The influence of drought stress on yield and forage quality of grasslands differing in functional composition

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A mi me gusta recordarlo

aunque a veces sea con dolor

después de todos estos años

no se olvide de su valor

- Ungestüm

Table of Contents

1 General Introduction

Grasslands cover about 25 % of all terrestrial ecosystems (Ojima *et al.*, 1993). Permanent grasslands cover more than 70% of the agricultural area worldwide (Panunzi, 2008), and about 30% or more of the agricultural area in Europe (Smit *et al.*, 2008) and in Germany (Statistische Ämter des Bundes und der Länder, 2011). They are a key component of ruminant forage supply and therefore a major resource for livestock and dairy production (Beever and Reynolds, 1994; Hopkins and Wilkins, 2006; Martin et al, 2005). They also provide various ecosystem services, such as soil protection, water quality, nutrient cycling, and conservation of biodiversity (e.g. Wrage *et al.*, 2011) and are superior to other crops in providing those services (Werling *et al.*, 2014). Soil fertility as well as botanical composition and biodiversity are key services necessary to maintain agricultural productivity and quality (Zhang *et al.*, 2007). It is paramount to understand the interplay of nutrient supply and diversity and its reaction to disturbance in order to maintain the current high level of productivity and quality of grassland forage and to accomplish a sustainable management of permanent grasslands. This will guarantee a continuous provision of both good forage supply and ecosystem services (Zhang *et al.*, 2007). A rising awareness for sustainability among farmers along with their preference for efficient adaption rather than crop change (Olesen *et al.*, 2011) underline the need for suitable management strategies of permanent grasslands.

1.1 Why diversity matters in grasslands

Managing old permanent grasslands is a complex task because they can react differently to external influences than sown-in grasslands due to their diversity, more complex root structure and general stability (Balvanera *et al.*, 2006; Flombaum and Sala, 2008; Zhang *et al.*, 2013). Unlike other crops, permanent grassland consists of a mixture of different plant species that all contribute to the final product. The properties of forage gained from grasslands are influenced by the quantity and quality each plant species contributes directly via its biomass. But grassland forage productivity and quality are also determined by the interactions of the plants in the sward and by their differing reaction to external influences. Therefore, diversity plays a key role in understanding swards and their ecosystem functioning, especially in terms of productivity and quality (Balvanera *et al.*, 2006; De Boeck *et al.*, 2008; Isbell *et al.*, 2011; Kahmen *et al.*, 2005; Kreyling *et al.*, 2008a; Pfisterer and Schmid, 2002; van Ruijven and Berendse, 2010).

Many studies have shown an increase of productivity with increasing species richness (e.g. Cardinale *et al.*, 2007; Hector *et al.*, 1999; Kahmen *et al.*, 2005; Tilman, 1996; Tilman *et al.*, 2006). Other field studies could not confirm this relationship (e.g. Loreau *et al.*, 2001; Mittelbach *et al.*, 2001; Mulder *et al.*, 2004), while some found a positive relationship only for a certain range of species number and biomass (Adler *et al*, 2011). Many examples of a positive impact of species richness on productivity come from young and sown-in grasslands (e.g. Naudts *et al.*, 2011; Pfisterer and Schmid, 2002), or comparatively species-poor pot experiments (e.g. De Boeck *et al.*, 2008; Dreesen *et al.*, 2014; Küchenmeister *et al.*, 2012; Van Peer *et al.*, 2004; Van Ruijven and Berendse, 2010). These conflicting results from artificial grasslands might arise from their specific or random assemblage of plant species. Studies on single agriculturally important species usually simplify the complex structures of plant-plant interactions that take place in permanent grasslands (Mariotte *et al.*, 2013). Another explanation for a positive effect of species richness on productivity, which can occur in both artificial and permanent grasslands, is the sampling effect (Kahmen *et al.*, 2005; Tilman and Downing, 1994) which states that more diverse swards have a larger chance to include a species that mainly contributes to the positive effect. Although the insurance hypothesis (Yachi and Loreau, 1999), which states that biodiversity insures ecosystems against decline in their functioning because a larger species richness provides a larger guarantee that some species will maintain functioning even if others fail, is widely accepted, the replacement of one biological entity by another to maintain ecosystem functioning under stressful conditions is a general characteristic of a stable plant community (Balvanera *et al.*, 2006; Díaz and Cabido, 2001; Wardle *et al.*, 2000; Wright *et al.*, 2006). Therefore, species identity, species composition, and dominance structures are more important for understanding grassland ecosystem functioning than species richness alone (Díaz and Cabido, 2001; Mariotte *et al.*, 2013; Sanderson, 2010; Sasaki and Lauenroth, 2011). Often, ecosystem processes and services depend more on functional composition than on species richness or identity (Cadotte *et al.*, 2011; Cardinale *et al.*, 2012). As a consequence, grouping species by their traits and functions is useful to better assess the influence of diversity on grasslands.

1.2 Functional diversity as a key to understand grassland functioning

The three main functional groups of plant species in temperate grasslands are grasses, forbs, and legumes (Schellberg and Pontes, 2012). Larger functional group richness generally leads to larger productivity (Grime *et al.*, 1997; Hector *et al.*, 1999; Hooper and Vitousek, 1998; Hooper and Dukes, 2004; Marquard *et al.*, 2009; Wardle *et al.*, 1997). This positive relationship between productivity and functional diversity has been found in permanent grasslands (e.g. Hector *et al.*, 1999; Mariotte *et al.*, 2013) as well as in experiments with sown-in swards or mesocosms (e.g. Díaz and Cabido, 2001; Küchenmeister *et al.*, 2013; Marquard *et al.*, 2009; Reich *et al.*, 2004; Tilman *et al.*, 1997).

While good yields are important for agricultural production, harvesting forage with low fibre content and high concentration of protein is desirable as well in order to produce high quality ruminant forage. Feed value and quality parameters for the main forage grasses and legumes as well as for some forbs are known (e.g. Baumont *et al.*, 2008; Fulkerson *et al.*, 2007; Wilman and Riley, 1993). The forage quality of permanent grassland swards is variable and the presence of dicots can both increase or decrease overall quality depending on the plant composition (Bruinenberg *et al.*, 2002; Donath *et al.*, 2004; Seither *et al.*, 2012). Many studies associate the variable influence of functional groups on quality with differences in maturation stage at the time of cut (Bruinenberg *et al.*, 2002; Buxton, 1996). Forbs generally have a better forage quality than grasses (Bruinenberg *et al.*, 2002; Duru *et al.*, 2008). While some studies could not confirm the positive influence of presence of forbs on forage quality (Hofmann and Isselstein, 2005; Sturludóttir *et al.*, 2014), the presence of legumes in artificial and permanent swards reliably increases the forage quality (e.g. Buxton, 1996; Nyfeler *et al.*, 2011; Sanderson, 2010; Sleugh *et al.*, 2000; Suter *et al.*, 2015; Zemenchik *et al.*, 2002).

Zemenchik *et al.* (2002) found that this positive effect of legumes even exceeds management factors such as nitrogen (N) fertilization.

Facilitation and better niche complementarity cause the positive effect of functional diversity: In a sward that consists of several functional groups, the species' niches among groups will complement each other. This leads to reduced competition and a better use of available resources, and subsequently to better growing conditions for plants in diverse swards than in species-poor swards or monocultures (Cadotte, 2013; Cardinale *et al.*, 2007; Flynn *et al.*, 2011; Hector *et al.*, 1999; Loreau and Hector, 2001; Naeem, 2002; van Ruijven and Berendse, 2005). Presumably, niche complementarity is smaller between species that belong to the same functional group than between species belonging to different ones (Díaz and Cabido, 2001). A well-known and thoroughly-investigated example for complementarity between species from different functional groups is the increase of whole-sward productivity, forage quality and resource efficiency in the presence of the legume functional group (Peyraud *et al.*, 2009; Sleugh *et al.*, 2000). Legumes are able to fix atmospheric nitrogen (N_2) symbiotically into water soluble compounds and transfer up to 320 kg N ha^{-1} yr^{-1} of plant available N to neighboring plants (Huguenin-Elie *et al.*, 2009; Pirhofer-Walzl *et al.*, 2012). N_2 fixation is generally regulated by a sink/source mechanism (as reviewed in Lüscher *et al*, 2014); in grass-legume mixtures, the N transfer from legumes to grasses can be stimulated by the grasses (Nyfeler *et al.*, 2011). This N transfer leads, together with below-ground niche complementarity, to a better N exploitation and larger plant uptake of available N and thus to a larger productivity and sometimes even transgressive overyielding as well as to better forage quality in swards that contain legumes (Husse *et al.*, 2017; Lüscher *et al.*, 2014; Mulder *et al.*, 2002; Peyraud *et al.*, 2009; van Ruijven and Berendse, 2005). Therefore, the interaction between legumes and other functional groups has been identified as one of the major mechanisms of ecosystem functioning in grasslands (Hooper *et al.*, 2005; Kirwan *et al.*, 2009; Nyfeler *et al.*, 2009; Spehn *et al.*, 2002; Temperton *et al.*, 2007). Yet, most observations of positive effects of legume presence derive from artificial mixtures with cultivars of only few species, mainly productive forage grasses like *Lolium perenne* with a well-researched legume partner like *Trifolium repens* and *Trifolium pratense*. Further research on interactions with species from functional groups other than legumes and grasses is needed to identify optimal strategies to use niche complementarity for enhancing resource exploitation and thus productivity (Lüscher *et al.*, 2014). The effect of richness of other functional groups than legumes and grasses in permanent grassland systems is not well understood (Morais and Cianciaruso, 2014), although mixture experiments point towards a positive effect (e.g. Husse *et al.*, 2017; Küchenmeister *et al.*, 2013; Skinner *et al.*, 2004; van Ruijven and Berendse, 2003).

The influence of functional group composition on productivity and quality is not always stable but can be modified by disturbance (Cadotte *et al.,* 2011; Díaz and Cabido, 2001). To have a resistant system, which was defined as a continued system functioning at a high level when the system is disturbed (Pimm, 1984), is an important property of agricultural grassland use. Another desirable aspect for farmers is a quick system recovery to the status prior to the disturbance, *i.e.* a very resilient system (Pimm, 1984). Therefore, identifying high-impact disturbances as well as the swards' level of resilience and resistance in terms of productivity and quality is vital for developing a sustainable management strategy for permanent grasslands.

1.3 Drought stress as a future challenge for grasslands

One of the most important and most severe disturbances of grassland systems is drought stress because it impairs plant growth and nutrient uptake (Beierkuhnlein *et al.*, 2011; Craine *et al.*, 2012; Fay *et al.*, 2011; Hoover *et al.*, 2014; Tilman and Downing, 1994). The definition of drought is a below-normal precipitation over a temporary period of time, with the dryness being relative to the local normal condition (Dai, 2011). In Central Europe, climate change will lead to a larger variability of temperatures and precipitation rather than to changes of their mean values (IPCC, 2013). Therefore, heat waves and accompanying drought events will become more frequent, more severe, and last longer starting from the second half of the 21st century (Beniston *et al.*, 2007; Easterling *et al.*, 2000; IPCC, 2013; Katz and Brown, 1992; Meehl and Tebaldi, 2004; Schär *et al.*, 2004). These droughts will occur during summer, which makes up the major part of the growing season in Central Europe (Frei *et al.*, 2006).

The duration of drought events in Lower Saxony will increase significantly (Haberlandt *et al.*, 2010). Smith (2011) defined an extreme climatic event as ‘‘an episode or occurrence in which a statistically rare or unusual climatic period alters ecosystem structure and/or function well outside the bounds of what is considered typical or normal variability.’’ The recent very hot and dry summers of 2003 and 2015 support the presumption that this predicted climate change is already affecting the frequency of European drought periods and extreme events (Dong *et al.*, 2016; Orth *et al.*, 2016; Sippel *et al.*, 2016; Stagge et al, 2017). An increase of the mean temperature, of CO_2 contents in the atmosphere, and the predicted extension of the length of the growing season are expected to increase plant productivity, but their positive effect will be antagonized by those frequent extreme drought events (Ciais *et al.*, 2005; Naudts *et al.*, 2011). Extreme temperatures and precipitation likely affect plants and ecosystem functions much stronger than shifts of mean values (Easterling *et al.*, 2000; Meehl *et al.*, 2000). For example, for plant production a lengthening of intervals between rainfall events is much more severe than a reduction of total precipitation quantity (Fay *et al.*, 2000; Swemmer *et al.*, 2007). Also, one severe drought can induce phenological shifts of a grassland community of the same magnitude as one decade of gradual warming (Jentsch *et al.*, 2009). That makes extreme events and their impact one of the main challenges that a successful grassland management will need to adapt to. The impact of drought events on yield and quality can be significant (Humphreys *et al.*, 2006), although most studies still target the influence of extreme events on productivity only (as reviewed by Jentsch *et al.*, 2007). The resistance and resilience of grasslands towards drought stress events and the upkeep of measurable forage quality parameters under drought stress are means to assess the sustainability of grassland forage provision in the face of drought as an aspect of climate change.

1.3.1 Drought stress impact on yields in grasslands of differing functional composition

In general, drought events reduce productivity of permanent grassland (Beierkuhnlein *et al.*, 2011; Craine *et al.*, 2012; Fariaszewska *et al.*, 2017; Fay *et al.*, 2011; Grime *et al.*, 2000;

Hartmann and Niklaus, 2012; Hoover *et al.*, 2014; Kahmen *et al.*, 2005; Zwicke *et al.*, 2013). Yet, contradicting results exist that found old permanent grasslands to be rather resilient against droughts (Gilgen and Buchmann, 2009; Jentsch *et al.*, 2011; Kreyling *et al.*, 2008b; Mirzaei *et al.*, 2008; Wieser *et al.*, 2008). Additionally, productivity was found to either decrease under drought or to decrease only inconsistently or after several recurring drought stress treatments (Bloor *et al.*, 2010; Evans *et al.*, 2011; Hartmann and Niklaus, 2012; Morecroft *et al.*, 2004; Zavalloni *et al.*, 2008). One possible reason for these diverging results is the different functional composition of the respective grasslands. The effect of functional diversity on productivity and quality of permanent grassland systems during drought events is still unclear and needs additional research (Craine *et al.*, 2013; Morais and Cianciaruso, 2014). Reports of increasing, stagnating, and decreasing productivity under drought with varying influence of different functional groups from Central European grasslands exist (Gilgen and Buchman, 2009; Grant *et al.*, 2014; Jentsch *et al.*, 2011; Khan *et al.*, 2014; Lanta *et al.*, 2012; Wardle *et al.*, 2000; Zwicke *et al.*, 2013). Some of these authors emphasize that the role of functional diversity needs further investigation, since no satisfactory explanation for the contradicting results could be deduced from the known facts (Grant *et al.*, 2014; Jentsch *et al.*, 2011). Forbs have a number of potential advantages over grasses during droughts (Fay *et al.*, 2003): Their deeper roots allow them to draw water from sources that are not available to the shallower-rooted grasses. Their main growth period, which is sooner or later during the growing season than that of the grasses, allows them to avoid the times when summer droughts are more likely to occur and to benefit from increased light transmittance. Evidence from North American prairie systems confirms that grass productivity followed variable precipitation patterns closely, while forbs remained unaffected (Nippert *et al.*, 2006). Previous results from artificial swards are inconsistent; forbs were of advantage or disadvantage for the swards' reaction to drought (Mariotte *et al.*, 2013, van den Berge *et al.*, 2014). Regardless of these findings, both prairies and artificial swards are thought to react differently to drought than agriculturally used permanent European grasslands and an experimental approach to test the influence of non-leguminous dicots on drought resistance and resilience of European grasslands is needed (Díaz *et al.*, 2003; Morais and Cianciaruso, 2014; Wardle *et al.*, 1999). The influence of legumes on drought-stressed swards has been more thoroughly tested and is better understood than that of the forbs. There are conflicting reports regarding the drought resistance and resilience capacity of legumes. Most studies test agriculturally relevant legume species like *Trifolium repens* for their productivity and the persistence of their facilitation effect towards companion grasses under drought stress. Severe and long droughts inhibited legume growth and productivity (Küchenmeister *et al.*, 2013; Lucero *et al.*, 1999). Other studies reported a very large drought resistance of legumes (Dumont *et al.*, 2015; Hofer *et al.*, 2016; Pfisterer and Schmid, 2002). Even though some species are known for their drought sensitivity, the inclusion of legumes in permanent grasslands is considered an important strategy for climate change mitigation and sward N supply, because they are quickly implemented, require little work-input, are robust and persistent, and generate no significant N_2O emissions (as reviewed by Suter *et al.*, 2015).

1.3.2 Drought stress impact on forage quality in grasslands differing in functional composition

In contrast to its uncertain impact on productivity, functional diversity is, apart from plant maturity, known to be one of the most influential drivers of grassland forage quality (Buxton, 1996; Gierus *et al.*, 2012; Hopkins and Wilkins, 2006; Michaud *et al.*, 2015). Functional groups differ in their initial forage quality (Bruinenberg *et al.*, 2002; Duru *et al.*, 2008) and their speed of maturation (Bruinenberg *et al.*, 2002; Buxton, 1996). The decline of dicot fodder quality with proceeding maturation is faster than that of grasses (Bumb *et al.*, 2016). Drought stress is known to affect the maturation process (Halim *et al.*, 1989) and forage quality (Buxton, 1996; Durand *et al.*, 2010) of grasses and dicots differently. Therefore, sward functional composition is expected to influence the drought response of forage quality parameters (Gardarin *et al.*, 2014). However, knowledge on how drought stress affects forage quality is mostly derived from mixture experiments of grass and legume cultivars. Their results include improved whole sward forage quality under drought stress (e.g. Dumont *et al.*, 2015; Jensen *et al.*, 2010; Küchenmeister *et al.*, 2013), negligible effects (e.g. Küchenmeister *et al.*, 2014), and decrease of quality (e.g. Walter *et al.*, 2012; White *et al.*, 2004) with increasing diversity. Results from permanent grasslands are scarce. Michaud *et al.*

(2015) found a significant impact of functional composition and drought on forage quality, but most studies investigate the effect of drought on other diversity parameters (e.g. Gardarin *et al.*, 2014) or the effect of drought on composition rather than the effect of functional composition on the swards' drought response (Grant *et al.*, 2014; Jung *et al.*, 2014; White *et al.*, 2014).

Grass-dominated swards are expected to be resilient to drought events (Deléglise *et al.*, 2015). There is evidence that the forage quality of grasses increases if grasses are exposed to drought stress (Fariaszewska *et al.*, 2017; Gibson *et al.*, 2016; Küchenmeister *et al.*, 2013). Due to the dominance of grasses in grasslands their influence on sward quality is expected to be large. Legumes still have the potential to positively influence sward quality under drought stress due to their facilitation effect. Like the results concerning the effect of drought stress on the whole sward quality, the results concerning the effect of drought stress on legume quality are inconsistent. Drought either increased legume forage quality (e.g. Dumont *et al.*, 2015; Peterson *et al.*, 1992), decreased it (e.g. Seguin *et al.*, 2002), or had no to negligible effects (e.g. Abberton *et al.*, 2002; Küchenmeister *et al.*, 2014). Results from permanent grassland on the influence of forbs on sward quality are also rare and inconsistent (Andueza *et al.*, 2015; Blonski *et al.*, 2004; Donath *et al.*, 2004; Grant *et al.*, 2014; Michaud *et al.*, 2015), and so are the results from artificial grasslands (Bruinenberg *et al.*, 2002; Hofmann and Isselstein 2005; Jing *et al.*, 2017; Khalsa *et al.*, 2014; Küchenmeister *et al.*, 2014).

However, combined effects on sward quality cannot be easily extrapolated from responses of single functional groups. Different functional groups not only react differently to drought stress, their presence or absence also influences abiotic parameters, thus affecting the response of other functional groups (Leimer *et al.*, 2014).

1.4 N availability impacts on the reaction of grasslands to drought stress

The interaction of functional groups is not only influenced by disturbances such as drought stress, but also by the amount of available N. After water status, sward nutrient status is the main factor determining the productivity of grasslands (Duru and Calvière, 1996; Vitousek and Howarth, 1991). Sward productivity (Durand *et al.*, 2010; Zwicke *et al.*, 2013), drought resistance and resilience (Evans *et al.*, 2011; Grman 2010), and forage quality (Durand *et al.*, 2010; Grant *et al.*, 2014; Lavorel *et al.*, 2011; White *et al.*, 2004) increase with additional N. Drought leads to reduced N uptake and thus blocks the use of soil mineral N, which as a result reduces yield and quality of sward forage (Gonzalez-Dugo *et al.*2005; Onillon *et al.*, 1995). This limited nutrient availability affects plants even sooner than the limitation of access to water (Kohli *et al.*, 2012).

The interacting effects of functional diversity, N availability, and drought stress on sward productivity and quality in permanent grasslands are hard to assess and not entirely understood. Systems with large N availability usually have small species and functional diversity (Loreau *et al.*, 2001). N fertilization is a common means of grassland management that can reduce functional diversity as it increases above-ground competition (Helsen *et al.*, 2014; Mpokos *et al.*, 2014; Suding *et al.*, 2008). This can affect the reaction of grasslands to drought stress (Grman *et al.*, 2010; Lamarque *et al.*, 2014). N fertilization impacts the drought reaction of grasses, forbs and legumes, which in turn affects the relationship between the functional groups. Grasses and forbs react to N fertilization with enhanced productivity, with grasses showing a faster and more pronounced growth than forbs when fertilized (Avolio *et al.*, 2014; Blonski *et al.*, 2004). Strong growth reactions of forbs to N fertilization have also been described (Reich *et al.*, 2003). However, legumes do not benefit as much from additional available N as the other functional groups (Nyfeler *et al.*, 2009).

Grass quality is increased by larger N availability (Buxton, 1996; Collins and Balasko, 1981; Peyraud and Astigarraga, 1998; Zemenchik *et al.*, 2002), but results concerning changes in forb and legume quality from permanent grasslands are scarce. There is evidence that diverse swards have a better N use efficiency than grass monocultures because of differing root lengths of forbs and grasses, allowing nutrient capture in various soil depths (Husse *et al.*, 2017; Kleinebecker *et al.*, 2014). However, effects of diversity may be diminished by N fertilization (White *et al.*, 2004). The question whether the positive influences of N fertilization can mitigate possible negative impacts of drought remains undecided (Grant *et al.*, 2014).

1.5 Research outline and main hypotheses

Given the inconsistency and limitations of available data as described above, there is need for an experimental study that focuses on the combined effects of sward functional composition, drought stress and fertilization on the productivity and quality of permanent grassland. Based on the known relations of sward functional composition, drought stress and fertilization we hypothesized that

i. The productivity of permanent grasslands under drought stress is affected by sward functional group composition as well as nutrient availability
ii. The forage quality of permanent grasslands under drought stress is affected by sward functional group composition as well as nutrient availability
iii. Different functional groups mutually influence their reactions to drought stress

To test these hypotheses, we selected three sites featuring typical permanent grasslands of Northern Germany to perform an experiment that induced drought stress by exclusion of natural precipitation with rain-out shelters for several consecutive weeks during spring and summer, respectively, for three consecutive years. Rain-out shelters like the ones we used in this experiment do not significantly influence plant productivity and were designed to have a minimal impact on the swards. (Vogel *et al.*, 2013). We modified the original sward functional composition by reduction of the forb and legume functional groups and compared the resulting grass-dominated swards to the original diverse swards. For complex research questions like ours, experimental methods that modify a given plant community are the most useful approach for detecting effects of competition, facilitation, and other interactions of diversity (Morais and Cianciaruso, 2014; Petersen *et al.*., 2012): Reduction of a diverse sward by a removal approach creates a comparable sward of simplified but otherwise equal properties and delivers better comparisons than comparing swards that naturally differ in diversity for initially unknown reasons. Yield and forage quality remain stable after herbicide application and are determined by other properties like functional diversity or management and not by herbicide application itself (Petersen *et al*., 2013). In addition, we applied an N fertilization regime (180 kg ha^{-1} $year^{-1}$) to test for the influence of enhanced N availability. This fertilization level is well within the common N fertilization regime of European grassland and can be classified as medium-intense land use (Allan *et al.*, 2014; Herzog *et al.*, 2006; Kleijn *et al.*, 2009; Klimek *et al.*, 2007; Socher *et al.*, 2012).

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2 Drought stress resistance and resilience of permanent grasslands are shaped by functional group composition and N fertilization

2.1 Abstract

Central European old permanent grasslands are of considerable economic importance and ecological value. So far, there are conflicting results on their resistance and resilience to future drought events. Especially the influence of functional diversity on these attributes has yet to be fully understood, as most studies originate from experimental grassland communities. During three consecutive years we induced spring and summer drought events on three permanent grassland sites typical for Northern Germany. We observed a larger drought resistance in swards that were grass-dominated than in functionally diverse swards. Grasses determined the drought resistance potential of a sward, and their performance was impaired by the presence of forbs and legumes. Fertilization increased the resistance to drought stress of swards either through direct positive effects on the productivity or indirect effects through changes of functional sward composition. Sward functional composition was not important for sward resilience. Grasses and whole swards were resilient to drought stress only if previously fertilized. We found that the grass functional group is responsible for the swards' resistance and resilience to drought, but its behaviour was partly regulated not only by the presence but also by the biomass share of the forb and legume functional group. The differences among the functional groups' share of the total sward biomass might be important determinants of responses to drought stress. We therefore hypothesize that there is no uniform reaction of permanent grassland to drought stress events.

2.2 Introduction

About 30% of the agricultural plant production area in Germany consists of permanent grasslands (Statistische Ämter des Bundes und der Länder, 2011). Unlike many other agricultural crops, permanent grassland contains a heterogenous plant community and therefore is a dynamic system with varying shares of species and plant functional groups. Thus, permanent grassland is also important for the conservation of phytodiversity (Cousins and Eriksson, 2008). Its economic importance and ecological value emphasizes the need to better understand its response to climate change, because studies targeting the drought resistance and resilience of grasslands in terms of above-ground biomass productivity have so far been conducted on experimental, sown-in grasslands with controlled environmental conditions (e.g. Dreesen *et al.*, 2014; Küchenmeister *et al.*, 2012; Naudts *et al.*, 2011; Pfisterer and Schmid, 2002). Following Pimm (1984) we define resistance in this context as the degree of productivity reduction during a drought event and resilience as the availability to recover during the period of ample water supply following a drought event. The expected larger future climatic variability in Central Europe (IPCC, 2011) will probably lead to more frequent and more severe drought events (IPCC, 2013).

In contrast to sown-in grasslands, old permanent grasslands can behave differently due to their phytodiversity, more complex root structure and general stability (Balvanera *et al.*, 2006; Flombaum and Sala, 2008; Zhang *et al.*, 2013). Some evidence from old permanent grasslands points towards a low resistance of above-ground biomass production to drought (e.g. Fay *et al.*, 2011; Grime *et al.*, 2000; Hartmann and Niklaus, 2012; Hoover *et al.*, 2014; Kahmen *et al.*, 2005; Zwicke *et al.*, 2013). In contrast, some authors have found old permanent grasslands to be rather resistant against droughts (e.g.; Gilgen and Buchmann, 2009; Jentsch *et al.*, 2011; Kreyling *et al.*, 2008; Mirzaei *et al.*, 2008; Wieser *et al.*, 2008) or to show a negative productivity response only inconsistently or after several consecutive years of drought stress treatment (Bloor *et al.*, 2010; Evans *et al.*, 2011; Morecroft *et al.*, 2004; Zavalloni *et al.*, 2008). Thus, the response of old permanent grassland to drought is apparently not only influenced by the severity or duration of the stress itself, but also by other factors such as diversity. Most diversity studies have been carried out on experimental grasslands and often studied species diversity with differing composition from or less species than commonly found in permanent grassland (Wrage *et al.*, 2012). The link between grassland diversity and productivity has often been found to depend less on the number of species but rather on - among others - functional diversity (Diaz and Cabido, 2001). Thus, studying the influence of functional diversity in permanent grassland promises both new insights on the functioning of diversity itself as well as applicability of research results by practitioners. So far, the role of functional diversity in permanent grassland systems during drought events is still unclear (Morais *et al.*, 2014) and needs to be better understood (Craine *et al.*, 2013). Increase, stagnation, and decrease of productivity under drought with varying influence of different functional groups have been reported (Gilgen and Buchman, 2009; Grant *et al.*, 2014; Jentsch *et al.*, 2011; Khan *et al.*, 2014; Lanta *et al.*, 2012; Wardle *et al.*, 2000; Zwicke *et al.*, 2013). However, none of these studies focused on functional groups and their influence on drought resistance or resilience of permanent grassland swards, nor do these studies give any satisfactory explanation for the contradicting results. In fact, some authors expressed the need to further investigate the role of biodiversity (Grant *et al.*, 2014; Jentsch *et al.*, 2011).

Another factor that has been known to increase resistance and resilience towards drought stress is nitrogen availability (Evans *et al.*, 2011). Nitrogen fertilization is common in agricultural grassland management, but has also been described to reduce functional diversity (Helsen *et al.*, 2013; Mpokos *et al.*, 2014; Suding *et al.*, 2005). This may influence the swards' response to drought stress (Grman, *et al.*, 2010; Lamarque *et al.*, 2014).

We aim to contribute to the predictability of the effects of droughts on sward productivity by conducting a three-year experiment on three typical old permanent grasslands in Northern Germany. We address the influence and the interaction of functional diversity and nitrogen fertilization on sward resistance during and resilience after drought stress.

We hypothesize that on these old permanent grasslands,

i. functional diversity increases resistance during drought stress,

ii. nitrogen fertilization affects the relationship between functional diversity and resistance.

iii. functional diversity increases resilience after drought stress,

iv. nitrogen fertilization affects the relationship between functional diversity and resilience.

2.3 Materials and methods

2.3.1 Study sites

The experiments were conducted on three permanent grassland sites typical for Northern Germany. The whole region is characterized by a temperate, sub-continental climate.

The south-eastern lowland site (SEL) is located near Göttingen in the Leine valley, the north-western lowland site (NWL) is located near Oldenburg in the Northern German Plain and the sub-mountainous site (SMS) is located in Silberborn near Uslar in the Solling mountain range. Site details are presented in Table 8-1.

2.3.2 Experimental design

In a three-year experiment (2011 – 2013) on all three sites, we investigated the effects of drought (with and without rain-out shelters), sward composition (with and without reduction of dicot species cover), and nitrogen fertilization (with 180 kg N ha-1 a-1 or without) in a completely randomized block design with four replicates. Treated plots had a size of 1.8 m by 1.8 m (3.24 m^2). All measurements and samples were taken from a core area of 0.4 x 0.4 m (0.16 m^2) in the center of each plot.

Spring and summer droughts of, on average, 36 days were induced by installing rain-out shelters with an inclined roof allowing rain to run off that covered the whole plot with UV-permeable greenhouse film (GeKaHo GbR, Gewächshausfolie SPR 5, 200my) at 1.5 m mean height. Measurements of photosynthetically active radiation (PAR) with the SunScan Canopy Analysis System on a sunny day in May 2012 around noon showed a significant difference (chi-squared = 44.9032, p-value <0.0001) of the ambient mean PAR between plots with and without shelters (n = 32; without shelter: 1757 ± 48 W m-^{2}, with shelter 1275 ± 86 W m^2). Due to the lower radiation intensity under the shelters we expect photosynthetic rates and thus assimilation to be smaller, leading to a similar effect as the drought stress treatment, although we cannot distinguish between the effects of water supply and PAR on plants.

Spring drought stress periods started at the end of April or the beginning of May, about seven to eight weeks after the start of the year's growing season. The start of the growing season was determined by five consecutive days with an average temperature > 5°C (Jones *et al.*, 2002). After the end of the spring drought stress period, the greenhouse films were removed for three weeks to allow rewetting of stress-treated plots by natural precipitation. Summer drought stress periods started around the end of June. Afterwards, natural precipitation was again allowed on all plots until the following spring. No irrigation was applied during the three years of experiments.

We follow the definition of a functional group as a group of species that share morphological, and perhaps physiological, traits (Lauenroth *et al.* 1978) and thus divided the species found in our swards into the functional groups grasses, forbs, and legumes. The functional group composition of swards was manipulated by the application of herbicides against dicotyledonous species (forbs and legumes). Thus, on half of the plots, Starane Ranger (100 g l-1 Fluroxypyr and 100 g l-1 Triclopyr, 2 l ha-1) and Duplosan KV (600 g l-1Mecoprop-P, 2 l ha-1) were applied one year before the start (2010) and in the course of the experiment (2012). Herbicide treatment resulted in two sward types: diverse swards with the original species composition and grass-dominated swards (Table 8-2).

On all plots, 200 kg ha-1 potassium chloride (in the form of 40 % K_2O) and 30 kg ha-1 triple superphosphate (in the form of 46 % P_2O_5) were applied at the beginning of the growing season to ensure plant nutrient supply. Additionally, to half of the plots 180 kg nitrogen (N) ha-1 were applied. N application was split into 90 kg N ha-1 at the beginning of the growing season and 45 kg N ha-1 after the each cut-. K application was split into 100 kg ha-1 at the beginning of the growing season and 100 kg N ha-1 after the first cut at the end of the spring drought stress period.

Whole plots were cut directly after each stress period and once again in the beginning of October at 7 cm stubble height. Biomass samples were taken only from the core area. Samples were sorted by functional group (grass, forbs, and legumes), dried at 60 °C for 48 hours and weighed. Aboveground production was determined as accumulated dry weight per year.

We cumulated the sample weights of the first two as well as of the third annual cuts over all three years and analyzed both datasets for the influence of sward type and fertilization level on sward biomass production during drought stress events.

2.3.3 Water and climate relations

The microclimate under the rain-out shelters and above control plots was surveyed by data loggers (CiK Solutions GmbH, Haxo-8 LogTag), which recorded values every 30 minutes from April until the last cut during all three years. Temperature and relative humidity showed no significant difference (Wilcoxon signed rank test with continuity correction, P = 0.1763) between treatments with and without shelters..

We did not apply barriers to prevent water run-off into the plots, to minimize disturbance to the swards' root system. Soil water content under rain-out shelters was monitored by gravimetric sampling. Even after major rainfall events, the core area from which all samples were taken proved to remain dry. During the induced droughts, which lasted on average 36 days, stressed plots received no rainfall. The control plots received natural precipitation. The rain-out shelters held back 73 ± 5% precipitation (SEL), 52 ± 8% (NWL), and 60 ± 5% (SMS), respectively, during the experimental period. On average over all locations and years, rain-out shelters held back 148 mm of the 240 mm precipitation (62 ± 8%) that fell from the beginning of the growing season until the end of the second drought stress treatment.

2.3.4 Statistical analysis

Statistical analyses were performed with R version 3.0.2 (R Core Team 2013) using a significance level of $\alpha \leq 0.05$ throughout. Two datasets each (spring and summer harvest weights, autumn harvest weights) of total plot biomass as well as grass functional group biomass were analyzed by applying linear mixed models using the additional software package nlme by Pinheiro *et al.* (2011). All models were tested for normal distribution (quantile-quantile-plots and Shapiro-Wilk test) and homoscedasticity (residual plots and Levene Test). If these criteria were not met, models were corrected by including a variance function or transformation. Fixed effects in the analyses of whole swards were sward type, fertilization, drought stress, year, and season. Site and block were included as nested random effects. In the fixed effects of the analyses of grass biomass sward type was substituted by the cumulative biomass share (%) of forbs and legumes, because forb and legume share as separate variables resulted in model overload.

All full models (initial models before optimization) included all possible interactions and were subsequently optimized according to the methods suggested by Pinheiro & Bates (2000) and Zuur *et al.* (2009) to obtain a model that optimally fit the data and had an AIC as low as possible. Fixed effects and their interactions that appear as not significant in the results tables were excluded from the model during optimization, which resulted in a better model fit to the data.

After all models were fit, we calculated and grouped post-hoc pairwise contrasts of the most influential variables and their interactions using the Tukey method for comparing families found in the packages lsmeans (Lenth 2016) and multcompView (Graves *et al.* 2015). Most influential variables were selected by the magnitude of the F-value.

2.4 Results

We present the data in two sets: First, the whole sward and the grass biomass data from cuts harvested directly after a drought stress period (spring and summer season, respectively) and reflecting the swards’ resistance to drought stress. Second, we show the whole sward and the grass biomass data from the autumn cuts originating from the regrowth period after drought stress events and reflecting sward resilience.

2.4.1 Drought stress resistance

2.4.1.1 Resistance of whole swards to drought stress

The resistance to drought was influenced by all tested variables, although all variables showed significant interactions in the analysis (Table 2-1). Whole sward spring and summer productivity was mostly influenced by fertilization, season, the interaction between sward and stress, and the stress-season interaction (F-values,^Table 2-1). Fertilization generally enhanced productivity. Spring yields were larger than summer yields. Stressed grass-dominated swards showed slightly larger biomass yields compared to controlled grass-dominated ones, but the difference proved not significant in the post-hoc analysis (Table 8-3). This means grass-dominated swards generally had a high resistance. Stressed diverse swards had significantly less biomass than the diverse control, pointing to lower resistance than grass-dominated swards. There was no significant biomass difference between the stressed diverse and both control and stressed grass-dominated swards (Figure 2-1). In spring, the control swards had on average less biomass than the stressed swards, but that difference was not significant. In summer, stressed swards had significantly less biomass than the control (Table 8-3). Thus, general sward resistance was higher in spring than in summer.

Other interactions were also significant (see Table 2-1), but their influence was not very large. For example, the year itself was just above the significance threshold, but appeared in significant interactions with sward, fertilization, and season. The post-hoc contrasts showed mostly a separation by the factors season and fertilization, as shown above, and only rarely actual differences among years were found. The interaction between fertilization and drought stress we aimed to explore was significant, but this result's explanatory power was also rather weak (Table 8-3). Nevertheless, fertilized swards showed higher resistance towards drought stress than non-fertilized ones.

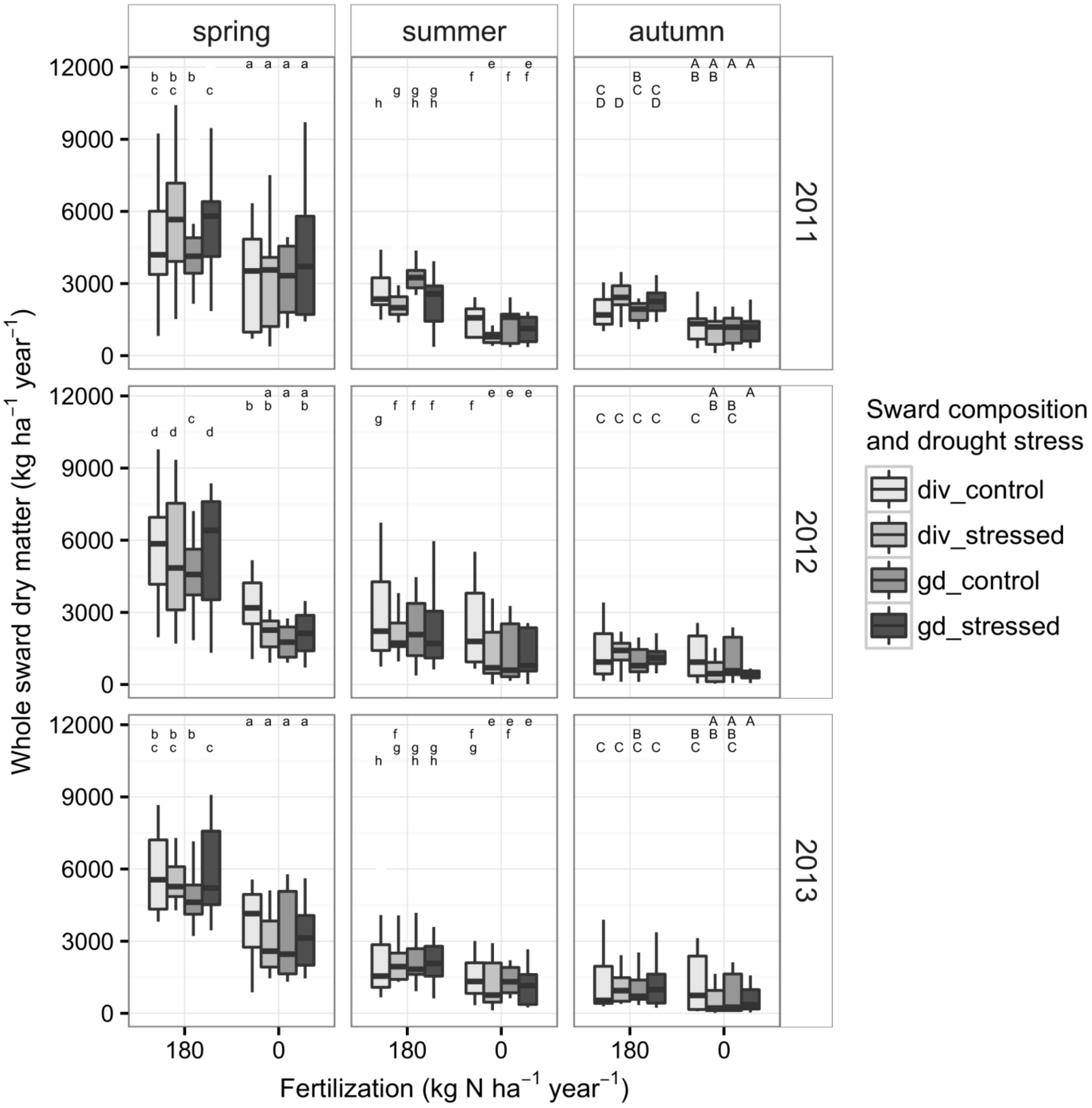

Figure 2-1 Whole sward biomass by season, year, sward type (diverse or grass-dominated), fertilization level (kg N ha-1 year-1), and drought stress treatment (control = control, stressed = drought stressed). Lower case grouping letters refer to the resistance analysis, capital grouping letters refer to the resilience analysis. For ease of readability the group visualizations were simplified. For grouping details see Table 8-3 and Table 8-4 in the supplement.

Table 2-1 F-test table of optimized linear nested mixed models of drought stress resistance measured as biomass of whole swards and the grass functional group. Explaining variables include sward type (sward, only for whole sward analysis), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:), as well as the cumulative biomass share of forbs and legumes (bsfl, only for the grass analysis). Variables marked n.s. were either not significant in the optimized model or excluded from the model during the model optimization process and are not significantly influencing the respective dependent variable. Possible interactions that do not appear were excluded from all models during the optimization process and are not listed for readability.

Whole sward	**F**	***P***	**Grass functional group**	**F**	***P***
sward	0.712	n.s.	bsfl	1.776	<0.0001
fert	21.99	<.0001	fert	21.222	<0.0001
stress	4.294	0.0387	stress	0.0001	n.s.
year	2.941	n.s.	year	11.663	<0.0001
cut	71.66	<0.0001	cut	63.152	<0.0001
sward:fert	n.s.	n.s.	bsfl:fert	3.676	n.s.
sward:stress	10.625	0.0012	bsfl:stress	0.003	n.s.
fert:stress	5.306	0.0216	fert:stress	0.013	n.s.
sward:year	5.453	0.0045	bsfl:year	9.032	<.0001
fert:year	3.997	0.0189	fert:year	4.825.	0.0084
stress:year	n.s.	n.s.	stress:year	2.36	n.s.
sward:cut	n.s.	n.s.	bsfl:cut	12.202	0.0005
fert:cut	1.951	n.s.	fert:cut	0.831	n.s.
stress:cut	11.171	0.0009	stress:cut	7.861	0.0052
year:cut	5.798	0.0032	year:cut	5.425	0.0046
sward:stress:year	n.s.	n.s.	bsfl:stress:year	4.432	0.0123
fert:stress:year	n.s.	n.s.	fert:stress:year	3.131	0.0445
sward:year:cut	n.s.	n.s.	bsfl:year:cut	3.267	0.0389
fert:year:cut	5.261	0.0055	fert:year:cut	3.107	0.0455

2.4.1.2 Resistance of functional groups to drought stress

In general, grasses dominated the swards and formed on average 68 – 99% (depending on treatment combination) of the total biomass share. On average, forbs and legumes combined had biomass shares between 0 – 25%.

Grass biomass was mainly influenced by dicot share, fertilization, year, and season. These effects interacted weakly but significantly with drought stress (Table 2-1). On average, grasses had a high drought resistance and did not decrease productivity during drought. Measured grass biomass during spring and summer decreased on average with increasing dicot share. During spring and summer, grass biomass of diverse swards decreased by 11% if drought stress occurred, showing low resistance to drought stress. In contrast, grass biomass of grass-dominated swards increased by 12% if drought stress occurred during spring and summer, pointing to very high resistance. During spring and summer, fertilization generally increased grass productivity and increased grass resistance to drought. General grass resistance in 2011 was good during both spring and summer season. In 2012 and 2013 high resistance of grasses was found only in spring and was slightly but significantly smaller during those years' summer drought period.

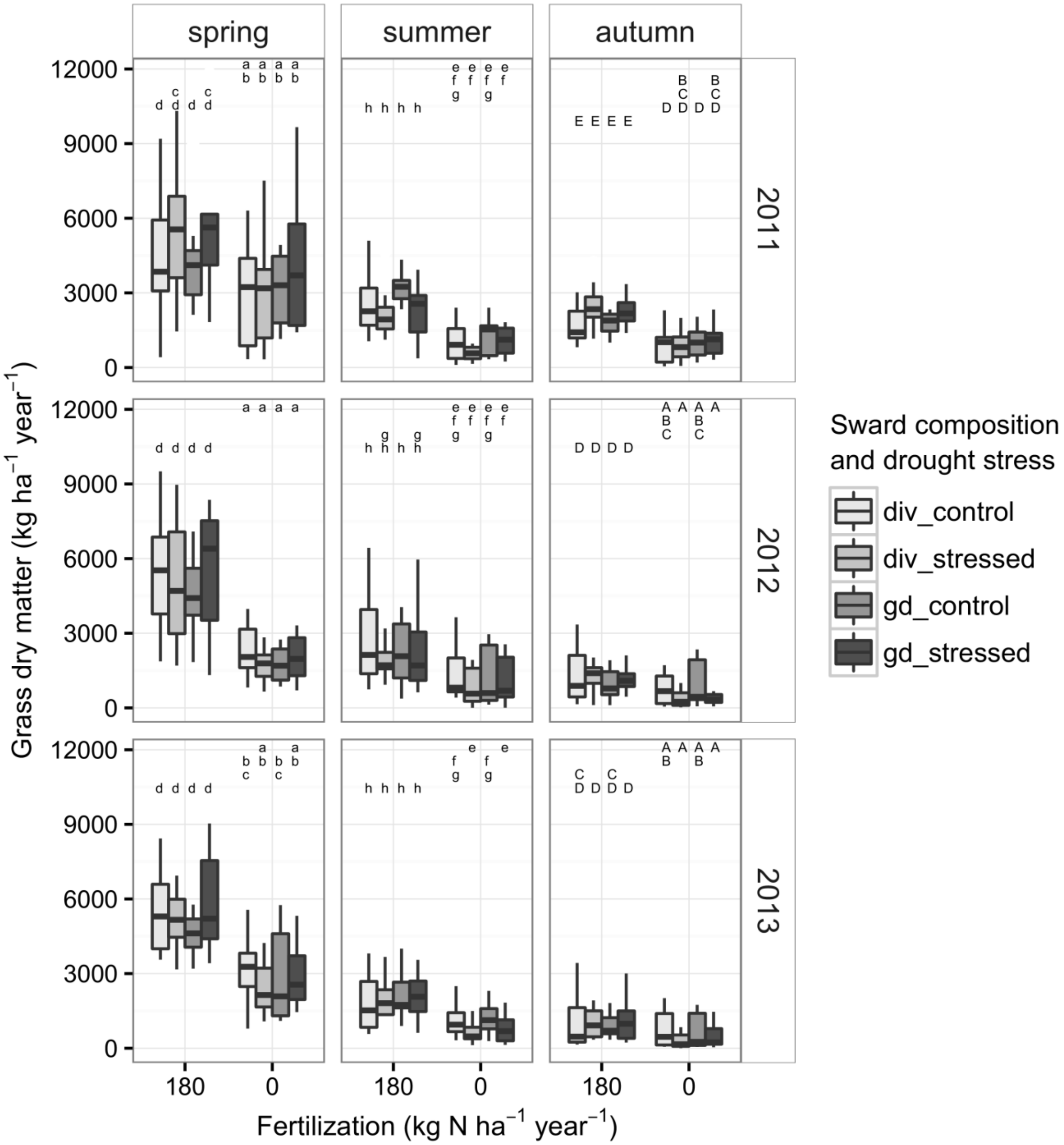

Figure 2-2 Grass biomass by season, year, sward type (diverse or grass-dominated), fertilization level (kg N ha-1 year-1), and drought stress treatment (control = control, stressed = drought stressed). Lower case grouping letters refer to the resistance analysis, capital grouping letters refer to the resilience analysis. For ease of readability the group visualizations were simplified. For grouping details see Table 8-5 and Table 8-6 in the supplement.

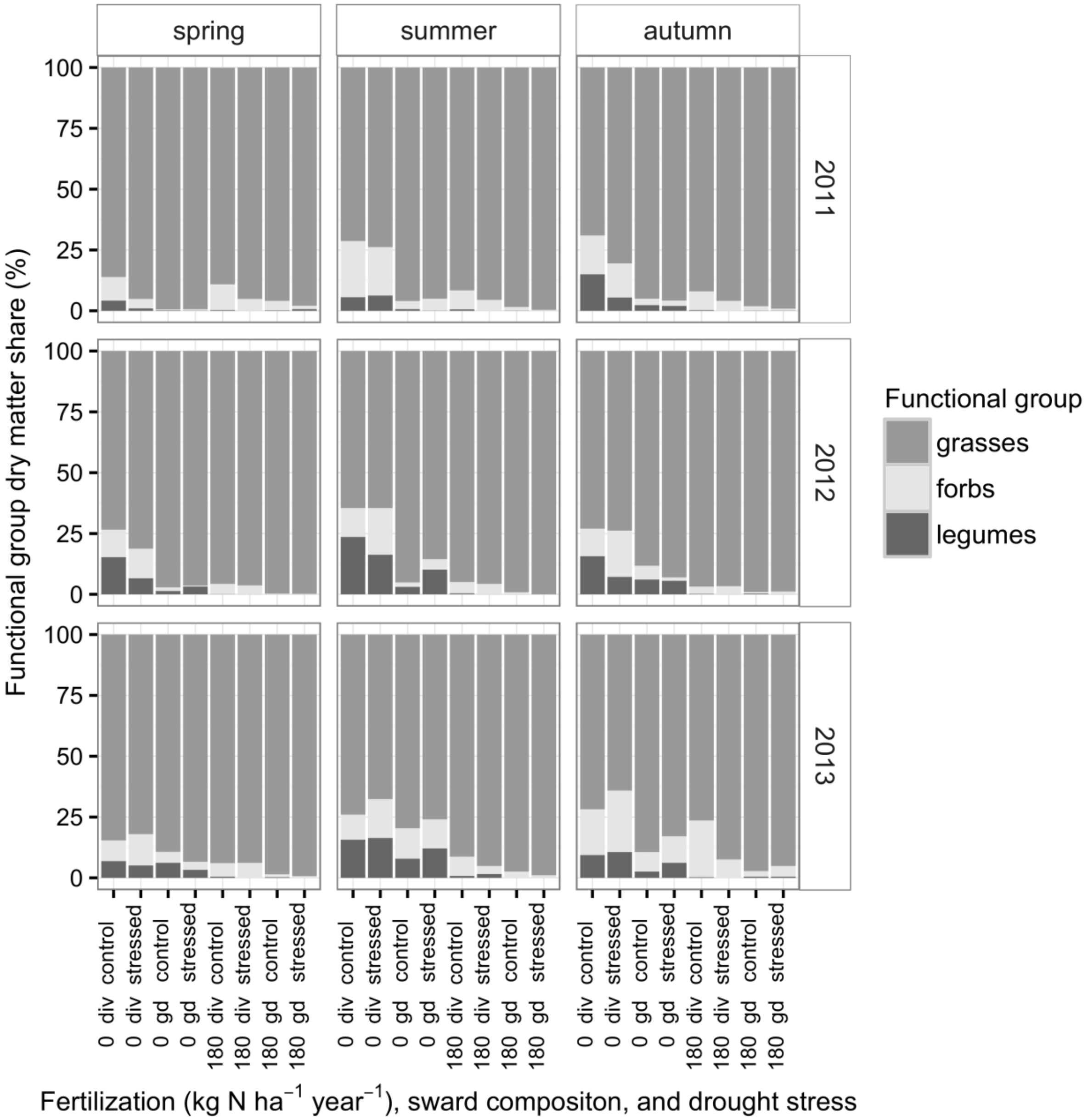

Figure 2-3 Forb (grey), grass (light grey), and legume (dark grey) biomass shares by season, year, sward type (diverse or grass-dominated), fertilization level (kg N ha-1 year-1), and drought stress treatment (control = control, stressed = drought stressed)

2.4.2 Drought stress resilience

2.4.2.1 Resilience of whole swards

Sward type did not significantly influence sward resilience. The main driver of resilience was fertilization level (Table 2-2). Fertilization generally led to a high resilience or even larger yields in previously stressed than control swards, and generally to a larger biomass than in unfertilized swards. Non-fertilized stressed swards had significantly smaller autumn biomass than the non-fertilized control and both control and stressed fertilized swards. Non-fertilized control swards were significantly less productive than both control and stressed fertilized swards. In 2011, autumn biomass was larger than in 2012 and 2013, but there was no difference among years concerning resilience.

Table 2-2 F-test table of optimized linear nested mixed models of drought stress resilience measured as autumn biomass of whole swards and the grass functional group. Explaining variables include sward type (sward, only for the whole sward biomass analysis), drought stress (stress), fertilization level (fert), year (year), biomass share of forbs and legumes (dicot share, only for the grass analysis) and their interactions (:). Variables marked n.s. were either not significant in the optimized model or excluded from the model during the model optimization process and are not significantly influencing the respective dependent variable. Possible interactions that do not appear were excluded from all models during the optimization process and are not listed for readability.

	F		***P***	
	whole sward	**grasses**	**whole sward**	**grasses**
sward/dicot share	3.428.	21.098	n.s.	<.0001
fert	16.906	20.474	0.0001	<.0001
stress	0.708	2.305	n.s.	n.s.
year	3.931	53.87	0.0208	<.0001
sward/dicot share:fert	n.s.	2.354	n.s.	n.s.
sward/dicot share:stress	n.s.	3.625	n.s.	n.s.
fert:stress	14.681	7.33	0.0002	0.0072
sward/dicot share:year	n.s.	11.255	n.s.	<.0001
fert:year	2.333	n.s.	n.s.	n.s.
stress:year	2.788	n.s.	n.s.	n.s.

2.4.2.2 Resilience of functional groups

Grass biomass of the late season was mainly influenced by dicot biomass share, fertilization, and year (Table 2-2). As during spring and summer, a large dicot biomass share resulted in less grass biomass. With each year, this relation became less pronounced and increasing grass biomass values were measured even with high dicot shares. Fertilization increased late season grass biomass. Fertilized grasses had a high drought resilience. Non-fertilized grasses showed lower resilience, as drought stressed non-fertilized grasses had significantly less biomass than the non-fertilized control (Figure 2-2). In the year 2011 grasses were more productive in the late season than in the late season of 2012 and 2013, but no differences in resilience occurred between years.

2.5 Discussion

Our results showed that sward composition is important for resistance towards drought stress (Figure 2-1 and Figure 2-3). Grass-dominated swards had a high resistance towards drought stress, as their productivity remained equal to the control. In comparison, diverse swards had lower resistance as their productivity declined under drought stress compared to the control. However, compared to their grass-dominated counterparts, the diverse swards in this study generally were equally productive. Nitrogen fertilization generally increased productivity and was found to support resistance against drought stress.

During the regrowth period after drought stress, sward composition was not important for sward resilience, but nitrogen fertilization increased resilience.

2.5.1 Resistance

The first hypothesis, that for old permanent grassland a sward with several functional groups is more resistant to drought than a sward with one dominating functional group, could not be confirmed by the results. We observed that the direct productivity response of diverse swards to drought stress was neutral to negative, representing their lower resistance towards drought stress than the grass-dominated swards', while the grass-dominated swards' response was neutral to positive (Figure 2-1), representing their good resistance. The year effect was rather weak, and none of our treatments led to a continuous change of sward biomass or resistance with time. This leads us to the conclusion that our fertilization regime as well as our sward management was fit to site, although changes can be expected to occur after additional years of treatments (Evans *et al.*, 2011). Larger yields in the spring season were not surprising given the typical annual productivity and growing curve of grasslands (Dierschke and Briemle, 2002). Lower resistance in the summer season compared to the spring season could have occurred because the swards already had to undergo one period of drought stress during early growing season and were thus not physically equipped to optimally resist yet another immediately following stress period (Walter *et al.*, 2011). The resistance of each functional group to drought stress was important for understanding differences we found in whole sward resistance, i.e. sward productivity during drought stress.

Grasses were the functional group with the largest biomass share (Figure 2-3) and had a resistance that matched or exceeded that of the whole sward. Decreased sward productivity was only measured in diverse swards, implying that in that case the grasses' drought resistance was steady, but the presence of drought sensitive forbs and legumes led to a decrease of the resistance of the whole sward.

From these results we conclude that primarily grasses, and not forbs or legumes, shaped the swards' drought stress resistance. Thus, the functional composition of swards was highly important for predicting the resistance of swards. The larger the initial plot biomass and/or the share of grasses in the swards, the higher resistance to drought stress was observed. However, cause and effect are hard to distinguish, because nitrogen fertilization was correlated with an increase of grass biomass share and also enhanced general biomass, which again correlated with a high share of grasses (data not shown). Our results do not support the assumption that the presence of forbs has a potentially high influence on sward resistance (Mariotte *et al.*, 2013). In contrast to Mariotte *et al.* (2013) we found the presence of forbs to have a negative effect. Forbs can have facilitating functions on grasses (Kahn *et al.*, 2014, Temperton *et al.*, 2007, van Ruijven and Behrendse, 2005). We assume that in our swards, possible facilitation of forbs and legumes on grasses could have been diminished by drought, leading to a decreased productivity and possibly resistance of grasses and thus contributing to the diverse swards' weaker resistance to drought, when compared to grass-dominated swards. However, based on our results from the fertilization treatment, we presume that the functional composition cannot be used as the only indicator to predict a sward's resistance.

2.5.2 Resistance, Sward type and Fertilization

The second hypothesis that fertilization affects the relationship between functional diversity and resistance to drought stress, could be confirmed by our results, although the effect size was rather weak (F-value of variable fert:stress Table 8-3). This result points to a possible stabilizing function of nitrogen availability on drought resistance (Evans *et al.*, 2011). The diversity effect and fertilization effect did not add up to create higher resistance in fertilized diverse swards. A possible explanation for this could be that nitrogen addition led to a decrease of forb and legume biomass, resulting in an even larger presence of grasses in the swards. This could originate from the competition – fertilization mechanism described by Helsen *et al.* (2013). N fertilization can induce a change of the functional group composition (e.g. Bai *et al.*, 2010; Cop *et al.*, 2009), which certainly happened during our experiment (Figure 2-3), although changes were by far not substantial enough to change the sward assemblage. Nevertheless, a continuation of our fertilization regime would be prone to shift the composition further in the direction of more productive assemblages that would be dominated by tall grasses such as e.g. Dactylis glomerata L. (Dierschke and Briemle, 2002).

While interactions between legumes and non-legumes can be one of the major functional mechanisms in grasslands (Hooper *et al.*, 2005), the increased presence of grasses with fertilization minimized the possibility to maintain a legume and forb effect in fertilized swards.

Resistance to drought stress is also known to increase with fertilization by its physiological effect on the plants' water regulatory system. N fertilization leads to increased protein contents and thus higher concentrations of Rubisco in the plant tissue, enabling plants to close their stomata sooner and conserve water during drought. Though not tested in this study, we assume that this direct effect of fertilization has also increased the drought resistance of our swards.

We conclude that nitrogen fertilization has direct as well as indirect effects on resistance through its effects on plant physiology and sward diversity (Dodd *et al.*, 1994; Grman *et al.*, 2010; Tilman 1996).

2.5.3 Resilience

The third hypothesis, that functional diversity increases resilience after drought stress could not be confirmed by our results. The productivity of control swards was on average slightly larger than the productivity of previously stressed swards. Functional diversity was not significant for changing resilience after drought. Findings from other grasslands also state that functional groups did not significantly influence resilience (Lanta *et al.*, 2012; Mariotte et al, 2013; Wardle et al, 2000), although Mariotte *et al.* (2013) found tendencies of lower resilience if forbs were removed from the sward. The tendency that decreasing diversity leads to a decrease in resilience is well-known from experimental grasslands and from experiments that address diversity as species number (e.g. Pfisterer and Schmid, 2002). The larger general productivity in 2011 might be a residual of a preceding year without experimental treatments or a response to the year's rather warm temperatures during the growing season (data not shown).

2.5.4 Resilience, Sward type and Fertilization

The fourth hypothesis that fertilization changes the relationship between diversity and the resilience to drought stress could not be confirmed by our results. However, fertilization itself was the main factor determining resilience. High sward and grass resilience after drought stress was found only in swards that were previously fertilized. In the non-fertilized swards, grasses could not compensate for the stress they had experienced earlier and produced significantly less biomass than controls. Thus, grasses and whole swards in the late season were resilient to drought stress only if previously fertilized. Limited nutrients have been recognized to even affect plants sooner than the lack of water (Kohli *et al.*, 2012). If the availability of N in the soil is much better due to fertilization, plant nutrition during dry conditions is easier to maintain on a sustainable level (Jacobsen *et al.*, 1996; Latiri-Souki *et al.*, 1998; Saneoca *et al.*, 2004), providing a higher plant resistance against stress and a better physical condition after the stress event, which allows the plants to maintain a high level of growing potential and thus a high resilience. To ensure resilience, the nitrogen level in the fertilized swards must have been sufficient to directly resist drought stress without exploiting the plants' resources, allowing them to return easily and fast to their pre-drought state. Another possibility is limited N uptake during drought stress due to the lack of water as a solvent. Thus, a large amount of N remained in the soil which then would have been abundantly available to the plants after drought stress ended and thus could support resilience, too. We see the limited soil nutrient availability and uptake that plants were exposed to during drought stress as the most probable reason for the differences in resilience because this relation has been found in other drought stress studies (Gonzalez-Dugo *et al.*, 2005; Onillon 1995).

We assume that the late season's swards' resilience was mostly determined by the grass functional group. The effect of forbs and legumes on whole swards could – due to their minor presence in the late season sward - only have been indirect. Forbs and legumes generally had a low resilience to drought stress, which was even smaller with fertilization (data not shown). While we measured over three consecutive years of drought stress, it seems possible that in the future the cover of forbs and legumes may be (severely) diminished in diverse swards fertilized before drought.

2.6 Conclusions

Our results show that the swards' resistance and resilience to drought stress was increased by nitrogen fertilization. We found high resistance and resilience of grasses to drought if the swards were fertilized or functionally not diverse. Further investigations will be needed to verify our hypotheses that the grass functional group is responsible for the swards' resistance and resilience to drought, but that its behavior is partly regulated not only by the presence but also by the biomass share of the forb and legume functional group. Fertilization could have direct and indirect effects via its influence on the functional group composition. We therefore conclude from our results and hints in literature (Dreesen *et al.*, 2014; García-Palacios *et al.*, 2012; Gilgen and Buchmann 2009; Mariotte *et al.*, 2013; Wardle *et al.*, 2000) that there might be no uniform grassland response to a drought event. The mitigation of potentially negative climate change effects in agronomic practice can only be achieved by a management and in particular N fertilization that is closely adapted to site conditions and sward functional group composition.

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3 The influence of drought stress on four forage quality parameters in old permanent grasslands

3.1 Abstract

Old permanent grasslands are ecologically valuable and contribute substantially to agricultural economics. So far, there are conflicting results on their reaction to future drought events in terms of ruminant forage quality. Especially the influence of functional diversity and its interaction with nitrogen (N) fertilization and cutting time is still unclear, because most studies so far dealt with artificial grassland communities. We investigated spring and summer drought periods on three old permanent grassland sites typical for Northern Germany during three consecutive years. We manipulated the initial functional composition of these grasslands and fertilized with N to simulate medium-intense utilization. Larger forage quality under drought stress was expected for diverse in comparison to grass-dominated swards, and for fertilized in comparison to unfertilized swards. Sward functional composition, N fertilization and time of the drought stress period (spring or summer) influenced forage quality parameters and also modified the drought stress response of swards. We observed increased crude protein contents in drought-exposed diverse swards and in grass-dominated swards that were fertilized. Neutral and acid detergent fibre contents were often stable in the face of drought and when they changed, the effect was small and interacted with stronger effects of time of season and sward composition. Drought stressed swards had larger water-soluble carbon contents than controls. We therefore hypothesize that future drought stress events will not necessarily substantially decrease forage quality of old permanent grasslands. They could even be beneficial for forage quality parameters under a sward management adjusted to N status and functional composition.

3.2 Introduction

Permanent grasslands make up 25% of the world's land ecosystems (Ojima *et al.*, 1993) and more than 30% of the agriculturally managed area in Europe and Germany (Statistische Ämter des Bundes und der Länder, 2011; Wilson *et al.*, 2012). Grasslands substantially supply ruminant production systems with high-quality forage. Important forage quality parameters are crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and water soluble carbohydrates (WSC). CP ensures N supply of ruminants. Large NDF contents decrease forage intake because NDF is an estimation of undesired total cell wall contents (cellulose, hemicellulose and lignin). ADF measures lignin and cellulose contents and indicates fibre digestibility. A large WSC content increases forage intake and also increases efficiency of protein utilization (Hopkins and Wilkins, 2006; Moorby *et al.*, 2006). Feeding ruminants with locally produced grassland material requires at least a constant minimum forage quality to ensure good animal performance and economic success even in the face of disturbance to the production system.

In Central Europe, the future climate will be characterized by larger variability, which includes more severe and more frequent droughts and other extreme events (IPCC, 2013). Considering the impact of such events on forage quality is important for agricultural production (Humphreys *et al.*, 2006). Water availability is known to influence forage quality parameters. However, reports of drought effects of forage quality are contradictory and for temperate regions and non-steppe ecosystems, most studies do not deal with old, permanent grassland but with cultivated grass and legume forages (Deleglise *et al.*, 2015; Dumont *et al.*, 2015). Moderate drought has been found to increase CP contents (Grant *et al.*, 2014; Jensen *et al.*, 2010; Peterson *et al.*, 1992; Sanaullah *et al.*, 2014). This increase is widely seen as a consequence of delayed maturity due to growth inhibition during the absence of water, a minimized N dilution effect, or a shift in the stem-leaf ratio (Buxton, 1996; Grant *et al.*, 2014; Peterson *et al.*, 1992). In contrast, drought can also have inconsistent or even negative effects on CP contents, a change that is mostly associated with high drought severity (Buxton, 1996; Deleglise *et al.*, 2015, Küchenmeister *et al.*, 2013; Wang & Frei; 2011). Drought is also known to decrease NDF and ADF contents (Bittman *et al.*, 1988; Bruinenberg *et al.*, 2002; Dumont *et* *al.*, 2015; Gibson *et al.*, 2016; Grant *et al.*, 2014; Küchenmeister *et al.*, 2013), but here, there are also contradicting reports (Buxton, 1996; Deleglise *et al.*, 2015; Durand *et al.*, 2010; Seguin *et al.*, 2002). Drought increases WSC contents (Küchenmeister *et al.*, 2013; Volaire and Lelievre, 1997). This is a protection mechanism that stabilizes membranes (Hincha *et al.*, 2007; Livingston *et al.*, 2009). Increasing WSC contents to make plants more drought resistant is even a breeding goal in forage grass breeding (Robins and Lovatt, 2016).

Apart from these relationships, there are other important influences on grassland forage quality: Time of the season, sward composition and N fertilization are bound to interact with the whole-sward's reaction to drought. The temporal cutting regime is highly influential for the nutritive value of herbage (Čop *et al.*, 2009a), and shifting cutting dates can result in significant differences of forage quality parameters (Buxton, 1996). These differences are due to plant maturation (Čop *et al.*, 2009b). Among species and also among functional groups, the initial forage quality parameters differ (Bruinenberg *et al.*, 2002; Duru *et al.*, 2008; Hatfield *et al.*, 2007). Furthermore, their speed of maturation and the extent of the resulting quality changes are different (Ergon *et al.*, 2017). The resulting decrease of digestibility with plant maturity over the course of the growing season is smaller in dicots than in grasses (Bumb *et al.*, 2016). Quality parameters among species and functional groups also vary with climatic conditions and management (Fales and Fritz, 2007; Gardarin *et al.*, 2014). Therefore, sward functional composition is expected to influence the drought response of forage quality parameters. N fertilization has been observed to be a highly influential factor for grassland quality parameters. It is known to increase CP contents of grasslands (e.g. Grant *et al.*, 2014; Keating and O'Kiely, 2000; Rasmussen *et al.*, 2008; Whitehead, 2000). It accelerates the decrease of forage quality that is caused by maturation (Duru and Ducrocq, 2002) because it enhances stem development (Duru *et al.*, 2000) and increases cell wall contents when dry matter is accumulated (Park *et al.*, 2017). Therefore, N fertilization increases fibre contents in mature grasses (Isselstein, 1993) and in swards in general. N fertilization has been found to decrease WSC contents in plants (Keating O'Kiely, 2000; Louahlia *et al.*, 2008; Nowakowski, 1962; Rasmussen *et al.*, 2008; Roche *et al.*, 2016), but also the opposite has been described (Collins and Balasko, 1981; DaCosta and Huang, 2006; Küchenmeister *et al.*, 2013). Interacting influences of drought stress and N fertilization on WSC contents are hardly described in literature, whereas some interactions of N fertilization with sward compositions are known. Ergon *et al.* (2016) found a diversity effect on forage quality that was suppressed when fertilization was present. They also described the phenomenon that N fertilization had a similar effect on forage quality as having a mixed sward with grasses and legumes.

So far, the interactions of N fertilization, sward composition and drought stress on forage quality of old permanent grassland still need to be understood. In a complex system like old permanent grassland, the prediction of combined effects on forage quality can neither be derived from simply combining effects of each single factor, nor from assuming that these grasslands will behave like sown-in grassland cultivations or even mesocosms (Balvanera *et al.*, 2006; Flombaum and Sala, 2008; Zhang *et al.*, 2013). Therefore, there is a need for experiments that target investigations of effect combinations of climate factors with other influencers (Dumont *et al.*, 2015). Due to the climatic predictions of more frequent summer drought events (Beniston *et al.*, 2007; IPCC, 2013; Meehl and Tebaldi, 2004; Schär *et al.* 2004), repeated drought events might become problematic (Humphreys *et al.*, 2006) and the reaction of recurring droughts has rarely been investigated in old permanent grasslands. Negative long-term effects of recurring drought will lead to decreased plant performance because even with adaptation to drought, plant resources will be depleted after some time (Lloret *et al.*, 2004; Lopez *et al.*, 2009; Walter *et al.*, 2012).

Here, we present a study that addresses those needs for combined research: We investigated the drought stress effect on three typical old permanent grassland sites in Northern Germany while considering the influence of the factors cutting time, sward composition and N fertilization.

We hypothesized that

i. the drought stress response of swards is also influenced by season, sward composition and fertilization level

ii. these influences will be different for each factor-level combination

3.3 Materials and methods

3.3.1 Experimental design

In a three-year experiment on three sites, we investigated the effects of drought (with and without rain-out shelters), sward composition (with and without reduction of dicot species cover), and N fertilization (with 180 kg N ha^{-1} a^{-1} or without) in a completely randomized block design with four replicates. Sites were chosen from typical grasslands in the temperate, subcontinental North of Germany. The south-eastern lowland site (SEL) is located south of Göttingen in the Leine valley, the north-western lowland site (NWL) is located near Cloppenburg in the Northern German Plain and the sub-mountainous site (SMS) is located in Silberborn in the Solling mountain range near Höxter. Site details are presented in Table 8-1.

Treated sward plots were quadratic and had a size 3.24 m^2. All measurements and samples stem from the core area of 0.4 x 0.4 m in the center of each plot. In each spring and summer, droughts of, on average, 36 days were induced by installing rain-out shelters that covered the whole plot with greenhouse film (GeKaHo GbR, Gewächshausfolie SPR 5, 200my) at 1.5 m mean height. Although highly light-permeable (even for UV radiation), films lowered radiation intensity significantly (SunScan Canopy Analysis System measurement of photosynthetically active radiation (PAR) on a sunny day in May around noon, $n = 32$; without shelter: 1757 ± 48 W m^{-2}, with shelter 1275 ± 86 W m^2, chi-squared = 44.9032, p-value < 0.0001). These values surpass the light saturation point of typical grassland species (Cooper and Tainton, 1968; Liu et al., 2016) and therefore we do not expect PAR radiation to be limiting leaf photosynthesis.

The microclimate under the rain-out shelters and above control plots was surveyed by data loggers (CiK Solutions GmbH, Haxo-8 LogTag), which recorded values every 30 minutes from April until the last cut during all three years. Relative humidity showed no significant difference (Wilcoxon signed rank test with continuity correction, $p = 0.1763$) between treatments with and without shelters, but temperature on the plots with shelters was significantly increased by 0.1°C (Wilcoxon signed rank test with continuity correction, $p < 0.0001$).

We did not apply barriers to prevent water run-off into the plots to minimize disturbance to the swards' root system. Soil water content under rain-out shelters was monitored by gravimetric sampling. Even after major rainfall events, the core area from which all samples were taken proved to remain dry. During the induced droughts, which lasted on average 36 days, stressed plots received no rainfall. The control plots received natural precipitation. The rain-out shelters held back 73 ± 5% precipitation (SEL), 52 ± 8% (NWL), and 60 ± 5% (SMS), respectively, during the experimental period. On average over all locations and years, rain-out shelters held back 148 mm of the 240 mm precipitation (62 ± 8%) that fell from the beginning of the growing season until the end of the second drought stress treatment.

Spring drought stress periods started about seven to eight weeks after the start of the year's growing season, which was determined by five consecutive days with an average temperature > 5°C (Jones *et al.*, 2002). Thus, stress periods started around the end of April or the beginning of May. Directly after each spring drought stress period, the greenhouse films were removed for three weeks to allow natural precipitation to rewet the stress-treated plots. Then, summer drought stress periods started around the end of June. After those, greenhouse films were removed until the following spring drought stress period. Plots were not irrigated during the three years of experiments.

We used the definition of Lauenroth *et al.* (1978) that a plant functional group is a group of species that share morphological, and perhaps physiological, traits. Therefore, we grouped the species found in our swards into the three functional groups grasses, forbs, and legumes. We manipulated the functional group composition of swards by the application of herbicides against dicotyledonous species (forbs and legumes) on half of the plots. Starane Ranger (100 g l^{-1} Fluroxypyr and 100 g l^{-1} Triclopyr, 2 l ha^{-1}) and Duplosan KV (600 g l^{-1}Mecoprop-P, 2 l ha^{-1}) were applied in the year before the experiment started and in the spring of the second year of the experiment. Hence, diverse swards with the original species composition were distinguishable from the grass-dominated swards on the herbicide-treated plots (Carlsson *et al.*, 2017, Table 8-1, Table 8-2). Each site's most abundant species are presented in Table 8-7

At the beginning of each growing season, 200 kg ha^{-1} potassium chloride (in the form of 40 % K_2O) and 30 kg ha^{-1} triple superphosphate (in the form of 46 % P_2O_5) were applied on all plots to ensure plant nutrient supply. Potassium application was divided into applying 100 kg ha^{-1}

at the beginning of the growing season and again after the end of the spring drought stress period. Additionally, half of the plots were fertilized with 180 kg ha^{-1} N (in the form of 46 % KAS). N fertilization was split into applying 90 kg N ha^{-1} at the beginning of the growing season and a further 45 kg N ha^{-1} after each cut which was preceded by a drought stress period.

Whole plots were cut to 7 cm stubble height directly when the greenhouse film was removed and once again in the beginning of October. Samples were taken from the core area only and the fresh material was separated by functional group (grass, forbs, and legumes). Those subsamples were dried at 60 °C for 48 hours and ground to pass a 1-mm screen. All subsamples of sufficient biomass to fill the cuvettes were measured with near infrared spectroscopy (NIRS) with a FOSS spectrometer. The spectra were analyzed by the institute VDLUFA Qualitätssicherung NIRS GmbH, Kassel, Germany (Tillmann, 2010) for their contents of CP, WSC, NDF and ADF. The VDLUFA calibration has been validated for many grasslands types of a multitude of management intensities. Then, whole sward values of all four parameters were estimated for each harvest and plot by calculating mean summed values of all samples with a H-value under 4, weighted by the share of the respective functional groups from each plot.

We analyzed the data from both drought stress periods from all three years for the influence of sward type and fertilization level on the estimated whole-sward CP content, estimated whole-sward WSC content, estimated whole-sward contents of ADF and NDF during drought stress events.

3.3.2 Statistical analysis

Statistical analyses were conducted with R version 3.0.2 (R Core Team, 2013). We used the significance level of $\alpha \leq 0.05$ throughout. Each dataset of whole sward CP, WSC, ADF, and NDF, respectively, was analyzed by applying a linear mixed model with the function lme() (software package nlme by Pinheiro *et al.*, 2011). All models were tested for normal distribution (quantile-quantile-plots and Shapiro-Wilk test) and homoscedasticity (residual plots and Levene Test). If necessary, models were corrected by transformation and/or including a variance function. The NDF model was fitted with the transformation log(100-n). Fixed effects in the analyses were year, cut, fertilization, sward type, and drought stress. Site and block nested in site were included as random effects.

All initial models included all possible interactions and were then subsequently optimized by stepwise exclusion of fixed effect interactions that appeared as not significant. They were removed if removal led to a lower model AIC. According to Pinheiro and Bates (2000) and Zuur *et al.* (2009) if a model cannot lose a fixed factor or an interaction thereof without increasing its AIC, it is a model that fits the data as well as possible.

We calculated and grouped post-hoc pairwise contrasts of the most influential variables and their interactions of these optimized models with the Tukey method found in the packages lsmeans (Lenth, 2016) and multcompView (Graves *et al.*, 2012). Influence of variables was determined by the magnitude of the F-value.

3.4 Results

3.4.1 Crude Protein

We measured significant influences of all tested parameters (year, season, fertilization level, sward type, drought stress), all but sward type as main effects that were significantly influential without interaction. While estimated crude protein content (CP) differed significantly between years, these differences were mainly found in scale (with 2011 being the year with largest CPs and 2013 the year with the smallest) and in effect size of other parameters (Figure 3-1). The overall tendencies remained the same in each year. CP was generally larger in the summer than in the spring season, also effects of other parameters tended to be more pronounced (Figure 3-1). Fertilization was the most important parameter to determine CP (F-value, Table 3-1). Fertilized swards always had larger CP than unfertilized swards (Figure 3-1, Table 8-8). Diverse swards that were unfertilized had smaller CP than grass-dominated swards, an effect that was significant in the summer cut, and in unfertilized swards in spring (Table 8-8). Drought stressed swards tended to have larger CP than control swards, but significant differences could only be found in the spring season in non-fertilized diverse swards and fertilized grass-dominated swards (Table 8-8).

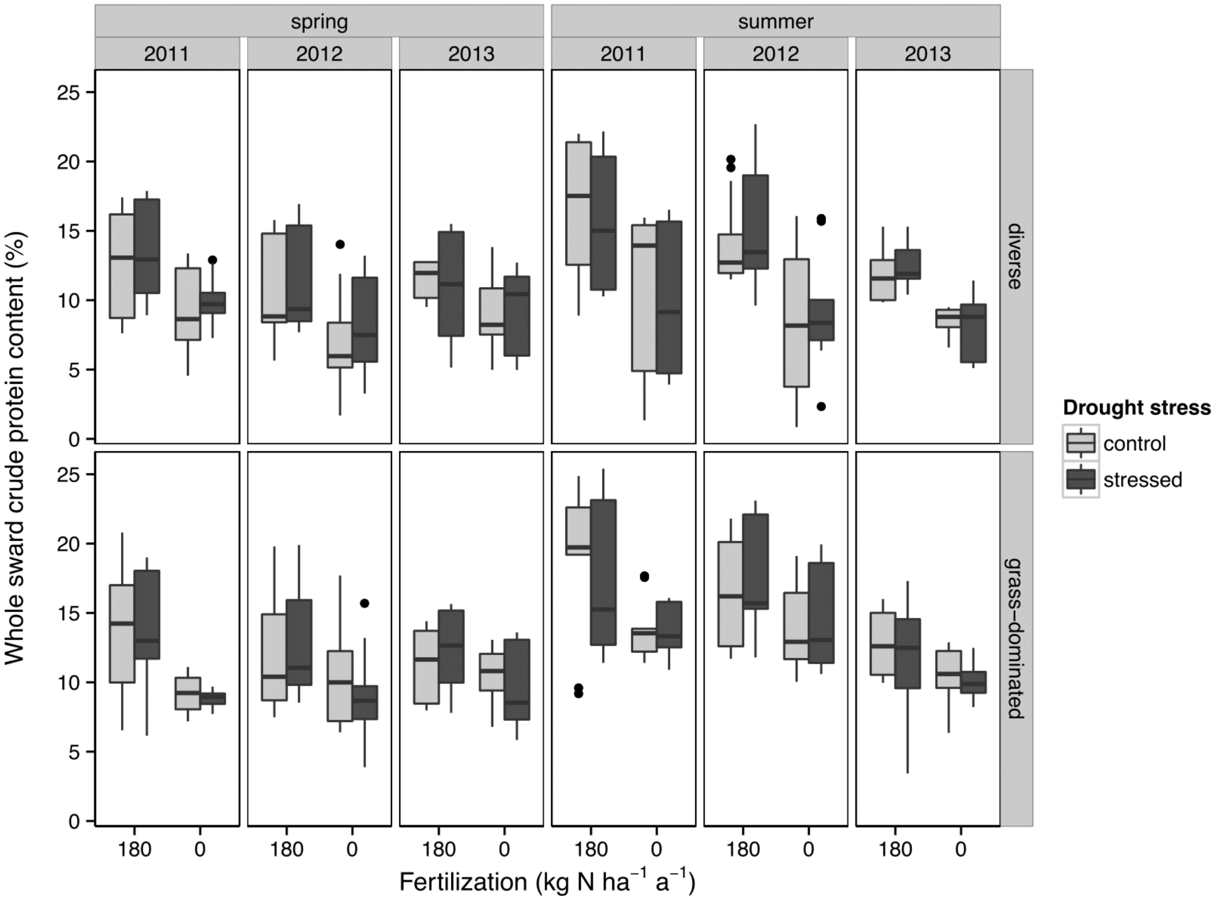

Figure 3-1 Crude protein contents of whole sward forage samples by cut (spring, summer), year (2011 – 2013). sward type (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed).

Table 3-1 F-test table of optimized linear nested mixed model of crude protein content of the whole sward. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
fert	26.19732	<.0001
sward	1.39128	0.2385
stress	21.00557	<.0001
cut	8.59048	0.0035
year	11.25936	<.0001
fert:sward	0.10638	0.7444
fert:stress	0.01358	0.9073
sward:stress	6.65058	0.0101
fert:cut	7.84267	0.0052
sward:cut	3.69161	0.055
stress:cut	9.95362	0.0017
fert:year	8.11591	0.0003
sward:year	12.52168	<.0001
stress:year	6.61614	0.0014
cut:year	7.21243	0.0008
fert:sward:stress	1.99627	0.158
fert:sward:cut	0.07510	0.7841
fert:stress:cut	1.84121	0.1751
sward:stress:cut	11.11492	0.0009
fert:sward:year	5.61756	0.0038
fert:stress:year	1.34756	0.2604
sward:stress:year	4.25392	0.0145
sward:cut:year	0.25632	0.774
stress:cut:year	4.58578	0.0104
fert:sward:stress:cut	10.58338	0.0012
fert:sward:stress:year	2.71533	0.0667
sward:stress:cut:year	2.65342	0.0709

3.4.2 Water Soluble Carbohydrates

We measured significant influences of all tested parameters: Year, season, fertilization level, sward type, drought stress (Table 3-2). Estimated water soluble carbohydrate content (WSC) was significantly larger in 2011 than in 2012 with 2013 being intermediate and not different from the others. The factor year added overall variability, but no development over time could be identified. Season was one of the most important factors. Mean WSC was larger and more homogenous among factor combinations in spring than in summer (Figure 3-2). Fertilization was significantly determining WSC, but aside from a generally slightly larger WSC content in fertilized swards, no clear trends could be identified. Diverse swards had lower WSC than grass-dominated swards, but this relationship was only significant for certain factor combinations and mostly masked by the variability between years and fertilization levels (Table 8-9). Stressed swards had a significantly larger WSC than the control swards (Table 8-9), this difference was more pronounced in spring than in summer (Figure 3-2).

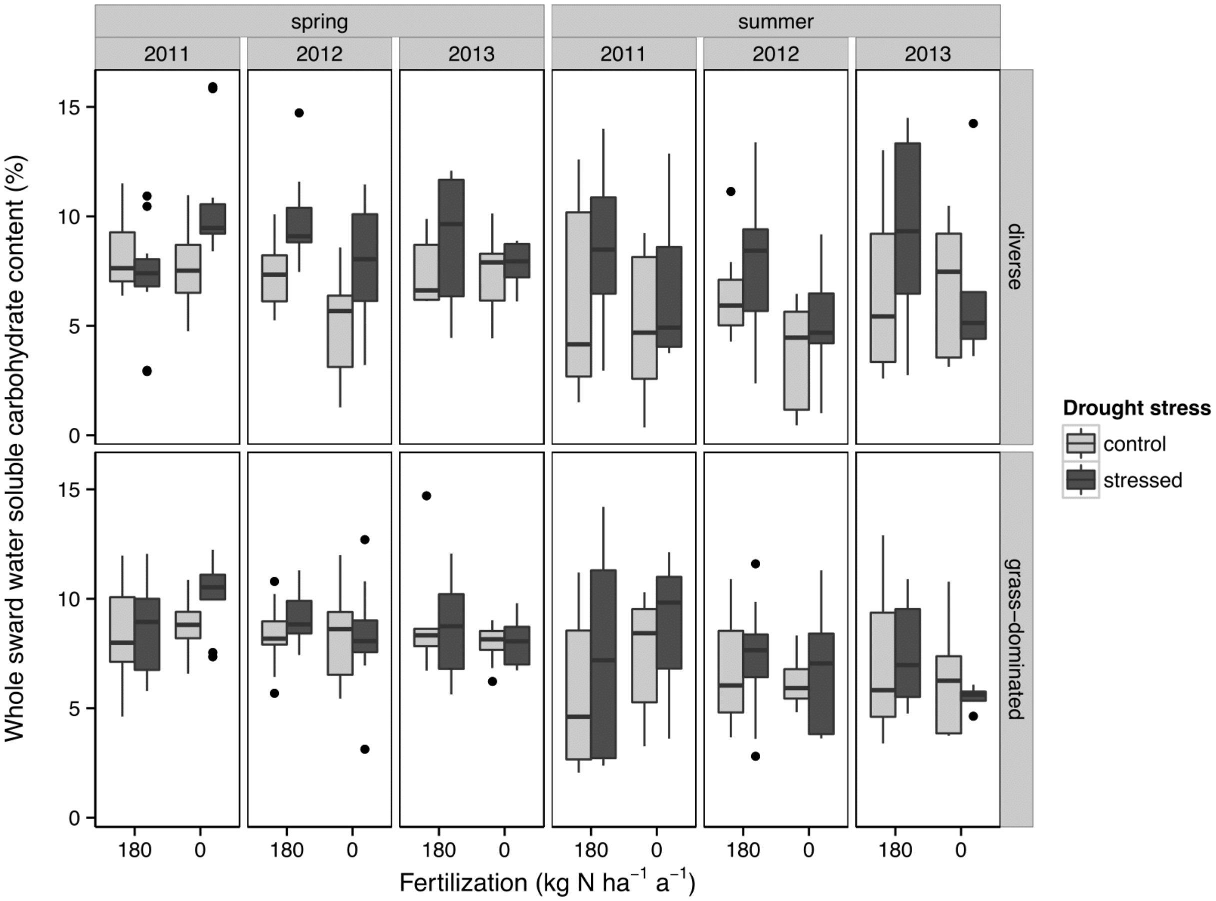

Figure 3-2 Estimated water soluble carbohydrate contents of whole sward forage samples by cut (spring, summer), year (2011 – 2013). sward type (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed).

Table 3-2 F-test table of optimized linear nested mixed model of water soluble carbohydrate content of the whole sward. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
fert	0.028	0.867
sward	0.12079	0.728
stress	22.77685	<.0001
cut	17.75918	<.0001
year	14.94107	<.0001
fert:sward	1.15205	0.283
fert:stress	17.49637	<.0001
sward:stress	0.09039	0.764
fert:cut	1.09313	0.296
sward:cut	5.86974	0.016
stress:cut	1.53416	0.216
fert:year	4.96413	0.007
sward:year	7.77564	0.000
stress:year	1.95699	0.142
cut:year	1.85443	0.157
fert:sward:cut	9.16388	0.003
fert:stress:cut	11.41385	0.001
fert:sward:year	4.48681	0.012
fert:stress:year	6.92346	0.001
sward:stress:year	3.93722	0.020
fert:cut:year	0.27545	0.759
sward:cut:year	3.05386	0.048
stress:cut:year	0.04475	0.956
fert:sward:cut:year	2.4304	0.089
fert:stress:cut:year	3.06822	0.047

3.4.3 Acid Detergent Fibre

All tested parameters showed significant influences: Year, season, fertilization level, sward type, drought stress (Table 3-3). Estimated Acid Detergent Fibre Content (ADF) varied among the years and in 2013 the overall mean ADF was larger than in the previous years. Variation of ADF from diverse swards was larger in 2011 and 2012. (Figure 3-3). ADF in spring was significantly larger than in summer (Table 8-10). Fertilized swards had larger ADF than non-fertilized ones with the exception of values from 2011, there the difference is present but not significant (Table 8-10). Diverse swards had smaller ADF than grass-dominated swards, although the effect was not always significant (Table 8-10). Also, variation of ADF tended to be larger in diverse swards (Figure 3-3). Drought stress did not affect ADF in the spring. In summer stressed swards had smaller ADF than controlled ones, but the effect was not significant if sward type was also considered (Table 8-10).

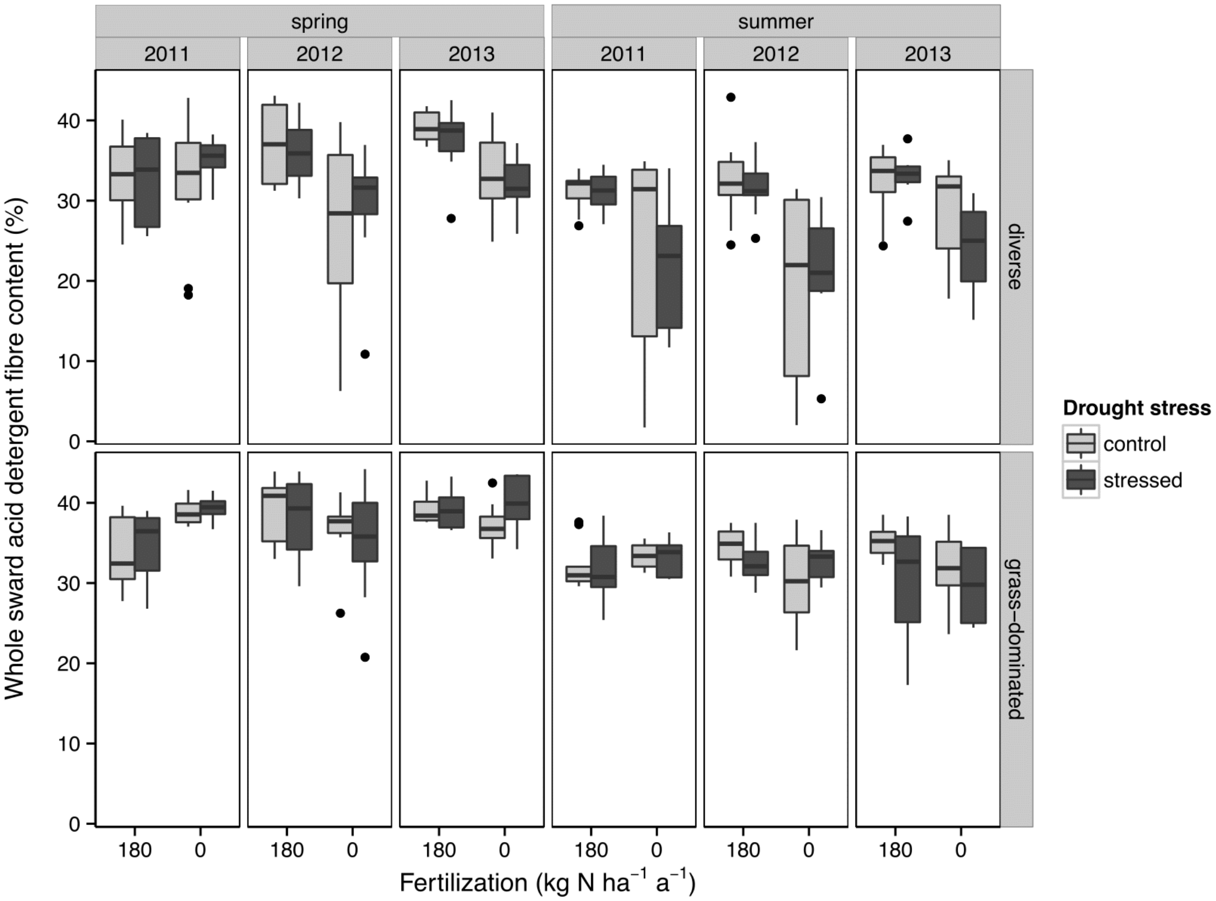

Figure 3-3 Acid detergent fibre contents of whole sward forage samples by cut (spring, summer), year (2011 – 2013). sward type (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} $year^{-1}$, unfertilized) and drought stress level (control, stressed).

Table 3-3 F-test table of optimized linear nested mixed model of acid detergent fibre content of the whole sward. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions. Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
fert	0.65757	0.4176
sward	27.52414	<.0001
stress	0.73222	0.3924
cut	23.2985	<.0001
year	10.39741	<.0001
fert:sward	19.06305	<.0001
fert:stress	0.00011	0.9916
sward:stress	0.17151	0.6789
fert:cut	5.41443	0.0202
sward:cut	1.76938	0.1838
stress:cut	13.2955	0.0003
fert:year	41.40115	<.0001
sward:year	2.04355	0.1301
stress:year	0.85325	0.4264
cut:year	0.53728	0.5845
fert:sward:stress	0.25302	0.6151
fert:sward:cut	2.80322	0.0944
fert:stress:cut	5.37803	0.0206
sward:stress:cut	4.14132	0.0421
fert:stress:year	2.43629	0.088
sward:stress:year	2.41938	0.0895
fert:cut:year	11.63834	<.0001
sward:cut:year	0.58508	0.5573
stress:cut:year	1.78924	0.1677
fert:sward:stress:cut	4.19766	0.0408
sward:stress:cut:year	4.08142	0.0172

3.4.4 Neutral Detergent Fibre

We measured significant influences of all tested parameters: Year, season, fertilization level, sward type, drought stress (Table 3-4). Estimated Neutral Detergent Fibre Content (NDF) varied among years and effect sizes of other parameters were larger in 2013 and 2012 than in 2011. NDF was significantly larger in spring than in summer (Tables 8-11). Variability of NDF values was larger in summer (Figure 3-4). NDF was significantly larger in fertilized than in unfertilized swards and significantly smaller in diverse than in grass-dominated swards (Tables 8-11). Variability of NDF values was larger in diverse swards (Figure 3-4). Drought stress appeared in significant interactions but only one clear significant difference among groupings by the other interaction parameters could be identified (in summer 2013, control swards had larger NDF values than stressed swards (Tables 8-11).

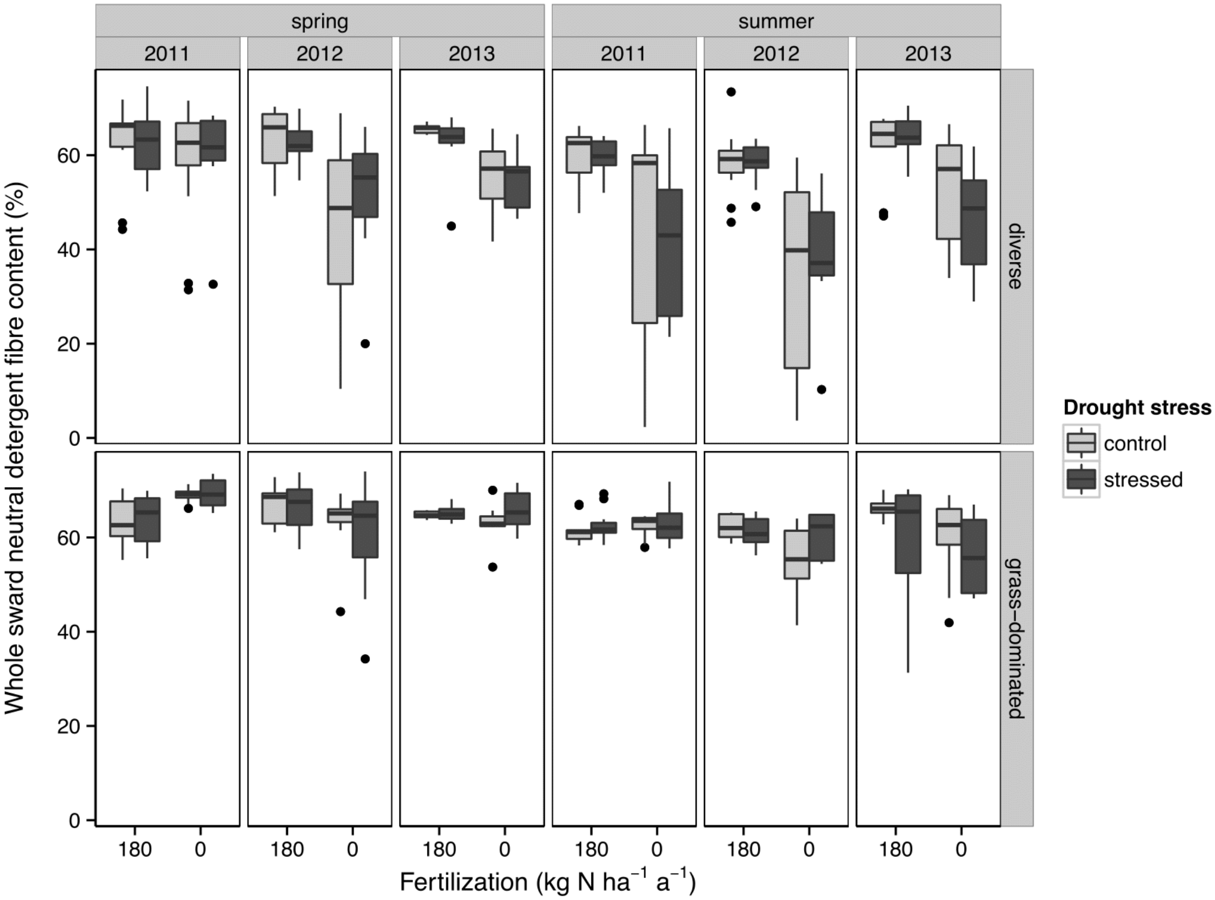

Figure 3-4 Neutral detergent fibre contents of whole sward forage samples by cut (spring, summer), year (2011 – 2013). sward type (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed).

Table 3-4 F-test table of optimized linear nested mixed model of neutral detergent fibre content of the whole sward. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions. Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
fert	4.437	0.0354
sward	24.973	<.0001
stress	0.954	0.329
cut	15.505	0.0001
year	17.612	<.0001
fert:sward	26.733	<.0001
fert:stress	1.524	0.2173
sward:stress	0.312	0.5764
fert:cut	5.293	0.0216
sward:cut	0.051	0.8212
stress:cut	5.148	0.0235
fert:year	27.804	<.0001
sward:year	4.022	0.0182
stress:year	0.794	0.4525
cut:year	1.272	0.2806
fert:sward:stress	1.456	0.2278
fert:sward:cut	0.468	0.4943
fert:stress:cut	4.121	0.0426
sward:stress:cut	5.425	0.0201
sward:stress:year	1.65	0.1926
fert:cut:year	4.809	0.0084
sward:cut:year	1.181	0.3075
stress:cut:year	1.108	0.3306
fert:sward:stress:cut	6.542	0.0107
sward:stress:cut:year	4.514	0.0112

3.5 Discussion

The response of grasslands in terms of forage quality to future climate events like droughts is not clear yet. We hypothesized that the drought stress response of swards was also influenced by cut, sward composition and fertilization level and that these influences will be different for each factor-level combination. We found that under a future climate in the tested conditions, the risk for a decrease in forage quality in terms of CP, NDF, ADF and WSC contents due to drought stress is rather low. Especially moderate drought stress could even improve CP and WSC contents of grassland forage. Our results show that drought stress was less important for the forage quality parameters NDF and ADF contents than cut and N fertilization. Drought affected CP and WSC contents more heavily than the fibre components, but cut and fertilization were at least equally important. Sward composition always played a role, but its impact was weaker and less differentiated. In the following, we discuss the influences of year, cut, fertilization and sward first and then the effects of drought stress and its interactions with other factors.

3.5.1 Effects of analyzed factors and their combinations on herbage quality parameters

We generally observed rather large NDF contents if compared to similar swards (Andrés *et al.*, 2005; Andueza *et al.*, 2010; Čop *et al.*, 2009b; Meisser *et al.*, 2013; Picon-Cochard *et al.*, 2004; Spanghero *et al.*,1999) and also mostly a little larger than the 550 to 600 g/kg recommended for the nutrition of dairy cows (Van Soest, 1994). This was possibly caused by the assemblage of species in our swards, many of which are not the preferred species for ruminant forage (Table 8-7), e.g. the high quality forage grass *Lolium* perenne was not among the main grasses, although it was present in the swards. Cutting three times a year is considered moderate to moderately intense usage of grassland. Intensively cut leys and grasslands that consist of a high proportion of sown-in *Lolium perenne* or *Festulolium* with a legume partner species reach much lower fibre contents (e.g. Østrem *et al.*, 2015; Schulze *et al.*, 2014) due to species-specific traits.

3.5.1.1 Year and cut

There was some variation of all measured factors among the years, but only NDF and ADF contents varied significantly. The fibre contents, however, showed no tendency among years. The variations could not be explained by any other measured parameter, so we propose that an unmeasured biotic or abiotic factor has been the cause.

Maturity stage explained the differences in CP, NDF, ADF and WSC contents among cuts. The plant material harvested in summer contained less stem and flower parts, thus its CP content was larger. In line with Čop *et al.* (2009a), we observed more pronounced effects of all treatments on CP content in the summer cut than in the spring cut, showing that the cutting regime is highly influential for the nutritive value of herbage.

The larger NDF and ADF contents in spring than in summer were unexpected. Although our time of cut was closely adjusted to local agricultural practice, a cut delay of only a few days in the end of May can result in significant differences due to the rapid increase of fibre content during that time of maturation (Buxton, 1996; Čop *et al.*, 2009b).

In line with other findings from grassland (Bruinenberg *et al.*, 2002; Čop 2009b), WSC contents were larger and also showed larger differences between treatments in spring than in summer. Generally, WSC contents are larger in spring than in summer due to plant maturity.

3.5.1.2 N fertilization

N fertilization generally enhanced CP contents of vegetation regardless of other treatments, a widely recognized phenomenon (Grant *et al.*, 2014; Keating and O'Kiely, 2000; Rasmussen *et al.*, 2008; Whitehead, 2000). We found larger NDF and ADF contents in fertilized than in unfertilized swards. In line with this, fertilization enhances stem development (Duru *et al.*, 2000), leads to an acceleration of the decrease of digestibility with time (Duru and Ducrocq, 2002) and increases fibre contents in mature grasses (Isselstein, 1993). Park *et al.* (2017) reported larger NDF and ADF contents under fertilization for their swards and argued that cell wall content of their swards increased with dry matter accumulation, which was enhanced by fertilization. As our sward dry matter increased with fertilization (Carlsson *et al.*, 2017), both considerations (faster development and larger dry matter accumulation under fertilization) are likely explanations for our results.

Fertilization generally led to an increase in WSC contents in swards. While it has been recognized that N fertilization decreases WSC contents in plants (Keating and O'Kiely, 2000; Louahlia *et al.*, 2008; Novakowski, 1962; Rasmussen *et al.*, 2008; Roche *et al.*, 2016), also the opposite has been described (Collins and Balasko, 1981; DaCosta and Huang, 2006; Küchenmeister *et al.*, 2013). No trend of the effect of sward composition on the influence of N fertilization on WSC contents was visible in this experiment. Likely, species composition or identity effects have to be considered to understand the effects of N fertilizer application on whole sward WSC contents.

3.5.1.3 Sward composition

The influence of sward composition on CP contents was only significant in interactions with drought and is thus discussed in 3.1.4. The share of forbs and legumes was not represented by the exact same species in each diverse sward, but included very different shares of e.g. clovers, plantain or dandelion, which all have very different and species-specific fibre contents (see Wilman and Riley, 1993). Legume species generally vary in fibre contents (Küchenmeister *et al.*, 2013). Therefore, the observed increased fibre variation in diverse swards if compared to grass-dominated swards was probably due to a species identity effect.

Diverse swards can contain less WSC than grass monocultures or intensively managed grass-dominated swards (Bruinenberg *et al.*, 2002; Ergon *et al.*, 2017). Explanations are decreased WSC storage because of increased growth caused by better N availability (Ergon *et al.*, 2016) and extended use of WSC for growth by grasses that are shaded (Evans *et al.*, 1996) in diverse compared to non-diverse swards. WSC contents in grasses are known to be larger than in forbs and legumes (Dewhurst *et al.*, 2003; Marshall *et al.*, 2004; Ulyatt *et al.*, 1988). Grasses formed the main proportion of our sward biomasses (Carlsson *et al.*, 2017; Table

8-7). A reduction in the grass WSC contents should therefore have a larger impact on whole sward WSC contents than a change in the dicot component.

3.5.1.4 Drought stress

We found that drought stress influenced CP contents under certain circumstances. Significant increases of CP contents under drought conditions were observed in the spring in diverse swards that were unfertilized and grass-dominated swards that were fertilized. If a drought is not severe, it can improve the quality of herbage (Jensen *et al.,* 2003), because it may slow maturation (Buxton, 1996; Halim *et al.*, 1989) as well as growth, leading to an accumulation of N in the tissue (Grant *et al.*, 2014; Jensen *et al.,* 2010; Sanaullah *et al.*, 2014).

Plants from different functional groups react independently and differently towards changed water availability (Walter *et al.*, 2012). It has been observed that dicot quality rises under drought stress (Peterson *et al.*, 1992). Therefore, the finding of enhanced CP contents in a drought exposed sward with a large forb and legume content was expectable. However, this effect of sward composition was only visible in the unfertilized swards. N fertilization is known to increase CP contents of grassland (Grant *et al.*, 2014; Whitehead, 2000) and may override an effect of sward composition.

Processes like facilitation and increased resource exploitation by temporal or spatial niche complementarity may lead to increased N yields in mixtures compared to monocultures (Suter *et al.*, 2015). One or several of these processes likely took place in our experiment and may explain why the CP contents of diverse unfertilized and grass-dominated fertilized swards were positively affected by drought. In unfertilized swards, the content of legumes and forbs was larger than in fertilized swards, and of course their content and thus contribution to the harvested material was also larger in the diverse swards. Therefore, even if we assume that grass quality under drought stress stagnated or even deteriorated, the enhanced CP contents of forbs and legumes alone might have been enough to create an improvement of whole-sward CP contents under drought conditions.

Decreasing fibre content during dry periods or under unstable water supply has been reported often (Bittman *et al.*, 1988; Bruinenberg, 2002; Gibson *et al.*, 2016; Grant *et al.*, 2014; Küchenmeister *et al.*, 2013), although contrary results exist (Buxton, 1996; Durand *et al.*, 2010; Seguin *et al.*, 2002). A decrease is mostly explained by delayed maturation and thus less cell wall material in the physiologically younger plant (Buxton 1996; Küchenmeister *et al.*, 2013), but could also be a result of lacking N supply, because N uptake and thus growth was inhibited under drought (Durand *et al.*, 2010). We fertilized our swards six to seven weeks before each drought was induced, so we can assume that N supply was sufficient during droughts.

Drought stress did not affect NDF and ADF contents in the spring. The first drought period was severe and should have slowed down plant maturation (Bittman *et al.*, 1988; Küchenmeister *et al.*, 2013). Our results show that this was not the case, as the NDF contents should have been decreased in the stressed plants, then. Resilience was large enough to cover the drought inhibition in the first drought period that the plants experienced that year (Carlsson *et al.*, 2017), but not in the second period, as there was a drought effect present. If plants did not fully recover after the first drought period and thus could not reach the maturation stage of the control swards until the second drought period, the stressed plants could have continuously been inhibited in their maturation and thus accumulated significantly less fibre during the second drought period. However, the extent of the drought effect on fibre contents in summer and the absolute difference between contents that were compared (control to stressed vs. spring to summer) leads us to the conclusion that the drought effect was small and overridden by stronger effects of season and sward composition.

In line with other publications, drought stressed swards had larger WSC contents than controls (DaCosta and Huang, 2006; Küchenmeister *et al.*, 2013). It is known that fructans accumulate during drought and protect cell membranes of plants from dehydration stress (De Roover *et al.*, 2000; Hincha *et al.*, 2007; Livingston *et al.*, 2009; Spollen and Nelson, 1994). There are even attempts to exploit this trait economically and breed grasses with large WSC contents to make them more drought resistant (Robins and Lovatt, 2016).

Thus, in terms of WSC contents, drought stress increased feeding value and also maybe N efficiency (Moorby *et al.*, 2006) of grassland material for ruminants. Large WSC contents in the forage have many advantages for ruminant production (Robins and Lovatt, 2016). Drought stress therefore does not have to be a disadvantage for grassland production in terms of WSC supply.

3.5.2 Consequences of drought stress for grassland production

Drought stress influenced CP contents only under certain circumstances. Both in rather extensively managed grasslands that had a larger diversity and were not fertilized and in rather intensively managed grasslands that were fertilized and had a smaller diversity, CP contents of the whole sward were stable or increased under drought stress. The influences of diversity and fertilizations can have similar positive effects on forage quality parameters, but can also be mutually exclusive in grassland (Carlsson *et al.*, 2017; Ergon *et al.*, 2017). Therefore, the forage quality of grasslands with a solid N supply, be it from legumes or fertilizer, will not necessarily be negatively affected by drought. Still, the N cycle in grasslands should be controlled closely to avoid other negative effects like leaching or gaseous N losses.

We found no effect of drought on NDF and ADF contents in spring, but a decrease of fibre contents in summer. We see sward maturation as the main reason and could show that maturation was influenced by sward composition and fertilization management. If sward management is adjusted to the composition and N regime, droughts should not have negative effects on ADF and NDF contents. Those contents are also highly dependent on species composition (Bruinenberg *et al.*, 2002; Ergon *et al.*, 2017), but sward composition can prove an easily applicable and useful tool for evaluating the expected forage quality. Whether the digestibility of material from diverse swards was really better than that from grass-dominated swards is speculative, because forbs and legumes might still be harder to digest than the grass component. This could still cancel out the low ADF contents of diverse swards. Grasslands produced more WSC under drought, which was to the extent found desirable for dairy cow nutrition. Thus, we found no negative effects of drought stress on WSC contents.

The general risk of recurring drought events for grassland forage quality parameters measured in this study was relatively low. This may be the case if the management is adapted to efficient N cycling on both more extensively and intensively managed permanent grasslands in Central Europe. According to our study, the first cut will be less affected by drought stress than the following in the same year. Under a future climate, the cutting time has to be even more closely adjusted to the advances in plant maturation to avoid quality disadvantages in terms of CP, NDF and ADF contents. Even the recurrence of droughts over three consecutive years did not establish a decline in quality parameters with time, but only variations between years that showed no trend.

If compared to the risk of decreased biomass yield that farmers may face with water shortages (Fay *et al.*, 2011; Grime *et al.*, 2000; Hartmann and Niklaus, 2012; Hoover *et al.* 2014; Kahmen *et al.* 2005; Zwicke *et al.*, 2013), the found risks of decreasing quality in terms of CP, NDF, ADF and WSC contents were relatively low. Drought, especially moderate drought events, can even have improving effects on CP and WSC contents.

3.6 References

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4 Nitrogen and water availability influence the effects of sward functional composition on three selected grass forage quality parameters

4.1 Abstract

Functional sward composition is an important factor for determining sward forage quality. Although there is much evidence on how functional groups contribute to whole sward forage quality, the mutual influence of functional groups on each other's forage quality in permanent grasslands remains mostly unassessed. We tested dicot biomass share in the sward for its influence on three grass forage quality parameters, also taking the management factor nitrogen (N)-fertilization and the disturbance factor drought stress into account. We found influences of sward composition and dicot biomass share on the forage quality of the grass functional group, but those were only significant in interactions with drought stress and N- fertilization level. Crude protein (CP) and water-soluble carbohydrates (WSC) of grass biomass were modified by the presence of dicots, while there were no effects on acid detergent fibre (ADF). Grass forage quality was modified by legume facilitation and by competition for N with forbs: Grasses from fertilized or diverse swards had a competitive advantage over dicots, leading to considerable stability of grass quality parameters in the face of drought stress. N availability can be improved both by managing functional diversity and by N-fertilization. A better understanding of the mechanisms of facilitation and competition between functional groups and their influence on grass quality parameters under distinct levels of N-availability might help to improve grassland management.

4.2 Introduction

More than a third of the agriculturally managed area in Europe and Germany consists of permanent grassland (Statistische Ämter des Bundes und der Länder, 2011). Grasslands provide herbage that is used as main forage **for** ruminants in dairy production (Hopkins and Wilkins, 2006). While yield is an important parameter for herbage production, the quality of herbage is equally important for efficient ruminant nutrition (Humphreys and Theodorou, 2001). Forage quality of grasslands is driven by plant maturity (Bruinenberg *et al.*, 2002; Buxton, 1996; Čop *et al.*, 2009) and environmental or management factors, but also largely determined by functional sward composition (Küchenmeister *et al.*, 2013; Michaud *et al.*, 2015). Functional groups and their relative biomass proportions are seen as more important for sward quality than species richness per se (Andueza *et al.*, 2015; Khalsa *et al.*, 2014). Including several functional groups in a sward, especially legumes, to enhance sward quality is a common and important measure in grassland management (Lüscher *et al.*, 2014). The beneficial presence of legumes on the quality of mixtures compared to grass-dominated monocultures has been widely shown and is caused by the addition of high-quality legume biomass to the whole-sward biomass as well as by the provision of N to neighboring plants (Lüscher *et al.*, 2014; Nyfeler *et al.*, 2011; Sleugh *et al.*, 2000;). Non-legume dicots are a common part of permanent grasslands, but their influence on whole sward quality is much less studied than the legumes'. Studies from permanent grassland that explicitly included the influence of forb occurrence on whole sward quality have only occasionally been conducted and produced contradictory results (Andueza *et al.*, 2015; Blonski *et al.*, 2004; Donath *et al.*, 2004; Grant *et al.*, 2014; Michaud *et al.*, 2015). Also results from artificial grasslands are inconsistent (Bruinenberg *et al.*, 2002; Jing *et al.*, 2017; Khalsa *et al.*, 2014; Küchenmeister *et al.*, 2013).

So far, there is limited knowledge on how the presence of dicots influences the grass component in terms of quality parameters in permanent grasslands (Ergon *et al.*, 2017). There are untested assumptions that their influence could be positive (Jing *et al.*, 2017). Only three studies aimed at enhancing the quality of the grass component of swards by incorporating legumes (Ergon *et al.*, 2017; Evans *et al.*, 1996; Gierus *et al.*, 2012). There are no studies examining the influence of non-leguminous dicots on grass quality in permanent grasslands. Understanding how grass quality is modified by functional diversity is important, because applied sward management normally concentrates on the grasses (Ansquer *et al.*, 2008; Duru *et al.*, 2010). Therefore, the gap of knowledge on biological influences on grasses is not only a drawback for the general understanding of the generation of grassland forage quality, but also for its consequences for grassland management.

Important influences on grassland forage quality that have to be considered by management are, apart from sward composition, N and water supply. Forage quality increases with good N supply (Buxton, 1996; Collins and Balasko, 1981; Peyraud and Astigarraga, 1998; Zemenchik and Albrecht, 2002), but interactions with sward functional composition remain mostly unexplored (Ergon *et al.*, 2017). Lack of water speeds up plant maturation, which in turn is the most influential variable for forage quality (Bruinenberg *et al.*, 2002; Buxton, 1996; Čop *et al.*, 2009). Drought also decreases N uptake and inhibits plant growth (Beierkuhnlein *et al.*, 2011; Fay *et al.*, 2011; Hoover *et al.*, 2014; Onillon *et al.*, 1995; Peterson *et al.*, 1992). This has consequences for forage quality, which can increase or decrease under drought stress (e.g. Bruinenberg *et al.*, 2002; Deleglise *et al.*, 2015; Dumont *et al.*, 2015; Grant *et al.*, 2014; Küchenmeister *et al.*, 2013; Livingston *et al.*, 2009; Rasmussen *et al.*, 2008). There is clear evidence that climate change and with it the more frequent occurrence of more severe drought events (IPCC, 2013; Lehner *et al.*, 2006; Schär *et al.*, 2004) is already affecting Europe (Stagge *et al.*, 2017). Experimental studies linking the above-mentioned knowledge gaps concerning the influence of forb and legume presence on the quality of the grass component of permanent swards to drought stress and N availability do not exist. So far, there are merely reports of non-experimental observations (Blonski *et al.*, 2004).

We present a study that focuses on the effect of sward functional composition on the quality of the grass component in old permanent grasslands under two different N-fertilization regimes, a drought stress treatment and a corresponding control under natural precipitation. Forage quality parameters of interest included crude protein (CP), acid detergent fibre (ADF), and water-soluble carbohydrates (WSC). Efficient use of local grassland herbage is only applicable for farmers if a minimum forage quality is achieved.

We hypothesize that

i. the presence of legumes and forbs has a measurable effect on the grass component quality

ii. this effect will change under the influence of drought stress

iii. the effect size will differ by N-fertilization level

4.3 Material and methods

4.3.1 Experimental design

This study was conducted on three typical grassland sites in temperate, subcontinental northern Germany. For three consecutive years, swards were cut once in spring (after on average 37 ± 5 days of drought treatment) and once in summer (after on average 35 ± 6 days of drought treatment). One lowland site was chosen south of Goettingen in the Leine valley, the second lowland site was on the Northern German Plain near Cloppenburg and the third site was a sub-mountainous grassland near Uslar in the Solling mountain range. In a completely randomized block design with four replicates, the effects of drought stress (with / without rainout shelters), sward composition (with / without removal of dicot species), and N-fertilization (180 / 0 kg N ha-1 a-1) on forage quality were measured.

Plots were covered with greenhouse film (GeKaHo GbR, Gewächshausfolie SPR 5, 200 my) on tilted rainout shelters of 1.5 m mean height to cause artificial droughts of, on average, 36 days during each year’s spring and summer. The films significantly reduced radiation intensity when covered plots were compared to the controls, although highly light and UV permeable film was chosen (SunScan Canopy Analysis System measurement of photosynthetically active radiation (PAR) on a sunny day in May around noon, n = 32, without shelter: 1757 ± 48 W m-^{2}, with shelter 1275 ± 86 W m-^{2}, chi-squared = 44.9032, p-value <0.0001). The experimental setup does not allow for a correction or differentiation of radiation effects. However, due to the relatively high PAR values under the shelter, which – on a sunny day - are well above the light saturation point of typical grassland species (Cooper and Tainton, 1968; Liu et al., 2016), we do not expect PAR radiation to be limiting leaf photosynthesis. Drought stress treatments began seven to eight weeks after the start of the growing seasons (five consecutive days with an average temperature > 5°C, Jones et al., 2002), which was around the end of April or the beginning of May. The films were removed after each spring drought stress period, and swards were rewetted by natural precipitation. After three weeks, around the end of June, the summer drought stress period was induced accordingly. After the end of the summer drought stress period until the beginning of the next year's spring drought stress period, the films were removed. Swards were never irrigated.

Plant species of the swards were grouped into three functional groups: grasses, forbs, and legumes. Functional group definition follows Lauenroth et al. (1978) and was based on similarities of morphological and physiological plant traits. Sward functional group composition of half of the plots was modified by herbicides that affect dicot species (the forb and legume functional groups). We used 100 g l-1 Fluroxypyr and 100 g l-1 Triclopyr, 2 l ha-1, and 600 g l-1 Mecoprop-P, 2 l ha-1 in the year before the experiment started and again in the second year and thus created a distinguishable functional composition of diverse swards (untreated original composition) and grass-dominated swards (treated with herbicides) (Figure 4-1; Carlsson et al., 2017).

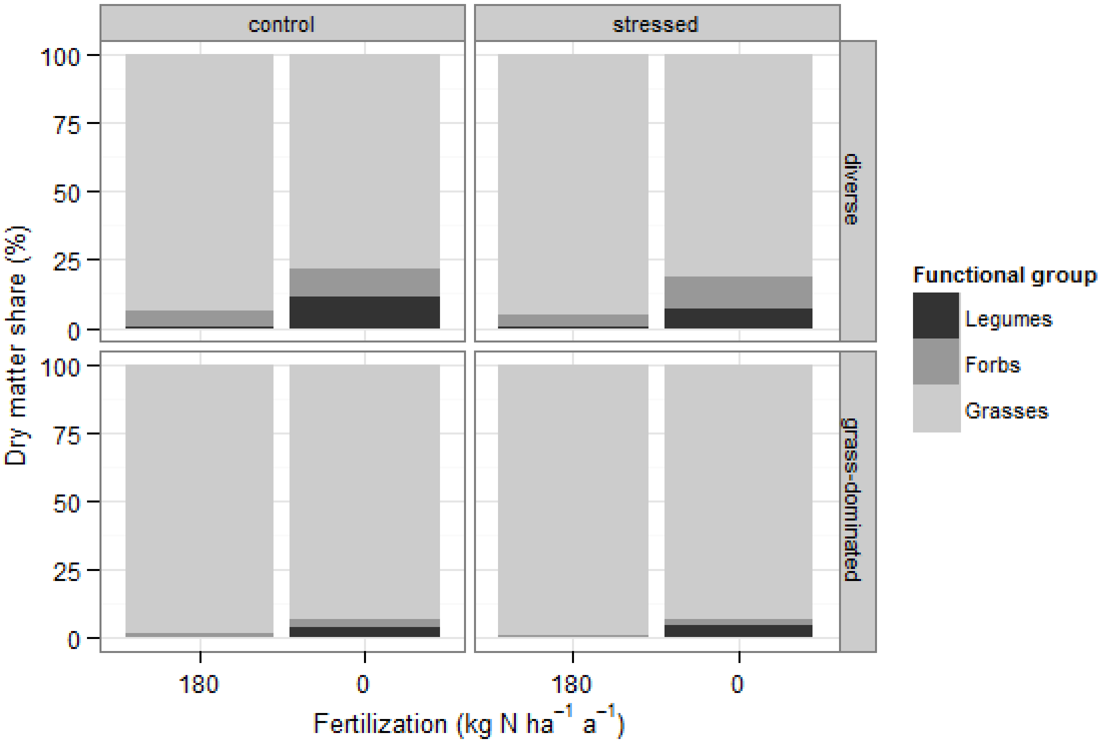

Figure 4-1 Mean biomass share of the three functional groups over all sites, years, and spring and summer cuts by sward composition (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed).

We fertilized all swards at the start of the growing seasons with 100 kg ha-1 potassium chloride (in the form of 40% K2O) and 30 kg ha-1 triple superphosphate (in the form of 46% P2O5) to avoid insufficient plant nutrient supply. As experimental treatment, half of the plots were fertilized with 90 kg N ha-1 (in the form of 46% KAS). At the start of the rewetting phase between spring and summer drought periods, again 100 kg ha-1 potassium chloride were applied to all plots and 45 kg N ha-1 to half of the plots. After the second cut in summer, 45 kg N ha-1 were applied again to half of the plots.

After each drought stress period, greenhouse film removal and cut were conducted simultaneously. Cutting height was 7 cm. A third cut was conducted without a preceding drought stress period at the start of October.

All treatments (drought stress induction, herbicide application, fertilization, cut) were applied to quadratic 3.24 m² plots. Samples were taken exclusively from the plot core area of 0.4 x 0.4 m in the plot center to avoid edge effects. Samples were separated by functional group and all subsamples were dried for 48 h at 60°C. Dry subsamples were ground to pass a 1-mm screen. All grass samples that yielded sufficient material to fill the cuvettes (402 of 512 samples) were analyzed with near infrared reflectance spectroscopy (NIRS) on a FOSS NIRSystems Scanning Spectrometer model 6500. The analysis was based on a large calibration dataset provided by the Institute VDLUFA Qualitätssicherung NIRS GmbH, Kassel, Germany (Tillmann, 2010). Their calibration has been validated for a wide range of intensively as well as extensively managed grasslands. For this study, we analyzed three quality parameters of the grass subsamples from both cuts after drought stress periods (spring and summer) from all three sites and three experimental years. Spectra were analyzed for the contents of crude protein (CP), water-soluble carbohydrates (WSC), and acid detergent fibre (ADF).

4.3.2 Design and effect of rain-out shelters

Temperature and relative humidity under rainout shelters were monitored and compared to the conditions above control plots (CiK Solutions GmbH, Haxo-8 LogTag data loggers). Each year, both values were measured twice an hour from April until the cut in October on all sites. Covered plots were on average 0.1°C warmer than control plots (Wilcoxon signed rank test with continuity correction, $P = < 0.0001$), but did not differ significantly in humidity (Wilcoxon signed rank test with continuity correction, $P = 0.176$).

As we worked on old permanent grassland, we avoided disturbance to the sward rooting system and thus did not install barriers to prevent water run-off from the shelter rims into the plots. Soil water content was monitored gravimetrically. Even after intense rainfall, the core area where sampling was conducted remained unaffected by water run-off. Stressed plots received no precipitation at all during the drought stress periods while the control plots did. The average water retention of rainout shelters at the different sites was 176 mm of the 242 mm precipitation (73 ± 5%, SEL), 127 mm of the 241 mm precipitation (52 ± 8%, NWL), and 142 mm of the 237 mm precipitation (60 ± 5%, SMS), respectively, during the experimental period. The measured average retention from the start of the growing season until the end of the summer drought stress period over all sites during the three years was 148 mm of the 240 mm precipitation (62 ± 8%).

4.3.3 Analytical methods

R version 3.0.2 (R Core Team 2013) was used with a significance level of $\alpha \leq 0.05$ for all statistical analyses. CP, WSC, and ADF contents of the grass subsamples were each analyzed by linear mixed modeling with the function lme() (software package nlme by Pinheiro et al., 2011). Year, cut, fertilization, sward composition, and drought stress were treated as fixed effects, whereas site and block nested in site were the random effects. ΔCP and ΔWSC were calculated by subtracting the respective forage parameter mean value of grasses from grass-dominated swards from the mean forage parameter value of grasses from diverse swards. Those data were also analyzed by linear mixed modeling, but instead of sward composition, the share of dicots in the diverse sward was included in the fixed effects. ΔADF was not analyzed, because the preliminary analysis of ADF showed no influence of sward composition on ADF.

Normal distribution (quantile-quantile-plots and Shapiro-Wilk test) and homoscedasticity (residual plots and Levene Test) was tested for and models were corrected by transformation and/or including a variance function (varIdent() function from package nlme) if necessary. Models were built with all four-way interactions possible and then improved by applying the stepAIC() function and removing fixed effects and their interactions that deteriorated the model AIC step by step according to the approaches of Pinheiro and Bates (2000) and Zuur et al. (2009) until the model could not be optimized further.

Grouped post-hoc pairwise contrasts of the most significant variables that contained sward composition as fixed effect were calculated with the Tukey method using functions from the packages lsmeans (Lenth 2016) and multcompView (Graves et al., 2015).

Due to technical issues, the data from the NWL site from 2013 are lacking and could not be included in the analysis.

4.4 Results

4.4.1 Crude Protein

Significant influences of sward composition on grass CP contents were found in interactions with stress, year, and fertilization and stress, respectively (Table 4-1). CP contents were largest in 2011 and smallest in 2013. Sward composition had no effect on grass CP contents in the non-fertilized swards. In the fertilized swards though, stressed grasses from grass-dominated swards showed larger CP contents than stressed grasses from diverse swards as well as larger CP contents than grasses from both grass-dominated and diverse control swards (Figure 4-2).

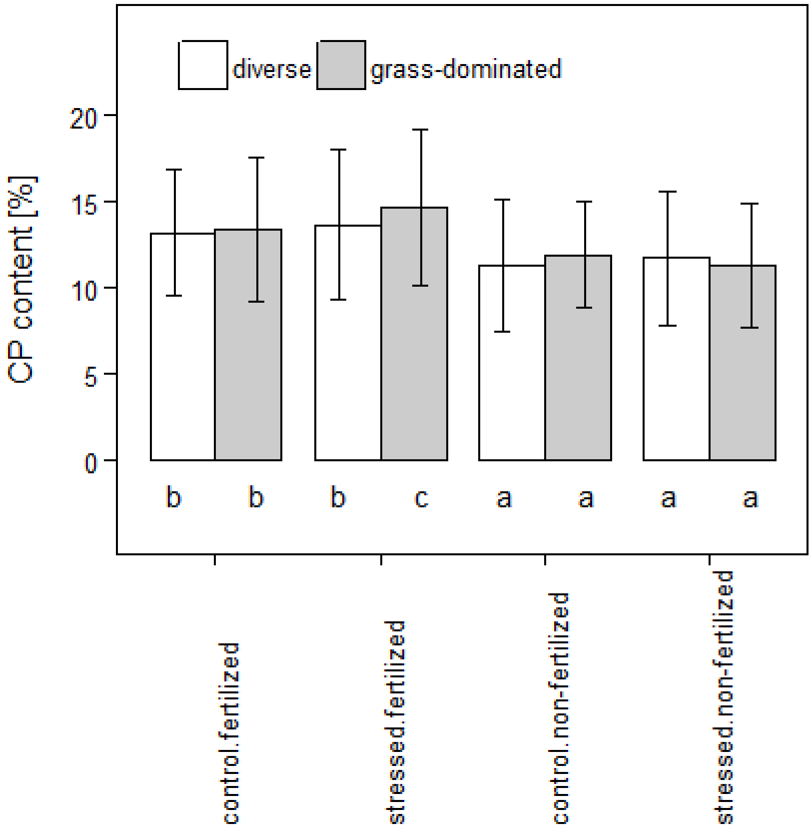

Figure 4-2 Crude protein contents of grass samples by sward composition (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed). Grouping derived from pairwise comparisons of the most significant parameters of the model presented in Table 4-1.

ΔCP is a measure for divergence of CP in grasses from swards differing in functional diversity. Large negative ΔCP values represent larger CP values in grasses from grass-dominated swards than in grasses from diverse swards. There were significant effects of dicot share on ΔCP (difference between the mean CP value of grasses from grass-dominated swards and that of grasses from diverse swards; as dicot share, we used that of the untreated diverse swards), but only in interactions with fertilization and drought stress, and fertilization and cut, respectively. The influence of fertilization and stress had a larger impact on explaining ΔCP than cut (Table 4-2). In the control swards with ambient rainfall, there was only a weak tendency of increased grass ΔCP content with increased dicot share. In stressed conditions, fertilized grass ΔCP content decreased, while the relationship of grass ΔCP content and dicot share remained the same in the non-fertilized grasses (Figure 4-3).

Table 4-1 F-test table of optimized linear nested mixed model of crude protein content of the grass functional group. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
(Intercept)	34.51249	<.0001
sward	2.30729	0.129
fert	24.00874	<.0001
stress	2.37691	0.1233
year	13.92317	<.0001
cut	67.94267	<.0001
sward:fert	1.501	0.2207
sward:stress	11.55533	0.0007
fert:stress	0.07973	0.7777
sward:year	9.57959	0.0001
fert:year	4.257	0.0143
sward:cut	0.01364	0.9071
fert:cut	2.85139	0.0915
stress:cut	0.23489	0.628
year:cut	30.89984	<.0001
sward:fert:stress	12.49961	0.0004
sward:fert:year	2.78416	0.0621

Table 4-2 F-test table of optimized linear nested mixed model of differences in grass crude protein content between diverse and grass-dominated swards (ΔCP). Explaining variables include dicot share [%] (dicot), drought stress (stress), fertilization level (fert), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
(Intercept)	0.483517	0.4878
dicot	0.959434	0.3287
fert	0.440169	0.5079
stress	22.903002	<.0001
cut	4.475945	0.0358
dicot:fert	1.619655	0.2048
dicot:stress	3.084138	0.0808
fert:stress	14.107006	0.0002
dicot:cut	4.84308	0.0291
fert:cut	4.192403	0.0421
dicot:fert:stress	2.166524	0.1429
dicot:fert:cut	3.903727	0.0498

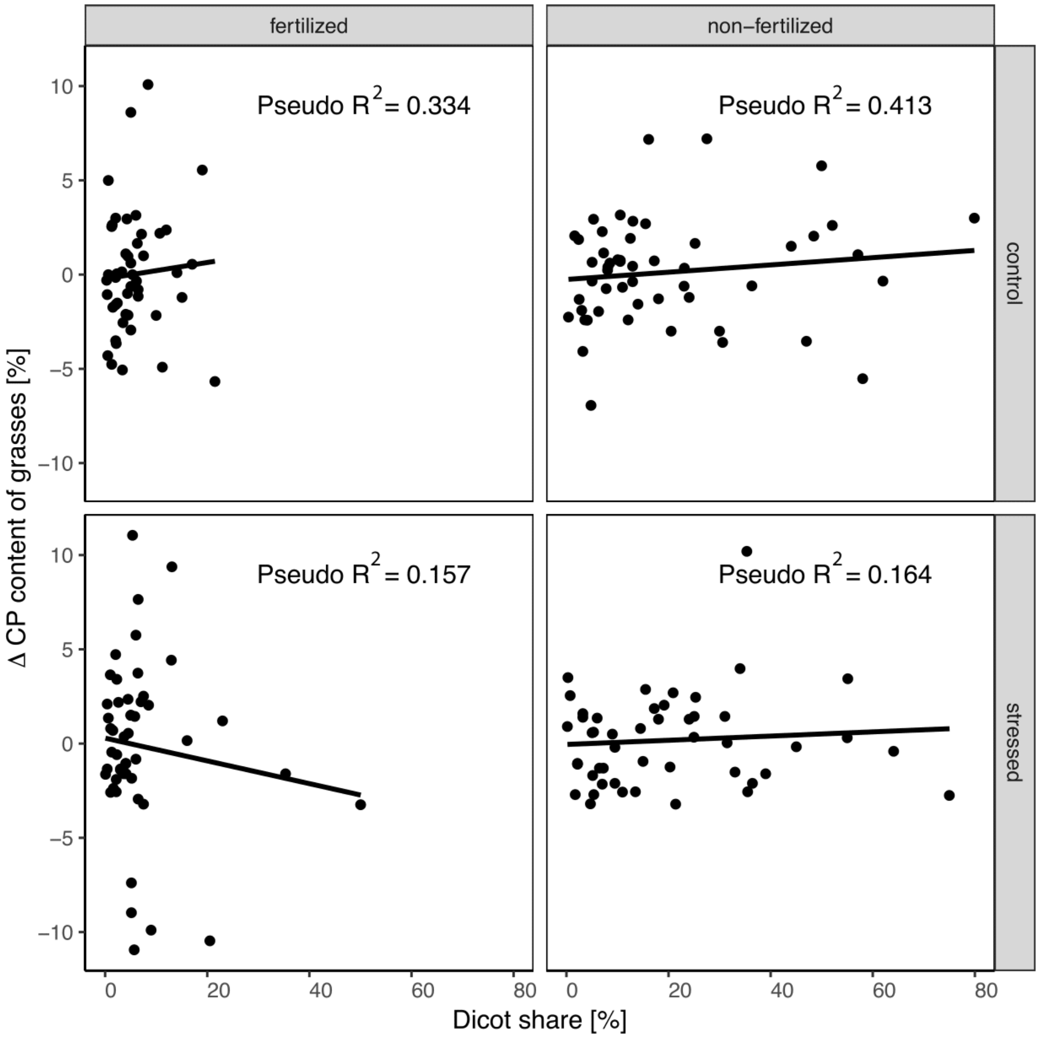

Figure 4-3 The difference of grass crude protein content (ΔCP) in diverse minus control swards in relation to the share of dicots in the respective sward presented by each N-fertilization level (180 kg ha^{-1} $year^{-1}$, non-fertilized) and drought stress level (stressed, non-stressed control).

4.4.2 Water Soluble Carbohydrates

Significant influences of sward composition on grass WSC contents were found in interactions with stress and year, respectively (Table 4-3). The mean WSC contents only showed significant differences in 2013, when the grasses from diverse swards had larger WSC contents than the grasses from grass-dominated swards. Grasses from the control swards that were not exposed to drought stress showed significantly larger WSC contents if the swards were grass-dominated and not diverse (Figure 4-4). On the contrary, grasses from stressed swards had significantly larger WSC contents if they grew in diverse swards than in grass-dominated ones (Figure 4-4). ΔWSC is a measure for divergence of WSC in grasses from swards differing in functional diversity. Large positive ΔWSC values represent larger WSC values in grasses from diverse swards than in grasses from grass-dominated swards. Analyses of the ΔWSC showed that year and cut had the most important influence on ΔWSC values (Table 4-4). The relation between ΔWSC and sward dicot share was generally weak.

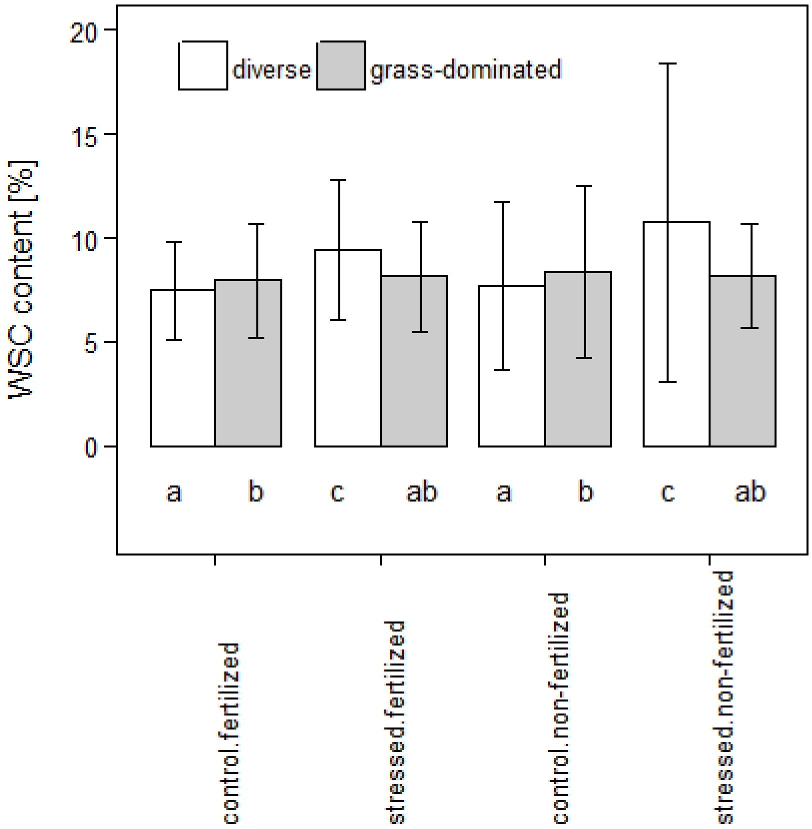

Figure 4-4 Water soluble carbohydrate contents of grass samples by sward composition (diverse, grass-dominated), N-fertilization (180 kg ha^{-1} year^{-1}, unfertilized) and drought stress level (control, stressed). Grouping derived from pairwise comparison of the most significant parameters of the model presented in Table 4-3.

Table 4-3 F-test table of optimized linear nested mixed model of water soluble carbohydrate content of the grass functional group. Explaining variables include sward composition (sward), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
(Intercept)	398.4117	<.0001
sward	0.5569	0.4556
fert	14.0701	0.0002
stress	15.4339	0.0001
year	29.6092	<.0001
cut	10.4504	0.0013
sward:fert	0.6706	0.413
sward:stress	11.4519	0.0007
sward:year	6.2579	0.002
fert:year	12.04	<.0001
stress:year	15.1303	<.0001
sward:cut	2.4196	0.12
fert:cut	1.367	0.2425
stress:cut	0.6337	0.4261
year:cut	0.84	0.4319
sward:fert:year	2.9239	0.054
sward:stress:year	2.525	0.0804
fert:year:cut	2.3817	0.0927
stress:year:cut	2.3613	0.0946

Table 4-4 F-test table of optimized linear nested mixed model of differences in grass water soluble carbohydrate content between diverse and grass-dominated swards (ΔWSC) of grass samples among sward compositions (diverse, grass-dominated). Explaining variables include dicot share [%] (dicot), drought stress (stress), fertilization level (fert), year (year), season (cut) and their interactions (:). Interactions that were excluded from the models during the optimization process are not listed for readability.

factor	**F**	***P***
(Intercept)	1.342513	0.2473
dicot	1.291889	0.2564
fert	0.010942	0.9167
stress	1.427919	0.2329
cut	0.533968	0.4654
year	0.900424	0.4073
dicot:fert	2.317219	0.1288
dicot:cut	3.622117	0.0578
stress:cut	0.75863	0.3843
dicot:year	3.322122	0.0372
stress:year	2.770661	0.0639
cut:year	2.216888	0.1104
stress:cut:year	3.337666	0.0366

4.4.3 Acid Detergent Fibre

We measured no significant influences of sward composition on grass ADF contents. Other parameters (fertilization, drought stress, year, and season) were influential and their results were in line with the results from a whole-sward analysis (data not shown).

4.5 Discussion

In contrast to what we expected from the literature (Ergon et al., 2017; Evans et al., 1996; Gierus et al., 2012; Lüscher et al., 2014; Petersen et al., 2013; Sturludottir et al., 2014), we found that the presence or absence of legumes and forbs had only significant effects on the three studied grass quality parameters when it interacted with drought stress or fertilization level. In the case of the interaction of sward composition and drought stress, the presence of legumes and forbs led to small but significant changes of CP and WSC contents of drought-stressed grasses. In the case of CP, those changes were modified by fertilization level.

4.5.1 Effect of sward composition on grass quality parameter contents

In the unstressed control swards, grasses from diverse swards did not generally show larger CP contents than grasses from grass-dominated swards. In contrast to that, Ergon et al. (2017) and Evans et al. (1996) found larger CP contents in grasses from grass-clover mixtures than in grasses from monocultures. In our diverse swards, the forbs contributed a lot more to the total dicot share than the legumes (Figure 4-1). In the grass-dominated swards, both functional groups' shares were comparably small (Figure 4-1). Thus, in our study the measured effects of dicot presence were probably caused to a substantial extent by the forb functional group rather than the legumes. While we know from grass-legume mixture experiments that legumes can enhance CP contents of grasses because they provide additional N to their companion plants (Sleugh et al., 2000), forbs may rather act as a sink for legume-generated N and thus were N competitive towards grasses.

In our study, grasses could apparently use N present in the soil well to enhance their CP content if they were accompanied by dicots and thus exposed to competition for N: In the absence of drought stress there was a slight, albeit not significant change of the grass ΔCP content with increased dicot share. Functional groups differ in their rooting depth; in Central Europe, forbs and legumes have deeper roots than grasses (Reich et al., 2003). In the case of drought, the deep-rooted species have access to deeper and wetter soil layers and thus a good drought resistance (Skinner et al., 2014), whereas the shallow-rooted plants have to increase their competitiveness for water in the upper soil layers (Fay et al., 2003). The N uptake of drought-stressed grasses increases with the size of their root systems (Jiang et al., 2000; Zemenchik and Albrecht, 2002). Due to the complementary use of resources by accessing different soil depths, N use efficiency of diverse swards is better than that of grass monocultures (Husse et al., 2017; Kleinebecker et al., 2004). The N use efficiency of swards is also closely related to plants' CP contents and is moderated by water availability (Gonzalez-Dugo et al., 2005; Onillon et al., 1995). This relation between different N use efficiencies in differently diverse swards and plants' CP contents may in turn explain why we must reject our first hypothesis for CP contents as we found no significant single effect of sward composition on grass CP contents, but significant effects of sward composition, drought stress and fertilization combined (hypotheses 2 and 3).

We found no effect of sward composition on grass ADF values. ADF is mainly affected by lignification (Hopkins and Wilkins, 2006; Moorby et al., 2006) and thus by plant maturity or indirectly by parameters that increase or decrease the speed of maturity, like e.g. drought stress (Bruinenberg et al., 2002; Buxton, 1996; Čop et al., 2009; Whitehead 2000). This is in line with the high significance of the "cut" parameter on ADF (data not shown). Due to a larger amount of stem material in the spring grass biomass, ADF values were significantly larger in the grass biomass from spring than in summer. The plant material from spring also grew from the beginning of the vegetation period until the first cut, which is a longer time span than from the first cut until the second cut. This caused plant material to be more mature in the spring cut than in the summer cut.. As in the case of CP contents, we must reject our first hypothesis for the other tested quality parameters (ADF, WSC), because we found no significant single effect of sward composition on grass quality parameter contents.

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4.5.2 Effects of sward composition, drought stress and fertilization on grass quality parameters

In drought-stressed swards, grasses from diverse swards showed larger CP contents than grasses from grass-dominated swards. In stressed conditions, fertilized grass ΔCP content decreased with increasing dicot shares, while grass ΔCP content did not change with dicot share in the non-fertilized grasses. This means that under drought stress in a non-fertilized sward, an increased dicot share had the same effect on grass CP content regardless if the grasses grew in a diverse or grass-dominated sward. However, in stressed but fertilized swards, an increase in dicot share decreased grass CP content (Figure 4-2; Figure 4-3). Those grasses were able to use their energy for growth even under drought stress, something which the dicots could not (Carlsson et al., 2017). More available N leads to fiercer competition for light and an increase of grass proportions in the sward (Helsen et al., 2014). That the effect of dicot share on grass CP content was only clearly visible under stressed conditions indicates that the reason for this effect is enhanced competition between forbs and grasses in times of drought stress.

Altogether, these findings support our second hypothesis of an influence of drought stress on CP contents and indicate that grasses with good N availability have a competitional advantage over dicots under drought conditions. As expected, we generally found larger CP contents in grasses from fertilized swards (Collins and Balasko, 1981; Grant et al., 2014; Keating and O'Kiely, 2000; Peyraud and Astigarraga, 1998; Rasmussen et al., 2008; Whitehead, 2000). In the fertilized swards, grass CP was also influenced by sward composition and drought stress. Apparently, sward composition and drought stress had stronger effects on grass CP above certain N levels as induced here by fertilization, which leads us to confirm our third hypothesis when it comes to CP contents.

There is, to our knowledge, only one study that presents data from old permanent grassland that we can fully compare our results to: With a different objective, Blonski et al. (2004) compared grassland sward components’ CP values from swards with additional N supply in a wet and a dry year. They also found increased CP values of the grass sward component in the dry environment and explained their result with a more intense competition for nutrients under dry conditions and the grasses' larger competitiveness compared to forbs. That explanation also fits well to our results and reasoning.

As hypothesized, in the case of WSC contents, drought stress interacted with effects of sward composition. The light competition mechanism also explains the increased WSC contents in grasses from diverse, stressed swards: With receiving more light and less need for investing resources (in the form of WSC) into growth under drought stress conditions, the grasses were able to accumulate WSC. Accumulation of WSC due to ongoing photosynthesis but halted plant growth under drought stress (Volaire and Lelievre, 1997; DaCosta and Huang, 2006; Küchenmeister et al., 2013; Thomas and James, 1999) and the use of WSC as energy resource for growth (Evans et al., 1997) are well-known mechanisms. Grasses have been shown to have better radiation use efficiency than legumes, especially under good N supply (Faurie et al., 1996; Reich et al., 2003). Especially in N-fertilized swards, legume performance is inhibited because of poor light conditions (Nyfeler et al., 2011). This most likely was also the case for our forbs, because most of the species were of small stature. Grasses in diverse swards apparently experienced less competition for light and thus less need to grow further, especially under drought stress. This reasoning is also supported by the results from our previous study on productivity and resistance (Carlsson et al., 2017). For WSC, we have to reject our third hypothesis, because no significant interactions of N-fertilization and sward composition could be found. While a decreasing effect of N-fertilization on WSC content of grasses is known (Laser and Opitz von Boberfeld, 2004), we did not identify a clear effect of N-fertilization on grass WSC, because the variability between years and fertilization levels was too large.

4.6 Conclusions

We conclude that grass quality in permanent grasslands was modified by the presence of other functional groups in combination with drought stress and N-fertilization. CP and WSC were modified by the presence of dicots, but effects on ADF could not be measured. The main drivers of this modification of grass quality parameters were facilitation by legumes and competition for N with forbs. Grasses with a good N supply had a competitive advantage over dicots, leading to increased grass resistance towards disturbances and resource shortages, e.g. drought stress. Further research is needed to better understand the mechanisms of facilitation and competition between functional groups and their influence on grass quality parameters under distinct levels of N availability.

4.7 References

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5 General Discussion

5.1 Climatic placement and evaluation of the environmental conditions during the experiment

5.1.1 Was the experiment successful in simulating predicted extreme events?

Precipitation frequency decreases over Central Europe (Beniston *et al.*, 2007). According to the CMIP5 multimodel simulation, sequences of consecutive days without precipitation will become longer in Central Europe (Sillmann *et al.*, 2013a) and Lower Saxony (Haberlandt *et al.*, 2010). Multimodel ensemble simulations such as CMIP5 combine several models and modeling approaches from various institutions and therefore provide authoritative predictions of future climate change, minimizing single model uncertainties (Gleckler *et al.*, 2008; Sillmann *et al.*, 2013b). Climatologists often define extreme events by one or all three of the following criteria: severity, intensity, and rarity. Severity describes the socio-economic consequences of the event. Intensity describes the deviation of the magnitude of an event from the norm. Rarity definitions vary, but according to Beniston *et al.* (2007), extreme weather events would be as rare as, or rarer than the 10th or 90th percentile. The severity of the artificial drought events was not part of this work. The intensity has to be defined over the duration of drought because in contrast to extreme rainfall events which can be qualified by their magnitude (in mm m^{-2}), the absence of rainfall cannot be less than 0 mm m^{-2} precipitation. The duration of an intense drought should therefore well exceed the average number of consecutive dry days on the site. For assessing the rarity criterion, not the minimum 90th, but the stricter 95th percentile threshold was used to ensure not to overestimate and falsely classify the many long dry spells in the datasets as extreme events when they were not. Precipitation data of the German Meteorological Office (DWD, 2018) show that during the period of 1971 – 2016 the longest dry period on the stations near our experimental sites was 26 days long (Deutscher Wetterdienst; Table 5-1). The number of consecutive dry days that still represented a dry period of normal length was calculated for each site by defining normal as lying within the 95% confidence interval calculated with the bootstrap method (R = 5000, CI type = BCa). This approach resulted in the mathematical definition of a dry period to be extreme for the respective site if it lasted for more than six (NWL) or eight (SEL, SWS) days.

Table 5-1 Dry period durations measured on the three German Meteorological Office (DWD) climate stations nearest to our experimental sites (exp site). Shown parameters are the longest period of consecutive days without precipitation during 1971 – 2016 (ldp), the number of periods of more than 20 consecutive days without precipitation during 1971 – 2016 (p >20d), the minimum (min ed) and maximum (max ed) duration of the experimental drought stress periods (in days), and the distance between experimental site and climate station (dist).

exp site	DWD station	ldp	p >20d	min ed	max ed	dist (m)
SEL	Göttingen	26	14	31	42	1400
NWL	Friesoythe-Altenoythe	22	2	28	46	18000
SWS	Holzminden-Silberborn	26	10	26	41	1900

Although nine consecutive dry days were mathematically considered to be an extreme event, from a plant's point of view, the lack of precipitation for about a week is likely not enough time to create a strong drought stress event, because there is still water available to the plant in the soil after several dry days. Therefore, extreme drought events that are relevant to plants have to be defined also via soil water availability. In the region of our experimental sites, long spring and summer drought periods are usually accompanied by heat waves or at least elevated temperatures (De Boeck *et al.*, 2010), which was not always the case during the time of our artificial drought periods. High temperatures increase evapotranspiration, which gradually depletes plant available water in the soil. Naturally occurring long drought events with accompanying heat will therefore impact plant available water sooner than our artificial drought. To assess this difference, we did not measure evapotranspiration, but soil water content. It indicates whether soil water is still available to plants and is a good indicator for whole sward drought stress. Determining soil water tension and soil moisture in a long-term field experiment is easier than determining whole sward evapotranspiration and does not interfere with the plants during the experiment. In diverse grasslands, it would also be a huge challenge to select spots for evapotranspiration measurements that are representative for the whole sward.

The measurements on the SEL site, which had loamy soil and therefore a much better water holding capacity than the other two sites, show that after an average of 21 days of drought treatment, no plant available water was left in the soil (pF = 4.2). Soil moisture was not measured daily, so it can be assumed that the permanent wilting point was reached several days earlier than our measurements indicated. On average, our swards experienced severe drought stress for 15 days, but likely even longer than that. Considering this and the data from the confidence interval analysis, the duration of a dry period that lasted more than 21 days (which was more than twice the mathematically defined duration) was defined as an extreme drought event for our sites.

Climate models predict that, for the region where our sites were located, the duration of dry periods (defined as consecutive days without precipitation) will increase by 2 to 7 days until the end of the 21st century (Pal *et al.*, 2004). Our artificial drought stress periods were well above this predicted future duration of dry consecutive days, e.g. the longest future period of consecutive days without precipitation is predicted to last 28 to 33 days (Table 5-1). The minimum drought stress duration at SWS was in the summer of 2012 and its short duration was unavoidable due to logistic reasons. Nevertheless, its duration was equal to the single longest dry period measured by the DWD in the area (26 days, measured once from 1971 to 2016). As described above, naturally occurring drought events with accompanying heat will impact plant available water sooner than our artificial droughts. We did not include temperature treatments, so we had to correct for drought intensity by long drought duration.

Our artificial drought periods lasted on average 36 days, with maximum durations longer than 40 days. The average duration of our artificial dry periods (21 or less until permanent wilting point + at least 15 days of absolute dryness) can therefore be called extreme. We therefore categorize our artificial drought periods as representative for extreme future dry periods predicted for the region.

5.1.2 Other important features of climate change for grasslands in Central Europe

Summer drought events in Central Europe were usually accompanied by elevated temperatures or heat waves (De Boeck *et al.*, 2010). An elevated mean temperature generally increases plant productivity and photosynthesis rate, and only has a decreasing effect on those parameters if it is accompanied by drought stress (as reviewed by Wu *et al.*, 2011). Grassland productivity and quality have been shown to be resistant to heat waves only as long as water supply was ensured (Hoover *et al.*, 2014; De Boeck *et al.*, 2006; De Boeck *et al.*., 2010; Rustad *et al.*, 2001; Reichstein *et al.*, 2007; White *et al.*, 2000), although some other authors see temperatures as more relevant than water supply (e.g. Bloor *et al.*, 2010). The single factor drought stress was thus preferred among the factors to be studied when it came to the impact of future climate on grasslands, although temperature is also one of the most important and most studied features of climate and climate modeling.

Climate models that are seen as relevant for plant production often include elevated CO_2 levels. CO_2 levels are predicted to increase until the end of the 21st century (Meinshausen *et al.*, 2011; Le Quéré *et al.*, 2016). Such an increase is thought to benefit plant growth and interactions of CO_2 levels with diversity and drought stress responses are important topics in grassland research (e.g. Reich *et al.*, 2001; Nowak *et al.*, 2004; Jentsch *et al.*, 2011; Milchunas *et al.*, 2005; Bloor *et al.*, 2010), but were not the focus of this study.

Including elevated temperature and elevated CO_2 levels would also have posed logistical constraints to the whole experiment. On two of the three sites there would hardly have been enough space to install the necessary additional plots with temperature and temperature x drought treatments, let alone for (additional) CO_2 enrichment facilities. Also, we would have needed more replicates to steady our statistical analysis if another treatment would have been included. Finally, enlarging the plot number by factor two or more would have entailed logistic shortcomings in terms of manpower as well as sample processing and storage capacities because cuts had to be conducted simultaneously on all sites and plots to create comparable data. Concentrating the experiment on one site but including temperature and/or CO_2 treatments would have limited the experiment to one specific grassland system. Part of our aim was to include different typical grasslands of the region to obtain more general results for the region. Initially, finding suitable sites for the experiment was a challenge, because most suitable and accessible sites were poor in species and especially forbs and legumes (Küchenmeister, F., personal communication). Also, because drought stress experiments are sensitive to water run-off after rainfall, some accessible sites had to be disregarded because of too steep inclination.

5.2 The influence of drought stress on grassland yield

We hypothesized that the productivity of permanent grasslands under drought stress is shaped by functional group composition as well as nutrient availability. We found significant influences of drought stress on grassland yield, which was affected by functional diversity and nitrogen fertilization level, confirming our hypothesis. In contrast to many other studies (Craine et al., 2012; Beierkuhnlein et al., 2011; Hoover et al. 2014; Fay et al. 2011; Kahmen et al. 2005; Grime et al. 2000; Hartmann and Niklaus, 2012; Zwicke et al. 2013), we found good yields during drought events and in their aftermath, pointing to a generally large resistance and resilience of grasslands to drought stress. While N fertilization increased both drought resistance and resilience of grasslands, we found that functional diversity was only relevant for sward resistance, not resilience (see 2.4).

We identified two kinds of functional diversity effects throughout the experiment:

1. Additive effects

Additive effects were present when the magnitude of the influence of the functional group on the whole sward response was in relation to its biomass share in the sward. The presence and traits of the grass functional group determined the whole sward reaction to drought stress. These effects are in accordance with the mass ratio hypothesis, which states that the dominant species and its biological traits are the driving force of ecosystem functions such as, among others, productivity, and that disturbance initially benefits these species before the whole system stabilizes again (Grime, 1998). Our experiments did not last long enough to yield relevant results concerning the second aspect of the mass ratio hypothesis. It is likely that a shift in functional group composition, which is caused by recurring drought stress events, will only be verifiable after more than our three consecutive years of treatment (Evans *et al.*, 2011; Morecroft *et al.*, 2004).

2. Interactive effects

Interactive effects were effects of an experimental factor that caused changes in one functional group's performance which in turn affected the performance of another functional group, e.g. competition and facilitation. In contrast to what was observed in terms of forage quality, interactive effects played only a minor role in the swards' yield reaction towards drought stress. Therefore our third hypothesis that functional groups mutually influence their reaction to drought stress could not be fully confirmed in terms of yield (but for quality, see 5.4). Facilitation by forbs on grasses was not detected on a significant level, although there were tendencies of decreased facilitation of forbs towards grasses if swards were exposed to drought stress.

The spring and summer yields of the whole swards under drought stress were stable or decreased if swards were diverse, and were stable or increased if swards were grass-dominated. We could show that this was the consequence of additive effects of the grass functional group. The grasses had larger resistance to drought stress than the dicots and had a considerably larger biomass share in the grass-dominated swards. As the decrease of productivity was only detected in diverse swards, the grass share in swards determined the whole sward productivity. N fertilization was found to support resistance and resilience, especially in grasses. It also decreased the dicot share in fertilized swards, which is a common phenomenon (Helsen *et al.*, 2014; Mpokos *et al.*, 2014; Suding *et al.*, 2005). N fertilization had direct and indirect effects on drought resistance and resilience of whole swards via direct improvement of the N availability and N metabolism of plants under drought stress (Grman 2010; Evans *et al.*, 2011) and via its effects on the grass biomass share.

There was no observable effect of functional composition in the aftermath of a drought stress period in autumn. Dicots had a smaller biomass share in the late season than during spring and summer periods. Their late-season regrowth potential apparently was smaller than that of grasses. The lack of dicot influence in the late season can likely be deduced to an additive effect. If there is only a minor representation of a functional group in the sward, its influence on the whole swards as well as on other functional groups can be considered to be negligible.

In autumn, N fertilized, previously drought stressed swards had larger yields than their non-stressed, fertilized counterparts. We did not observe this compensatory effect in non-fertilized swards. Our experimental design allows no statements about whether the compensatory effect and thus the large resilience of fertilized swards stems from enhanced robustness during drought that was caused by fertilization prior to the drought event, or if the N uptake during drought stress was inhibited and the plants benefitted from larger remaining resources of N in the soil after the end of the drought stress periods.

5.3 The influence of drought stress on forage quality

We hypothesized that the forage quality of permanent grasslands under drought stress is shaped by functional group composition as well as by nutrient availability. Apart from the large influence of time of season on forage quality parameters, we found significant influences of drought stress on grassland forage quality. Those were only visible in the interactions with functional composition and N fertilization level (see 3.4). Therefore, we could confirm our second hypothesis. Forage quality parameters were, if effects were present, in most cases positively affected by drought. In contrast to the observations regarding yield results, the increase of forage quality under drought was expected and this result is supported by the majority of related studies (e.g. Dumont *et al.*, 2015; Jensen *et al.*, 2010; Küchenmeister *et al.*, 2013).

An interesting pattern shows up in the results for forage quality: Forage quality under drought stress was increased either in grass-dominated, fertilized swards or in diverse, unfertilized swards. We interpret this pattern as an effect of increased N availability. This increase was either generated directly by N fertilization or indirectly by facilitation of dicots. Both N-increasing mechanisms did not occur jointly, neither did they add up to diverse, fertilized swards showing the maximum forage quality parameter values. This exclusion of effects might result from the fact that N fertilization decreased the dicot share in the swards (Helsen *et al.*, 2014; Mpokos *et al.*, 2014; Suding *et al.*, 2005) and thus influenced the additive effects of each functional group and its biomass share on the whole sward performance. Also, N fertilization influenced the interactive effects between functional groups, namely competition and facilitation between the functional groups. Based on these considerations, the influence of functional group composition on the drought response of swards might in fact be evoked by N availability.

5.4 Interactive effects of functional groups

We hypothesized that different functional groups influence each other's reaction to drought stress. For the productivity reaction of swards towards drought stress this could not be fully confirmed. But we found influences of drought stress on the forage quality of the grass functional group, which were only significant in interactions with functional composition and nitrogen fertilization level (see 4.4). Therefore, the third hypothesis could be confirmed for the forage quality of swards. Analogous to what was discussed above and in 4.5, these interactions might represent the influence of N availability. Therefore, functional diversity can indicate N availability in the sward and *vice versa*. N availability can be improved by managing functional diversity.

5.5 Implications for mitigating climate change by sward management in Central Europe

The results show that grasslands in Central Europe have a large resistance and resilience towards drought stress. To acquire and conserve productive grasslands that provide high quality forage even during and after drought stress events, a successful management has to consider N availability. We could show that N availability in grasslands depends on functional diversity as well as on the N fertilization level. Functional diversity is helpful for developing a more sustainable concept for sward management. The biomass share of the grass functional group largely determines productivity and forage quality. It can be influenced by managing functional diversity, *i.e.* the forb and legume share as well as by directly managing the N availability by fertilization. Depending on a specific sward's functional diversity and N availability status, future drought stress might pose no threat to the yield and forage quality delivered. However, this experiment cannot provide new insights on how other important factors that influence grassland functioning will impact grasslands of differing functional diversity under drought stress: Management factors (e.g. time and frequency of cut, fertilization with other nutrients than N, level of N fertilization, grazing regimes), ecological features (e.g. species identity effects, plant functional traits like e.g. leaf dry matter content, herbivory, invasive species, nutrient and water cycling) or other aspects of future climate (e.g. heat waves, CO_2 levels, prolonged vegetation period, changes in winter climate) can all interact with the factors studied here.

However, according to our results, functional diversity has the ability to help prevent negative consequences of drought stress on productivity and forage quality. This is good news in terms of nature conservation, because diverse, permanent grasslands thus have a large potential to be used as agronomic resources.

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6 Summary

The present study aimed at unraveling the interacting influences of functional group composition, drought stress and N fertilization on sward productivity and forage quality in old semi-natural grassland in Northern Germany. The study was conducted in the framework of the KLIFF project at the University of Goettingen, which focused on climate impact and adaptation research in Lower Saxony. The experimental sites were located in a semi-natural permanent grassland in the Leine valley, in the north-eastern lowlands, and in the Solling mountain range, each with a decades-long history of extensive agricultural use. The three experimental factors (sward composition, fertilization, and drought stress) led to 8 different treatments and were set in a completely randomized block design with 4 replications of each treatment at each site.

In the first chapter the impact of drought stress on resistance and resilience of grasslands, measured by their productivity, was investigated. Drought resistance in swards that were grass-dominated was larger than in functionally diverse swards. Grasses determined the drought resistance potential of swards. The presence of forbs and legumes impaired their resistance. Fertilization increased the resistance to drought stress of swards either through direct positive effects on the productivity or indirect effects through changes of functional sward composition. The presence of dicots was not important for sward resilience. Grasses and whole swards were resilient to drought stress only if previously fertilized. Sward resistance and resilience was shaped by the presence of the grass functional group. Regulating mechanisms on grass performance of the presence as well as of the biomass share of the forb and legume functional group were identified. The differences among the functional groups' share of the total sward biomass might be important determinants of productivity changes under and after drought stress.

In the second chapter the impact of drought stress on forage quality parameters of grassland was investigated. Forage quality was determined by sward functional composition, nitrogen fertilization level and time of the drought stress period (spring or summer), which all also influenced the drought stress quality response of swards. Drought stressed diverse swards

and grass-dominated swards that were fertilized had increased crude protein contents. Drought stress did not induce major changes of neutral and acid detergent fibre contents. Water-soluble carbon contents were larger in drought stressed swards than in controls.

The results of the first two chapters, and mainly the apparent importance of grass performance for productivity and quality, led to a more thorough investigation on how the grass functional group was influenced by forbs and legumes. The third chapter examines how the forage quality of grasses (not whole swards) was shaped by the presence and biomass share of the forb and legume functional groups. Grass quality was not generally modified by the presence of other functional groups, but effects were visible in combination with drought stress and N fertilization. Grass crude protein and water soluble carbohydrate contents were modified by the presence of dicots, while effects on fibre contents were negligible. Facilitation by legumes and competition for N with forbs were identified as the main drivers of these influences on grass quality parameters. Grasses with a good N supply had a competitive advantage over dicots, leading to large resistance of grasses against drought stress.

The results showed that grasslands had a high resistance and resilience towards drought stress. This resulted in stable yields and stable, if not increased forage quality during and after drought stress. Sustainable management has to consider N availability. N availability in grassland can be assessed via functional diversity as well as N fertilization level. Therefore, functional diversity is a helpful concept for sward management. The biomass share of the grass functional group largely determines productivity and forage quality. It can be influenced by managing functional diversity, and thus the forb and legume share, as well as by directly managing the N availability by fertilization. Depending on functional diversity and N availability status, future drought stress might pose no threat to the yield and quality of grassland forage.

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8 Appendices

8.1 Experimental setup

All three experimental sites were located in Lower Saxony, Germany (Figure 8-1 Location of the three experimental sites in Germany.(Figure 8-1). The climatic conditions and other site characteristics are presented in Table 8-1 and Table 8-2. The experimental design of each site is presented inFigure 8-2, Figure 8-3, and Figure 8-4.

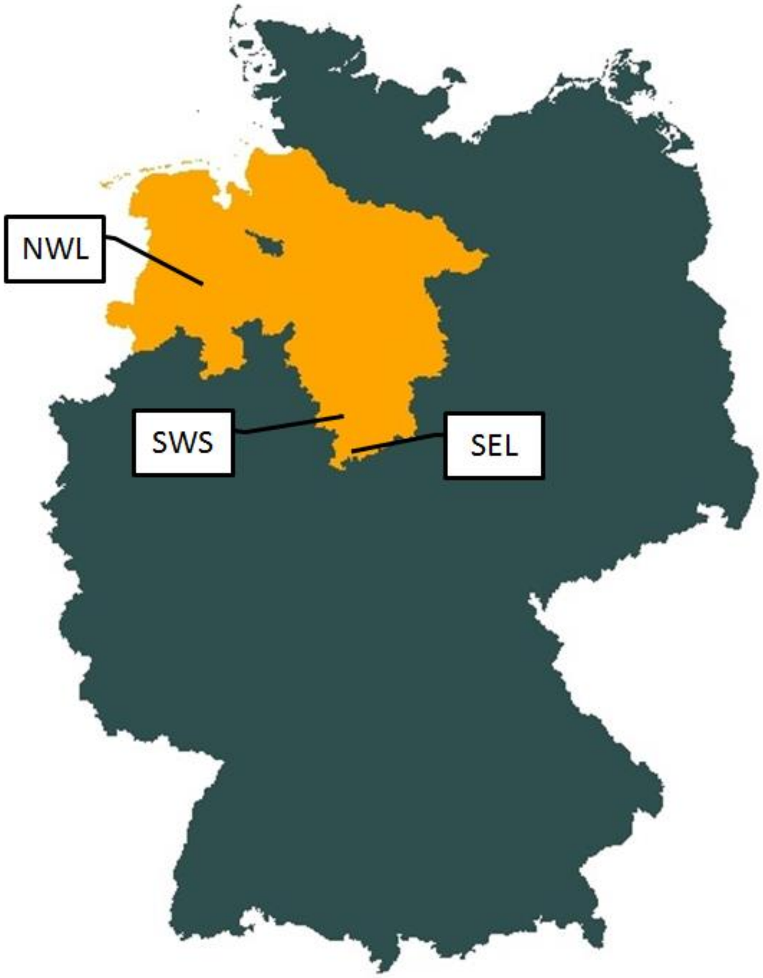

Figure 8-1 Location of the three experimental sites in Germany. SEL: South-eastern lowland site, NWL: north-western lowland site, SMS: sub-mountainous site.

Table 8-1 Climatic conditions and characteristics of the three study sites. SEL: South-eastern lowland site, NWL: north-western lowland site, SMS: sub-mountainous site.

site characteristics		**SEL**	**NWL**	**SMS**
geographical coordinates North		51°29’46’’	52°54’04’’	51°44’54’’
geographical coordinates East		9°55’57’’	7°54’04’’	9°32’38’’
elevation above sea level (m)		150	35	490
soil type		fluvic cambisol	podzol	haplic cambisol
soil texture (% sand in dry matter)		30	80 - 90	30
Plant association		Arrhenatheretum	Festuco-Cynosuretum	Lolio-Cynosuretum
usage		hay meadow	hay meadow	extensive cattle grazing
minimum age (years) of grassland		30	20	90
long term averages (1981 – 2010)	annual rainfall (mm)	651	784	1119
	mean annual temperature (°C)	9.2	9.6	7.4
Average rainfall in the vegetation period during the experiment (mm)	control	384	357	357
	stressed	208	230	215

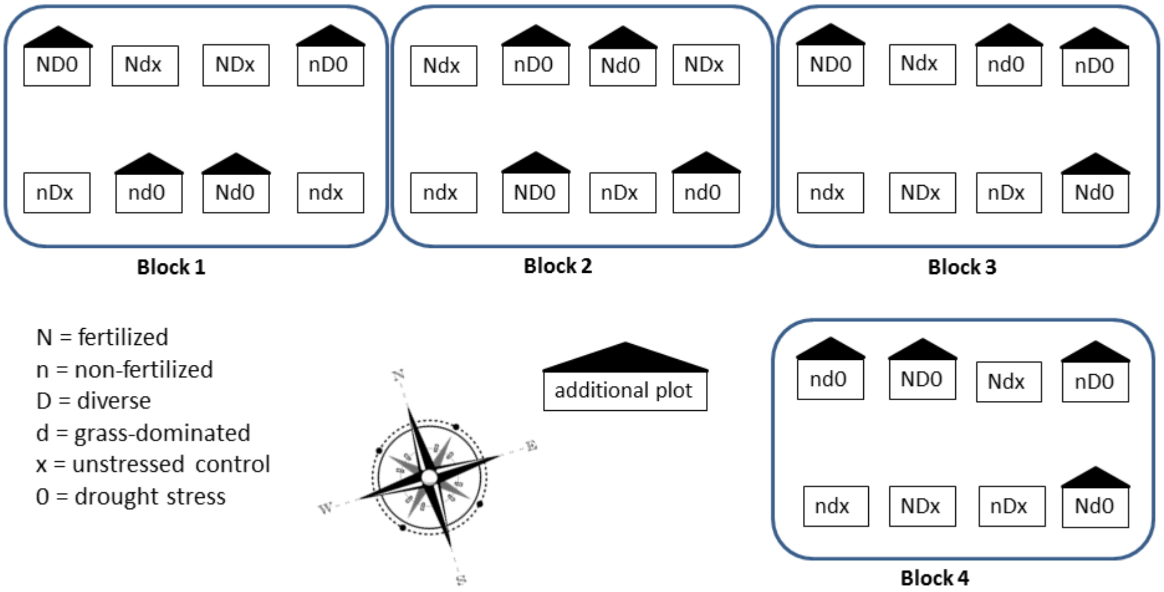

Figure 8-2 Experimental design at the SEL site. Additional plot was used for soil moisture measurements.

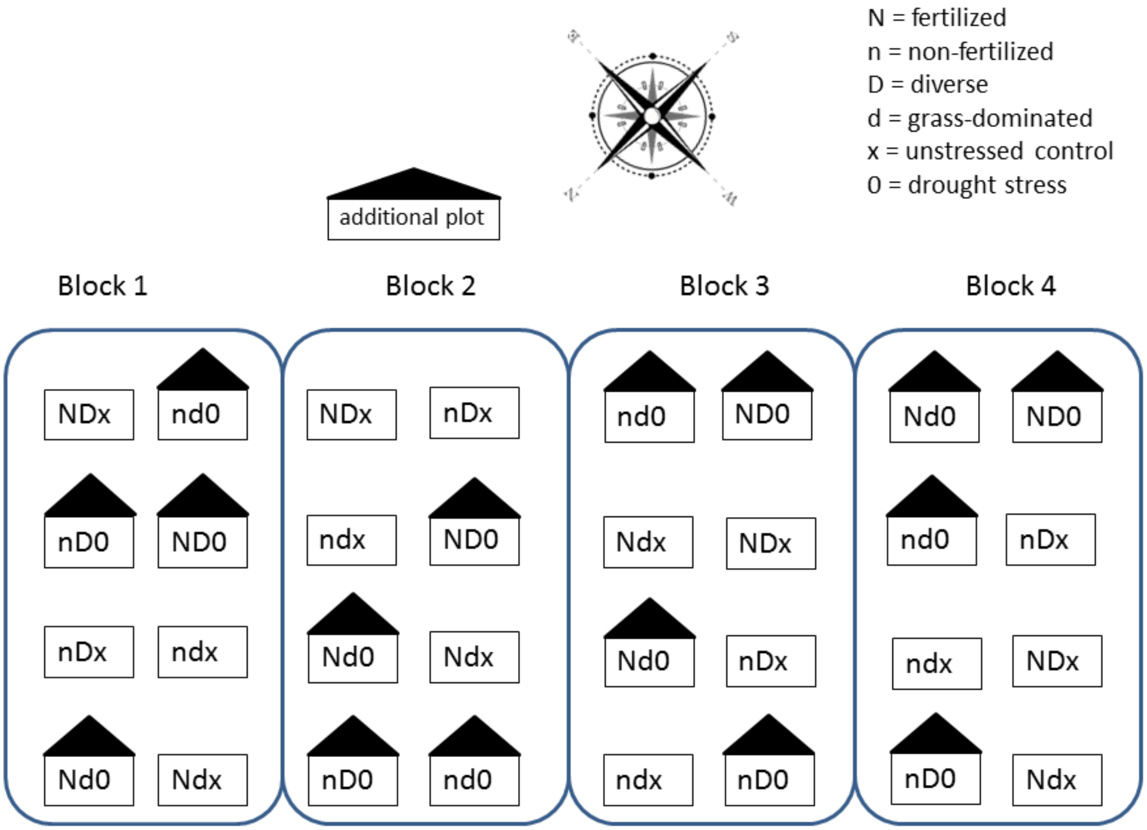

Figure 8-3 Experimental design at the NWL site. Additional plot was used for soil moisture measurements.

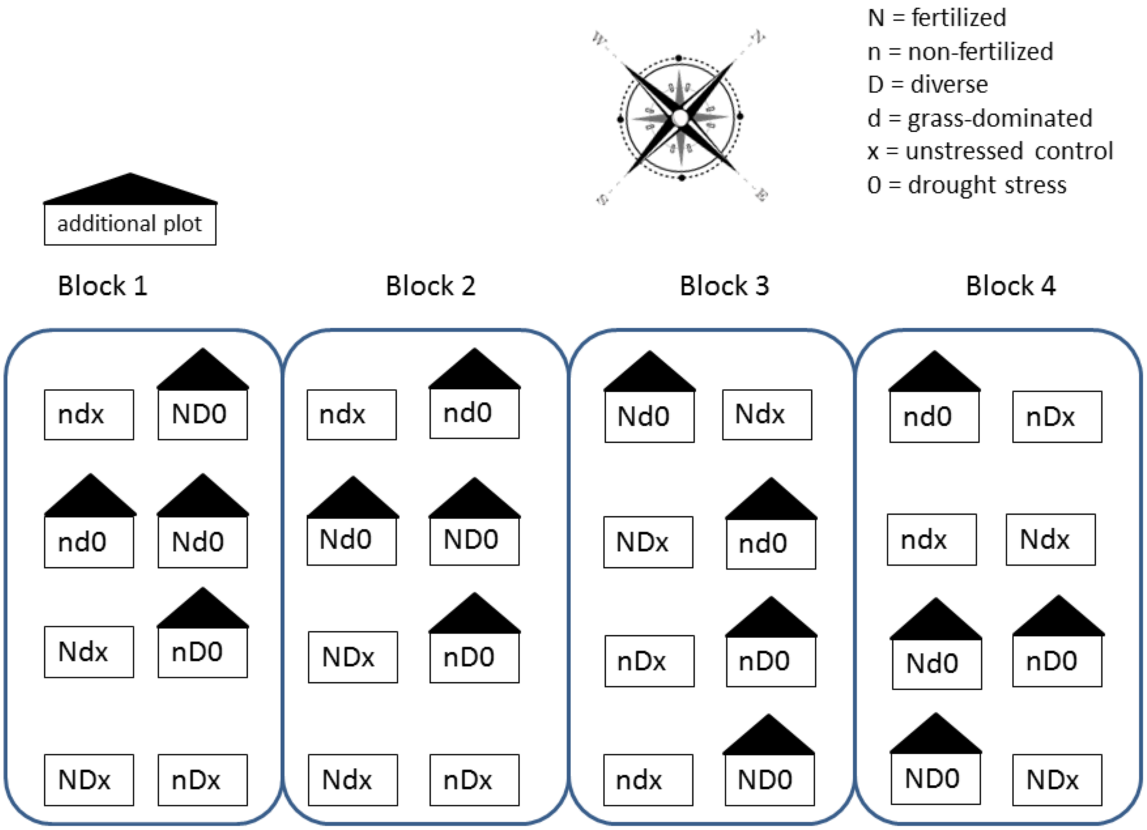

Figure 8-4 Experimental design at the SWS site. Additional plot was used for soil moisture measurements.

8.2 Supplementary material Chapter 2

Table 8-2 Average annual dicot yields, average species numbers, and maximum species number by sward composition and site (SEL: South-eastern lowland site, NWL: north-western lowland site, SMS: sub-mountainous site). Shown are averages and standard deviations per site, year, and sward composition.

sward composition	**measure**	**SEL**	**NWL**	**SMS**
diverse	Average annual non-legume dicot yield [g kg^{-1}]	77 ± 145	32 ± 169	4 ± 9
	Average annual legume yield [g kg^{-1}]	46 ± 181	95 ± 196	0.1 ± 0.3
	Maximum species number	21	14	12
	Mean species number	11 ± 3	9 ± 2	7 ± 3
grass-dominated	Average annual non-legume dicot yield [g kg^{-1}]	16 ± 71	1 ± 2	0.1 ± 0.3
	Average annual legume yield [g kg^{-1}]	2 ± 9	16 ± 56	0 ± 0
	Maximum species number	14	12	11
	Mean species number	7 ± 3	9 ± 1	5 ± 3

Table 8-3 Tables of post-hoc pairwise least-square means (lsmean) comparisons in optimized linear nested mixed model of drought stress resistance measured as biomass of whole swards. Explaining variables include interactions of sward type (sward: div = diverse, gs = grass-dominated), drought stress (stress: s = stressed, c = unstressed control), fertilization level (fert: kg N ha^{-1} $year^{-1}$), year (year), and season (cut), We also give confidence margins (level used: 0.95, upper and lower CL), and groupings by significant differenced (group).

cut	**year**	**fert**	**sward**	**stress**	**lsmean**	**lower.CL**	**upper.CL**	**group**
summer	2011	0	div	s	14.103	4.601	23.606	1
summer	2012	0	gd	s	15.062	5.559	24.565	123
summer	2013	0	div	s	15.227	5.724	24.730	124
summer	2011	0	gd	s	15.727	6.224	25.230	12345
summer	2013	0	gd	s	15.927	6.424	25.429	12345678
summer	2012	0	div	s	16.120	6.618	25.623	123790
summer	2012	0	gd	c	16.514	7.011	26.017	12369A
summer	2011	0	gd	c	17.179	7.676	26.682	1234567890AB
summer	2013	0	gd	c	17.379	7.876	26.881	1234567890AB
summer	2011	0	div	c	17.751	8.249	27.254	234567890ABC
summer	2013	0	div	c	18.875	9.372	28.378	3567890ABCD
summer	2012	0	div	c	19.768	10.266	29.271	458BCDE
summer	2013	180	div	s	20.167	10.664	29.669	68ABCDEFGH
spring	2012	0	gd	c	20.290	10.787	29.792	780BCDEGIJ
summer	2012	180	gd	c	20.453	10.950	29.955	0BCDEFIK
summer	2012	180	gd	s	20.553	11.050	30.056	90ABCDEFGHIJKL
summer	2013	180	gd	c	20.766	11.263	30.269	BCDEFGHIJKLM
summer	2013	180	gd	s	20.866	11.363	30.369	ABCDEFGHIJKLM
spring	2012	0	gd	s	21.090	11.587	30.592	BCDEFGHIJKLMN
summer	2011	180	div	s	21.465	11.962	30.968	BCDEFGHIJKLMO
summer	2012	180	div	s	21.611	12.108	31.114	CDEFGHIJKLMNOP
spring	2012	0	div	s	22.148	12.645	31.651	DEFGHIJKLMNOP
summer	2013	180	div	c	22.263	12.760	31.765	DEFGHIJKLMNOP
summer	2011	180	gd	c	22.989	13.486	32.492	DEFGHIJKLMNOP
summer	2011	180	gd	s	23.089	13.586	32.592	EFGHIJKLMNOP
spring	2012	0	div	c	23.544	14.041	33.047	FHKLMNOPQ
summer	2011	180	div	c	23.561	14.058	33.064	EFGHIJKLMNOP
summer	2012	180	div	c	23.707	14.204	33.210	GHJLMNOPQ
spring	2011	0	div	s	23.900	14.397	33.403	EFGHIJKLMNOPQ
spring	2013	0	gd	c	24.129	14.626	33.632	FHKLMNOPQ
spring	2013	0	div	s	24.229	14.726	33.732	IJKLMNOPQ
spring	2011	0	gd	c	24.724	15.221	34.227	MNOPQ
spring	2013	0	gd	s	24.929	15.426	34.432	OPQR

spring	2011	0	div	c	25.297	15.794	34.799	N PQR
spring	2011	0	gd	s	25.524	16.021	35.027	OPQRS
spring	2013	0	div	c	25.625	16.123	35.128	PQRS
spring	2012	180	gd	c	27.756	18.254	37.259	QRST
spring	2011	180	gd	c	28.904	19.401	38.407	RSTU
spring	2011	180	div	c	29.476	19.973	38.979	STU
spring	2011	180	div	s	29.632	20.129	39.135	STU
spring	2013	180	gd	c	29.706	20.203	39.209	STU
spring	2012	180	gd	s	30.108	20.606	39.611	TU
spring	2012	180	div	c	31.011	21.508	40.514	U
spring	2012	180	div	s	31.167	21.664	40.670	U
spring	2013	180	div	c	31.202	21.700	40.705	TU
spring	2011	180	gd	s	31.256	21.753	40.759	TU
spring	2013	180	div	s	31.358	21.855	40.861	TU
spring	2013	180	gd	s	32.058	22.555	41.561	TU

Table 8-4 Tables of post-hoc pairwise least-square means (lsmean) comparisons of most influential variables in optimized linear nested mixed model of drought stress resilience measured as biomass of whole swards. Explaining variables include interactions of sward type (sward, only for whole sward analysis), drought stress (stress), fertilization level (fert), and year (year), We also give confidence margins (level used: 0.95, upper and lower CL), and groupings by significant differenced (group).

fert	**stress**	**sward**	**year**	**lsmean**	**lower.CL**	**upper.CL**	**group**
0	stressed	gd	2013	10.6	1.011	20.190	1
0	stressed	gd	2012	11	1.409	20.588	1
0	stressed	div	2013	11.42	1.828	21.007	12
0	stressed	div	2012	11.82	2.226	21.405	123
0	control	gd	2013	13.16	3.573	22.752	1234
0	control	div	2013	13.98	4.390	23.569	1234
0	control	gd	2012	14.2	4.612	23.791	1234
180	control	gd	2013	14.96	5.371	24.550	234
0	control	div	2012	15.02	5.429	24.608	234
0	stressed	gd	2011	15.03	5.438	24.617	234
180	control	gd	2012	15.66	6.071	25.250	34
0	control	gd	2011	15.77	6.180	25.359	34
180	control	div	2013	15.78	6.188	25.367	45
180	stressed	gd	2013	15.78	6.190	25.369	345
180	stressed	gd	2012	15.84	6.250	25.429	45
0	stressed	div	2011	15.84	6.255	25.434	45
180	control	div	2012	16.48	6.889	26.067	45
0	control	div	2011	16.59	6.998	26.176	45
180	stressed	div	2013	16.6	7.008	26.186	456
180	stressed	div	2012	16.66	7.068	26.246	456
180	control	gd	2011	19.4	9.810	28.989	567
180	control	div	2011	20.22	10.627	29.806	67
180	stressed	gd	2011	22.04	12.449	31.628	7
180	stressed	div	2011	22.86	13.266	32.445	7

Table 8-5 Tables of post-hoc pairwise least-square means (lsmean) comparisons in optimized linear nested mixed model of drought stress resistance measured as biomass of grass functional group. Explaining variables include interactions of sward type (sward, only for whole sward analysis), drought stress (stress), fertilization level (fert), year (year), and season (cut), We also give confidence margins (level used: 0.95, upper and lower CL), and groupings by significant differenced (group).

cut	stress	fert	year	lsmean	lower.CL	upper.CL	group
summer	stressed	0	2013	14.038	4.942	23.133	1
summer	stressed	0	2011	14.687	5.321	24.054	12
summer	stressed	0	2012	14.937	5.566	24.308	12
summer	control	0	2011	16.390	7.009	25.770	1234
summer	control	0	2012	16.491	7.164	25.818	123 56
summer	control	0	2013	17.003	7.918	26.088	2345 7
summer	stressed	180	2012	19.355	9.993	28.718	345678
summer	control	180	2013	19.916	10.907	28.924	5678
summer	stressed	180	2013	19.992	10.951	29.033	345678
spring	control	0	2012	20.392	11.107	29.677	4 789
spring	stressed	0	2012	20.539	11.282	29.795	6 89
summer	stressed	180	2011	20.604	11.124	30.084	3456789
summer	control	180	2012	21.410	12.065	30.755	890
summer	control	180	2011	22.157	12.770	31.544	890
spring	stressed	0	2011	22.966	13.506	32.427	890
spring	control	0	2011	22.968	13.585	32.351	890
spring	stressed	0	2013	23.074	14.085	32.062	90
spring	control	0	2013	24.339	15.344	33.334	0A
spring	control	180	2011	27.533	18.167	36.900	AB
spring	stressed	180	2011	27.680	18.183	37.178	AB
spring	stressed	180	2012	28.365	18.946	37.785	B
spring	control	180	2013	28.532	19.484	37.580	B
spring	control	180	2012	28.720	19.323	38.117	B
spring	stressed	180	2013	30.309	21.240	39.377	B

Table 8-6 Tables of post-hoc pairwise least-square means (lsmean) comparisons in optimized linear nested mixed model of drought stress resilience measured as biomass of grass functional group. Explaining variables include interactions of sward type (sward, only for whole sward analysis), drought stress (stress), fertilization level (fert), year (year), and season (cut), We also give confidence margins (level used: 0.95, upper and lower CL), and groupings by significant differenced (group).

fert	stress	year	lsmean	lower.CL	upper.CL	group
0	stressed	2013	10.19	1.492	18.885	1
0	stressed	2012	10.73	2.056	19.413	1
0	control	2013	12.01	3.315	20.697	12
0	control	2012	12.55	3.866	21.237	123
0	stressed	2011	14.24	5.563	22.913	234
180	control	2013	14.54	5.885	23.201	34
180	control	2012	15.09	6.387	23.792	4
180	stressed	2013	15.19	6.469	23.903	4
180	stressed	2012	15.73	6.932	24.531	4
0	control	2011	16.06	7.368	24.742	4
180	control	2011	18.59	9.904	27.281	5
180	stressed	2011	19.23	10.437	28.033	5

8.3 Supplementary material Chapter 3

Table 8-7 Mean rank of the ten main contributing species in the sward on the three sites; South-eastern lowland (SEL), North-western lowland (NWL) and Sub-mountainous site (SWS), respectively. Ranks were calculated with species biomass share estimations from vegetation relevees. Relevees were conducted each year for each plot once during each drought stress period and once in the late season during September. Rank 1 corresponds to the largest estimated biomass share in the sward.

site	**species**	**mean rank**	**standard deviation**
SEL	Arrhenatherum elatius	2.13	1.83
	Alopecurus pratensis	2.56	2.15
	Dactylis glomerata	6.4	5.15
	Cerastium holosteoides	8.41	2.97
	Taraxacum officinale agg.	8.87	6.76
	Agrostis capillaris	9.98	3.41
	Achillea millefolium	10.33	3.75
	Ajuga reptans	10.83	3.24
	Capsella bursa-pastoris	11.75	3.78
	Lolium perenne	12.69	7.69
NWL	Dactylis glomerata	1.52	0.65
	Poa pratensis	2.73	8.41
	Trifolium repens	4.61	3.9
	Taraxacum officinale agg.	5.85	8.51
	Festuca rubra	6.7	13.72
	Veronica arvensis	7.16	7.24
	Rumex acetosella	7.99	4.95
	Hypericum maculatum	8.12	7.29
	Crepis biennis	10.59	13.97
	Senecio jacobaea	10.98	9.27
SMS	Agrostis capillaris	2.41	6.58
	Dactylis glomerata	4.43	13.38
	Ranunculus repens	7.97	37.06
	Veronica chamaedrys	8.65	62.07
	Alopecurus pratensis	9.02	11.67
	Poa pratensis	9.78	106.72
	Cerastium holosteoides	10.01	9.23
	Festuca rubra	10.19	38.9
	Cirsium vulgare	11.16	5.07
	Deschampsia cespitosa	12.63	10.73

Table 8-8 Pairwise comparison of the most significant parameters of the optimized linear nested mixed model of crude protein content of the whole sward. Most significant parameters are season (cut), fertilization level (fertilization kg N ha^{-1} year^{-1}), sward type (diverse, grass-dominated), and drought stress level (control, stressed). Comparisons are based on least square modelled means (lsmean), standard error (SE), lower and upper margins of confidence levels (lower.CL, upper.CL) are given. Significant group affiliation is indicated by letters (group).

cut: spring

fertilization	sward	stress	lsmean	SE	lower.CL	upper.CL	group
0	diverse	control	4.095837	0.6813706	1.164136	7.027538	a
0	gd	stressed	4.558086	0.6816827	1.625042	7.49113	b
0	diverse	stressed	4.570435	0.6816022	1.637737	7.503132	b
0	gd	control	4.857777	0.6823528	1.92185	7.793704	bc
180	diverse	control	5.17216	0.6819157	2.238114	8.106207	cd
180	gd	control	5.330761	0.6820707	2.396047	8.265474	d
180	diverse	stressed	5.464966	0.6813548	2.533334	8.396599	de
180	gd	stressed	5.763132	0.6812347	2.832016	8.694249	e

cut: summer

fertilization	sward	stress	lsmean	SE	lower.CL	upper.CL	group
0	diverse	control	4.413017	0.6864407	1.459501	7.366532	a
0	diverse	stressed	4.573721	0.6870031	1.617785	7.529657	a
0	gd	control	5.613368	0.6864745	2.659707	8.56703	b
0	gd	stressed	5.91051	0.6891286	2.945429	8.875591	bc
180	diverse	control	6.026632	0.6852062	3.078428	8.974837	bc
180	diverse	stressed	6.380658	0.6852143	3.432419	9.328897	cd
180	gd	control	6.544953	0.685998	3.593342	9.496564	d
180	gd	stressed	6.679856	0.6848625	3.73313	9.626581	d

Table 8-9 Pairwise comparison of the most significant parameters of the optimized linear nested mixed model of water soluble carbohydrate content of the whole sward. Most significant parameters are fertilization level (fertilization kg N ha^{-1} $year^{-1}$), sward type (diverse, grass-dominated), season (cut), and year. Comparisons are based on least square modelled means (lsmean), standard error (SE), lower and upper margins of confidence levels (lower.CL, upper.CL) are given. Significant group affiliation is indicated by letters (group).

fert	**sward**	**cut**	**year**	**lsmean**	**SE**	**lower.CL**	**upper.CL**	**group**
0	diverse	summer	2012	4.457	0.499	2.308	6.605	a
0	gd	summer	2013	5.583	0.716	2.501	8.665	abc
0	diverse	summer	2011	5.827	0.517	3.600	8.053	ab
0	gd	summer	2012	6.319	0.491	4.206	8.433	abc
0	diverse	summer	2013	6.495	0.703	3.471	9.519	abcdef
0	diverse	spring	2012	6.540	0.403	4.804	8.275	bc
180	gd	summer	2011	6.626	0.537	4.315	8.936	bcd
180	gd	summer	2012	6.830	0.473	4.796	8.863	bcd
180	diverse	summer	2012	7.124	0.467	5.116	9.132	bcde
180	diverse	summer	2011	7.207	0.521	4.966	9.447	bcdefg
180	gd	summer	2013	7.209	0.612	4.577	9.841	bcdefgh
0	diverse	spring	2013	7.541	0.497	5.404	9.679	bcdefgh
180	diverse	summer	2013	7.652	0.627	4.955	10.350	bcdefgh
180	diverse	spring	2011	7.706	0.425	5.876	9.536	cdefgh
0	gd	summer	2011	7.771	0.568	5.326	10.217	bcdefgh
0	gd	spring	2013	7.830	0.487	5.735	9.925	bcdefgh
180	diverse	spring	2013	8.077	0.497	5.939	10.215	cdefgh
0	gd	spring	2012	8.234	0.397	6.528	9.941	defgh
180	gd	spring	2011	8.450	0.430	6.600	10.299	defgh
180	diverse	spring	2012	8.568	0.403	6.833	10.303	efgh
180	gd	spring	2013	8.671	0.497	6.533	10.809	defgh
180	gd	spring	2012	8.744	0.399	7.026	10.463	fgh
0	diverse	spring	2011	9.037	0.418	7.239	10.836	h
0	gd	spring	2011	9.142	0.469	7.126	11.159	gh

Table 8-10 Pairwise comparison of the most significant parameters of the optimized linear nested mixed model of acid detergent fibre content of the whole sward. Most significant parameters are fertilization level (fertilization kg N ha^{-1} year^{-1}), sward type (diverse, grass-dominated), drought stress level (control, stressed), and season (cut). Comparisons are based on least square modelled means (lsmean), standard error (SE), lower and upper margins of confidence levels (lower.CL, upper.CL) are given. Significant group affiliation is indicated by letters (group).

fert	sward	stress	cut	lsmean	SE	lower.CL	upper.CL	group
0	diverse	stressed	summer	7085.015	1196.247	1937.981	12232.05	a
0	diverse	control	summer	10641.603	1175.414	5584.206	15699	b
0	gd	stressed	summer	16168.399	1243.398	10818.488	21518.31	c
180	diverse	stressed	summer	16464.592	1150.666	11513.677	21415.51	c
180	gd	stressed	summer	16587.967	1140.903	11679.057	21496.88	c
0	gd	control	summer	16900.522	1175.97	11840.733	21960.31	c
0	diverse	control	spring	17107.174	1258.044	11694.249	22520.1	c
180	diverse	control	summer	17107.391	1147.589	12169.714	22045.07	c
0	diverse	stressed	spring	17630.789	1275.933	12140.892	23120.69	c
180	gd	control	summer	19472.957	1164.487	14462.575	24483.34	cd
180	diverse	stressed	spring	22945.107	1259.043	17527.884	28362.33	de
180	diverse	control	spring	24419.501	1289.189	18872.571	29966.43	ef
180	gd	control	spring	25798.443	1297.072	20217.593	31379.29	ef
0	gd	control	spring	26057.102	1312.19	20411.206	31703	ef
180	gd	stressed	spring	26109.22	1251.155	20725.936	31492.5	ef
0	gd	stressed	spring	27163.316	1273.64	21683.284	32643.35	f

Tables 8-11 Pairwise comparisons of the most significant parameters of the optimized linear nested mixed model of neutral detergent fibre content of the whole sward. Most significant parameters are fertilization level (fertilization kg N ha^{-1} $year^{-1}$), sward type (diverse, grass-dominated), year, drought stress level (control, stressed), and season (cut). Comparisons are based on least square modelled means (lsmean), standard error (SE), lower and upper margins of confidence levels (lower.CL, upper.CL) are given. Significant group affiliation is indicated by letters (group).

fert	sward	lsmean	SE	lower.CL	upper.CL	group
180	gd	3.584	0.012	3.532	3.636	a
0	gd	3.611	0.015	3.546	3.676	ab
180	diverse	3.630	0.014	3.569	3.691	b
0	diverse	3.893	0.018	3.814	3.972	c

fert	year	lsmean	SE	lower.CL	upper.CL	group
180	2013	3.590	0.016	3.521	3.660	a
180	2012	3.615	0.014	3.555	3.675	ab
180	2011	3.615	0.014	3.554	3.676	ab
0	2011	3.659	0.018	3.581	3.737	b
0	2013	3.752	0.022	3.657	3.847	c
0	2012	3.845	0.018	3.767	3.923	d

fert	sward	stress	cut	lsmean	SE	lower.CL	upper.CL	group
180	gd	stressed	spring	3.537	0.017	3.463	3.611	a
0	gd	control	spring	3.540	0.025	3.431	3.648	a
0	gd	stressed	spring	3.540	0.024	3.438	3.643	a
180	gd	control	spring	3.550	0.018	3.471	3.628	a
180	diverse	control	spring	3.574	0.024	3.472	3.677	ab
180	gd	control	summer	3.594	0.018	3.517	3.671	ab
180	diverse	stressed	spring	3.608	0.023	3.510	3.706	abc
180	gd	stressed	summer	3.654	0.017	3.580	3.727	bc
180	diverse	stressed	summer	3.662	0.023	3.563	3.760	bcd
180	diverse	control	summer	3.676	0.023	3.578	3.773	bcd
0	gd	stressed	summer	3.676	0.027	3.558	3.794	bcde
0	gd	control	summer	3.688	0.025	3.582	3.793	cde

0	diverse	stressed	spring	3.772	0.032	3.634	3.910	de
0	diverse	control	spring	3.802	0.032	3.666	3.938	e
0	diverse	control	summer	3.963	0.034	3.818	4.107	f
0	diverse	stressed	summer	4.036	0.034	3.888	4.183	f