werden.

Jedoch waren die funktionellen Gruppen wichtige und bestimmende Faktoren der Leistung unter nicht Wasser limitierten Bedingungen und Trockenstress. Die Leguminose hatte besonders auf Ertrag, agronomische Wassernutzungseffizienz und Rohprotein einen positiven Einfluss, jedoch erhöhte sie auch den Wasserverbrauch und die saisonale Variabilität. Gräser stabilisierten den Ertrag und den Wasserverbrauch und erhöhten die wasserlöslichen Kohlenhydrate sowie die Faserfraktionen, während sie den Ertrag und die agronomische Wassernutzungseffizienz unter den stickstofflimitierten Bedingungen

unseres Experiments verringerten. Die funktionelle Gruppe Kräuter zeigte ähnliche Ergebnisse bezüglich Ertrag und Wassernutzung, aber sie erhöhte das Rohprotein.

Unsere Ergebnisse demonstrieren, dass die vorhergesagte Zunahme von Trockenstressereignissen die Produktion reduzieren und die Wassernutzung ändern wird. Änderungen im Futterwert werden dabei weniger wichtig als der Ertragsrückgang sein. Für die Produktion, die Wassernutzung und den Futterwert wird die Artenanzahl weniger relevant sein als die funktionelle Zusammensetzung von Grünland. Deshalb wird eine angepasste Grasnarbenzusammensetzung für die Sicherung der Produktion von wiederkäuenden Nutztieren unter den Bedingungen des erwarteten Klimawandels Bedeutung erlangen.

Curriculum Vitae

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Education and Professional Career

Since 2011	Cooperation with FU Berlin, Department of Veterinary Medicine, Institute of Parasitology; Project: Interactions of drought, plants and soil with *Cooperia oncophora*
Since 2009	PhD student at University of Göttingen; Thesis: Sustainable grassland herbage production under drought stress – the role of plant species number and functional group composition (in English)
2008-2009	Research assistant at University of Göttingen; Project: Suitability of plant species for establishing swards for free range poultry management
2008	Research consultant Arpolith GmbH at National Agriculture and Animal Resources Research Centre, Riyadh, Kingdom of Saudi-Arabia
2006-2009	Master student at University of Göttingen, Agricultural Science/Crop Science; Thesis: Water stress and crop growth: Testing the effectiveness of two soil enhancers (in English); M.Sc.
2003-2006	Bachelor student at University of Göttingen, Agricultural Science/Crop Science; Thesis: Stable isotope in pasture systems: A prediction of possible nutrient losses? (in German); B.Sc.
2002-2003	Compulsory Military Service; awarded: Saxon Order of Flood Relief; German Armed Forces Flood Service Medal
2002	A-Levels (Abitur) at Johann-Gottfried Seume Gymnasium Vacha

Teaching Experience

Advised Thesis:	4 B.Sc.-Thesis and 3 M.Sc.-Thesis
Teaching Assistant 2008-2009:	Scientific working and presentation skills in crop production (in German) at University of Göttingen, Agricultural Science/Crop

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Mentor for A-level students at Johann-Gottfried Seume Gymnasium Vacha

Country Experience

09/2012	Field trip trough Kyrgyzstan; Course in pasture and water management in Kyrgyz agriculture; Kyrgyz State Agriculture University, Bishkek
08/2011	Field trip trough Siberia, Altai region; Course in Economic and Social Geography of Altai State University
01/2008; 04/2008-06/2008	Research project for Arpolith GmbH at Kingdom of Saudi-Arabia, Riyadh, Ministry of Agriculture, Department of Research and Development, National Agriculture and Animal Resources Research Centre
07/2006-08/2006	Field trip trough European Russia; Course in Soil Science, Geography and Agriculture University of Bayreuth

Language Skills

German	Native speaker
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Further Qualifications

Professional Membership:	Association of German Engineers (VDI)
Relevant Courses:	Treatment of archaeological objects (in German); Dendrochronology (in German); Measurements of biodiversity (in German); Statistics and STATISTICA; Scientific writing
Internships:	Work on farm Agrarvereinigung e.G. Rhönpforte Sünna, Germany Work at brewery Rhönbrauerei Kaltennordheim, Germany
Hobbies:	Rennsteig cross run (12 participations); Fulda 84km March (4 participations); travelling; history and culture; old trades

Publications

Peer-reviewed Journals

Küchenmeister, K., Küchenmeister, F., Kayser, M., Wrage-Mönnig, N., Isselstein, J., 2013. Influence of drought stress on nutritive value of perennial forage legumes. *International Journal of Plant Production*, in press.

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Conference Proceedings

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Other publications

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Acknowledgement