# Effects of plant diversity on bioenergy parameters in grassland biomass



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# Preface

This thesis was submitted to the Faculty of Organic Agricultural Sciences of the University of Kassel on October 15th, 2012 in order to fulfil the requirements for the degree 'Doktor der Agrarwissenschaften' (Dr. agr.). The oral defense took place on December 20th, 2012 in Witzenhausen.

This dissertation is based on three papers which were published by or submitted to international refereed journals. A list of the original papers including the chapter in which they appear in this dissertation will be given on the following page. A list of other publications (e.g. contributions to conference proceedings) is given in chapter 12.

Witzenhausen, January 2013

# List of papers

Chapter 4:   KHALSA, J., FRICKE, T., WEISSER, W.W., WEIGELT, A. and WACHENDORF, M. (2012): Effects of functional groups and species richness on biomass constituents relevant for combustion - Results from a diversity grassland experiment. *Grass and Forage Science*, 67, 569-588.

Chapter 5:   KHALSA J., FRICKE T., WEIGELT A. and WACHENDORF M. (2012) Effects of species richness and functional groups on chemical constituents relevant for methane yields from anaerobic digestion: results from a grassland diversity experiment. *Grass and Forage Science, doi: 10.1111/gfs.12028*

Chapter 6:   KHALSA J., FRICKE T., SALVARO DE SOUZA M.A. and WACHENDORF M. (2013) Solid fuel production from extensive grassland biomass through hydrothermal conditioning and mechanical dewatering: effects of species richness and functional group composition.

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# Abbreviations

ADL:        Acid detergent lignin

AST:        Ash softening temperature

CA:         Crude ash

CF:         Crude fibre

CL:         Crude lipid

CP:         Crude protein

$CH_{4\ area}$:   Area specific methane yield

$CH_{4\ sub}$:    Substrate specific methane yield

E:          Shannon-evenness

$E_{area}$:   Area specific energy yield

$E_{sub}$:    Substrate specific energy yield

FGC:        Functional-group composition

FGR:        Functional-group richness

GE:         Gross energy yield

gr:         Grasses

HHV:        Higher heating value

IFBB:       Integrated generation of solid Fuel and Biogas from Biomass

lg:         Legumes

MF:         Mass-flow

PC:         Press-cake

PF:         Press-fluid

PM:         Parent-material

PMC:        Mash of parent material and water

NfE:        Nitrogen-free extract

sh:         small herbs

SR:         Species richness

th:         tall herbs

VS:         Volatile solids

# 1   Summary

Extensive grassland biomass for bioenergy production has long been subject of scientific research. The possibility of combining nature conservation goals with a profitable management while reducing competition with food production has created a strong interest in this topic. However, the botanical composition will play a key role for solid fuel quality of grassland biomass and will have effects on the combustion process by potentially causing corrosion, emission and slagging. On the other hand, botanical composition will affect anaerobic digestibility and thereby the biogas potential.

In this thesis aboveground biomass from the Jena-Experiment plots was harvested in 2008 and 2009 and analysed for the most relevant chemical constituents effecting fuel quality and anaerobic digestibility. Regarding combustion, the following parameters were of main focus: higher heating value (HHV), gross energy yield (GE), ash content, ash softening temperature (AST), K, Ca, Mg, N, Cl and S content. For biogas production the following parameters were investigated: substrate specific methane yield ($CH_{4\ sub}$), area specific methane yield ($CH_{4\ area}$), crude fibre (CF), crude protein (CP), crude lipid (CL) and nitrogen-free extract (NfE). Furthermore, an improvement of the fuel quality was investigated through applying the Integrated generation of solid Fuel and Biogas from Biomass (IFBB) procedure.

Through the specific setup of the Jena-Experiment it was possible to outline the changes of these parameters along two diversity gradients: (i) species richness (SR; 1 to 60 species) and (ii) functional group (grasses, legumes, small herbs and tall herbs) presence. This was a novel approach on investigating the bioenergy characteristic of extensive grassland biomass and gave detailed insight in the sward-composition - bioenergy relations such as: (i) the most relevant SR effect was the increase of energy yield for both combustion (annual GE increased by 26% from $SR_{8\rightarrow16}$ and by 65% from $SR_{8\rightarrow60}$) and anaerobic digestion (annual $CH_{4\ area}$ increased by 22% from $SR_{8\rightarrow16}$ and by 49% from $SR_{8\rightarrow60}$) through a strong interaction of SR with biomass yield; (ii) legumes play a key role for the utilization of grassland biomass for energy production as they increase the energy content of the substrate (HHV and $CH_{4\ sub}$) and the energy yield (GE and $CH_{4\ area}$); (iii) combustion is the conversion technique that will yield the highest energy output but requires an improvement of the solid fuel quality in order to reduce the risk of corrosion, emission and slagging related problems. This was achieved through applying the IFBB-procedure, with reductions in ash (by 23%), N (28%), K (85%), Cl (56%) and S (59%) and equal levels of concentrations along the SR gradient.

# 2   General introduction

The goal of the European Union (EU; 27 member states) to bring the contribution of renewable energies up to 20% of the total energy mix by the year 2020 (*status quo* in 2008 was 10%; European Commission, 2010) and the need to explore renewable energy sources as substitutes for fossil fuels, has brought attention to different kinds of biomass (from agriculture, forestry and waste) that can be used for anaerobic digestion or combustion.

In the EU, agricultural biomass is expected, in the long term, to have the largest energy potential over biomass from forestry and waste (European Environment Agency, 2006). Regarding anaerobic digestion, 10.9 million tons of oil equivalents (Mtoe) were produced as biogas in the year 2010 across the EU (number one producer was Germany with 6.7 Mtoe), which was a 31.3% increase compared to the year before (EurObserv'Er, 2011a). Regarding combustion of solid biomass, 79.3 Mtoe were produced as primary energy in 2010 (Germany was the number one producer with 12.2 Mtoe), with more than half of the production being from logwood, while other plant materials and waste account for only 8% (EurObserv'Er, 2011b).

The increase in energy production from biomass goes along with an increased cultivation of energy crops, such as rapeseed (bio-diesel), maize (biogas) and miscanthus or short rotation coppice (combustion). Especially the biogas production has caused a major shift in agricultural practices. In Germany, for example, 18% of the arable land is now covered with energy crops, of which 45% is for biogas-production, 43% for bio-diesel (and other plant oils), while only 0.3% are used for combustion (FNR, 2012). This trend in land-use change, which is not restricted to Germany, has triggered a global discussion about the competition with food production and possible negative impacts on the environment (Fargione *et al.*, 2008; Petersen, 2008; Tilman *et al.*, 2009).

As part of a sustainable scenario for energy production loss of biodiversity through land-clearing or intensification and loss of valuable ecosystem services has to be avoided. Therefore, more biomass from less-favoured, low-productivity sites that were abandoned from agricultural use should be utilized (Tilman *et al.*, 2009), such as extensively managed grasslands.

Traditionally, the biomass from semi-natural, extensively managed grasslands was used for animal nutrition. However, in developed countries forage quality of extensive grassland cut is decreasingly suitable for animals with high milk and meat performance which makes farmers

abandon their grassland (Isselstein *et al.*, 2005), especially in mountainous areas. For some regions in Germany it was estimated that up to 25% of permanent grassland will be abandoned in the near future (Rösch *et al.*, 2007). A direct feedback from changing the management of these sites toward abandonment (or even toward intensification) will be a decline in species diversity (Mitchley *et al.*, 2001). Extensive grasslands are known to be hotspots of biodiversity in rural areas and are valuable to society due to their aesthetics and recreational functions as well as their ecosystem services; i.e., groundwater collection, prevention of erosion and soil degradation (Wilkins and Vidrih, 2000). Often extensive grassland sites are under environmental protection schemes which are subsidised by the EU (e.g. NATURA 2000; European Commission, 2012). The management of these sites requires a regular defoliation either through grazing or cutting in order to maintain the species richness (SR) and avoid succession. Hence, the conversion of the biomass to energy may provide an opportunity for (i) a regular management and maintenance of these valuable ecosystems, and for (ii) a sustainable energy production. Furthermore, it may provide economic incentives for the farmers to manage high nature value, low input sites beyond agro-economic subsidies.

However, to which extend extensive grassland biomass is currently used for energy production remains unclear. In Germany, grass silage is one of the commonly used co-substrates (used in 40% of the biogas plants) after maize (which is used in 90% of all plants) (Weiland, 2006; FNR, 2009) but the contribution of extensive grassland is unknown, as well as the amount of extensive grassland biomass that is utilized for energy production through combustion.

The suitability of permanent grassland biomass for energy production depends on some key parameters such as energy content, fuel quality, and energy yield, which have been widely studied (Prochnow *et al.*, 2009a, 2009b). For combustion, fuel quality of the biomass is of great importance. It is a major determinant of the life span of the combustion plant and for the choice of type of plant. While the carbon (C), hydrogen (H) and oxygen (O) content in wood from spruce accounts for about 99% of the total biomass, it comprises only 90-93% of grassland biomass depending on the species or species composition (Hartmann, 2009). The remaining fraction consists of elements that contribute to ash formation and/or become volatile and cause emissions. The ash content is negatively correlated with the energy content (Jenkins *et al.* 1995) and will thus determine the energy potential of the substrate.

An ideal substrate for biogas production should be rich in degradable carbohydrates (e.g. sugars), lipids and proteins, and at the same time be poor in hemicelluloses and lignin (El

Bassam, 1998). Also, the ratio of fibre to protein, often expressed by the C/nitrogen (N) ratio, is important for high methane yields, as too high fibre contents will limit energy availability (Buxton and Redfearn, 1997) and too high protein concentrations may lead to process failure owing to ammonia accumulation (Zubr, 1986).

In general extensive grasslands can be very heterogeneous in the number of occurring species and the species composition which creates a wide range of variation in some of the bioenergy parameters. This is a clear disadvantage over wood chips or maize silage. Thus, to understand how the composition of species (or the functional groups they belong to) and species richness (SR) affects bioenergy parameters, is the key to understand the energy potential of extensive grassland biomass.

A team of scientists found, by comparing grassland biomass from sites that differed in plant SR, that trends along the SR gradient in, for example, crude fibre (CF) and crude protein (CP) content can be observed (White *et al.*, 2004). This is relevant information regarding the anaerobic digestibility of such biomasses, as CF and CP will affect the substrate specific methane yields ($CH_{4\ sub}$) directly. A study by Florine *et al.* (2006) on different grassland sites with different SR found variation in, for example, ash and C content, while the higher heating value (HHV) did not differ. Also, it has been shown in several experiments that biomass yield can be higher in high SR grasslands, than low SR grasslands (Hector *et al.*, 1999; Tilman *et al.*, 2001), even when fertilizers are applied and management is intensified (Weigelt *et al.*, 2009). This is highly relevant for the gross energy yield, which is calculated as the product of biomass yield and energy content.

Other studies have shown that functional groups play an important role for bioenergy parameters. Tonn *et al.* (2010) observed that herbaceous species were higher in ash content than grasses and legumes, which will make high abundances of herbaceous species less favourable for combustion. Furthermore, it can be expected that legumes play an important role regarding CP, $NO_x$ emissions and biomass yield, by supplying the system with additional N through fixing atmospheric N via symbiotic root bacteria, and by accumulating high concentrations of N in their tissue (Tomm *et al.*, 1995).

To obtain a better understanding on how the heterogeneity of extensive grassland biomass affects bioenergy parameters relevant for both combustion and anaerobic digestion, this thesis analysed artificial grassland biomass that were established along the two diversity gradients functional group composition (FGC) and plant SR. Furthermore, the improvement of solid

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fuel quality was investigated and put in relation to the diversity gradients. The solid fuel improvement was done according to the Integrated generation of solid Fuel and Biogas from Biomass – IFBB (Wachendorf *et al.*, 2009). Therein the parent material (PM) is being separated into a press-cake (PC) which will have an improved fuel quality (Richter *et al.*, 2010) and can be used for combustion, and a press-fluid (PF), which will be rich in highly digestible constituents (Richter *et al.*, 2009) and can be used for anaerobic digestion. As the PM is dependent on the FGC and SR, the solid fuel quality of PC and the digestibility of the press-fluid may vary accordingly.

# 3 Research objectives

The underlying assumption of this study was that changes in bioenergy parameters, relevant for combustion and anaerobic digestion, along a SR and functional-group gradient can be expected and are best investigated through an experimental set-up with artificially designed diversity gradients that reduce the variance in site-specific biotic and abiotic factors.

The Jena-Experiment (see set-up in the Appendix) with its 82 main plots representing different combinations of functional-group richness (FGR), FGC and SR, all in the context of extensive managed grassland, gave a promising base for such investigations. The experimental set-up evolved from a line of ecology experiments (Hector *et al.*, 1999; Tilman *et al.*, 2001) and was established with the intention to address the criticism provoked by previous diversity studies (Roscher *et al.*, 2004). Rather than dividing the species simply in monocotyledonous and dicotyledonous species, the plant functional groups were chosen more specifically, for example, legumes were regarded as a separate functional group as they can have disproportionate effects on ecosystem processes (Spehn *et al.*, 2002). Furthermore, instead of having one functional group containing all herbaceous species, they were divided into two functional groups, i.e. small herbs and tall herbs. Also, the design of the experiment was aimed at disentangling the effects of SR, FGR and the presence of individual functional groups as much as possible.

The specific objectives of this study were

(i) to assess how SR and the functional groups may affect fuel quality (relevant for combustion) and the concentrations of chemical constituents (relevant for anaerobic digestion),

(ii) to assess how SR and the functional groups may affect the higher heating value (calculated from C,H and N content) and the $CH_{4\ sub}$ (calculated from the Weender constituents),

(iii) to assess how SR and the functional groups may affect the respective gross energy yields,

(iv) to assess how the solid fuel quality will differ before and after an improvement through applying the IFBB procedure, and if the diversity gradients are still relevant for the fuel.

# 4 Effects of functional groups and species richness on biomass constituents relevant for combustion: results from a grassland diversity experiment.

**Abstract** Grassland biomass has been identified as a potential energy source. The combustion of mature and fibrous biomass, as it occurs in extensive grasslands with low cutting frequencies, is one possible conversion technique. This study tested the relationship between plant diversity and biomass constituents relevant for combustion, as they determine energy content, energy yield and emission and corrosion risks. The biomass from a biodiversity experiment, with a species richness (SR) gradient of 1-60 species from Central European mesophilic grasslands divided into four functional groups (grasses, legumes, small and tall herbs), was harvested twice a year (in 2008 and 2009). The higher heating value (HHV) was estimated from carbon, hydrogen and oxygen contents to give insight into the energy potential of the species mixtures. The potential risk of emission and corrosion was assessed by analysing ash content, potassium, calcium, magnesium, nitrogen, sulphur and chloride content.

HHV was independent of SR, and the overall mean was 18.13 MJ kg$^{-1}$ DM. Biomass and gross energy yield (GE) were positively affected by SR. The presence of legumes in a mixture resulted in increased HHV, biomass yield and GE, irrespective of the level of SR. Annual GE varied between 59 (average of monocultures) and 152 GJ ha$^{-1}$ year$^{-1}$ (mixture of 60 species).

The concentration of ash forming elements was generally high, suggesting a pre-treatment of the biomass prior to combustion. Emission and corrosion related constituents were clearly affected by the different functional groups, and sulphur and nitrogen both declined with SR.

The results of this study show that high SR in experimental grassland communities is beneficial for the energy output and that legumes play a key role for the energy potential. However, identifying a functional group as being solely beneficial or disadvantageous for fuel quality was difficult.

## 4.1 Introduction

The need to explore renewable energy sources has brought attention to different kinds of biomass (from agriculture, forestry and waste). In the European Union (twenty-seven member states), agricultural biomass is expected to have the largest energy potential in the long term (European Environment Agency, 2006). However, with increasing cultivation of energy crops, such as maize (*Zea mais* L., grown for anaerobic fermentation) and miscanthus (*Miscanthus x giganteus*, grown for combustion), a discussion about the competition with food production and their negative impacts on the environment (e.g. loss of biodiversity, greenhouse gas emissions owing to land-use change) has arisen (Fargione *et al.*, 2008; Petersen, 2008, Tilman *et al.*, 2009).

In the context of grassland utilization, competition with food production and environmental impacts can be minimized if biomass is taken from areas where agricultural management is receding and from land that would otherwise be abandoned. For some regions in central Europe, it has been estimated that up to one quarter of permanent grassland will be abandoned in the near future due to structural changes in agricultural practices (Rösch *et al.*, 2007), which will mainly affect extensive grasslands on less favourable soils. Hence, the utilization for energy production may provide an opportunity for regular management and maintenance of such grasslands. It could provide a way of protecting landscapes that are valuable to society due to their aesthetics and recreational functions and help to maintain their ecosystem services; i.e., nature and species conservation, groundwater collection, prevention of erosion and soil degradation (Wilkins and Vidrih, 2000), especially when species diversity is high (Minns *et al.*, 2001).

The preservation of extensive grassland usually requires 1-2 cuts per year, resulting in a fibre-rich substrate that will result in relatively low methane yields during anaerobic fermentation (Lemmer and Oechsner, 2001; Prochnow *et al.*, 2005), but still contains energy that can be accessed by other conversion techniques. Combustion has been considered as promising technique to convert fibre-rich, extensive grassland biomass into energy (Prochnow *et al.*, 2009a; Rösch *et al.*, 2009).

If combustion is chosen as a conversion technique, fuel quality becomes of great importance. It is a major determinant of the life span of the combustion plant and for the choice of type of plant (regarding combustion chamber layout, equipment with filter and condensation units, grate methods, etc.). While the carbon (C), hydrogen (H) and oxygen (O) content in wood

from spruce (*Picea*) accounts for about 99% of the total biomass, it comprises up 95% of the biomass from miscanthus and only 90-93% of grassland biomass depending on the species or species composition (Hartmann, 2009). The remaining fraction consists of elements that contribute to ash formation and/or become volatile and cause emissions. The major ash forming elements in biomass are silicon (Si), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na) and phosphorus (P) (van Loo and Koppejan, 2008). These ash-forming elements, when exposed to high temperature, either contribute to the melting and deposition of ash on the surface of the combustion chamber (slagging) or contribute to vaporization and subsequently condense in the cooler regions (fouling). However, some elements play multiple roles in these processes, which cannot easily be disentangled.

According to Hartmann (2009), the average ash-softening temperature (AST; the temperature at which a cube of pressed ash shows the first signs of softening) of wood (1426°C for spruce) is relatively high, whereas it is much lower for hay from landscape management (1061°C) and declines further for wheat straw (998°C) and miscanthus (973°C).

Amongst others, the elements creating harmful emission and corrosion problems are nitrogen (N), chlorine (Cl) and sulphur (S). N concentration in the solid fuel shows a logarithmic correlation with the $NO_x$ emissions (van Loo and Koppejan, 2008) which have been identified as one of the major environmental impacts of solid biomass combustion (Nussbaumer, 2003). Cl is responsible for the formation of HCl and, together with alkali metals, for the formation of chlorides such as KCl or NaCl. Furthermore, high Cl concentrations are expected to contribute to the formation of polychlorinated dibenzodioxines and dibenzofuranes (PCDD/F) and their concentrations were found to be higher during the combustion of herbaceous biomass than during wood combustion (Launhardt and Thoma, 2000). The S contained in the biomass will mainly transform into $SO_x$. Whilst 40-90% of the S concentration is bound in the ash, the rest is emitted as $SO_2$ and to a minor extent as $SO_3$ (van Loo and Koppejan, 2008). As part of the $SO_x$ emission process, some of the $SO_2/SO_3$ will react with deposited alkali chlorides and thus replace, and consequently release, Cl as gaseous $Cl_2$. This leads to the formation of alkali sulphates such as $K_2SO_4$ or $NaSO_4$, while the Cl can repeatedly react with metal parts of the plant forming, for example, $FeCl_2$ which is the source of severe corrosion (Riedl *et al.*, 1999).

In extensive grassland, where a multitude of species occurs, the fuel quality can vary considerably depending on the individual species composition. Whilst perennial energy grasses such as switchgrass (*Panicum virgatum*), miscanthus, or reed canary grass (*Phalaris*

*arundinacea*) have been the focus of research on solid biofuels from grassland biomass (Christian *et al.*, 2002; Lewandowski *et al.*, 2003), there is a lack in knowledge on extensive grasslands and their characteristics regarding biofuel production (Prochnow *et al.*, 2009a). The energy content and fuel quality of grassland biomass have, so far, been studied over a range of different locations (Florine *et al.*, 2006; El-Nashaar *et al.*, 2009; Wachendorf *et al.*, 2009; Tonn *et al.*, 2010) creating insight into the effects of species composition and abiotic location properties on energy content and fuel quality. However, none of the above-mentioned studies had clear diversity gradients that could have outlined their results specifically as diversity effects. Florine *et al.* (2006) mentioned that the variance in chemical composition was due to the diversity of herbaceous plant species, and Tonn *et al.* (2010) subsequently found the difference between grasses and herbaceous species to be mainly responsible for the variance. In a study on forage quality, White *et al.* (2004) found fibre content to be increasing with increasing species richness (SR) in a late cut (while no trend was found in the early cut) when multiple grasslands with different species richness were compared. This could be a weak indication that, along a species-richness gradient, differences in higher heating value (HHV) might be found, as HHV is mainly affected by carbon and thus by fibre content. Furthermore, they found that crude protein content (N content × 6.25) decreased with increasing SR in both cuts, which would lead to less $NO_x$ emission because of less N content in the fuel. However, with their experimental approach, it cannot be addressed whether these effects are related to SR *per se* or an interaction with biotic and abiotic factors of the different sites.

Therefore, the hypothesis of this study is that changes along a species-richness and functional-group gradient regarding fuel quality are to be expected and are best investigated through an experimental set-up with artificially designed diversity gradients that reduce the variance in site-specific biotic and abiotic factors.

Thus, the intention of this study was to investigate (i) how SR may affect energy content, gross energy (GE) yield and fuel quality, (ii) how energy content and fuel quality differ among the functional groups and how they affect those parameters in mixtures, and (iii) to draw conclusions from the results regarding possible emission and corrosion risks and the potential energy output that can be expected.

This was approached by choosing an experimental layout that represents extensive grassland communities of the Arrhenatherion community through artificially assembled species mixtures. The biomass investigated in this study came from a large-scale biodiversity

experiment, which was also established to address the criticism provoked by previous diversity studies (Roscher *et al.*, 2004). Therefore, the choice of functional groups was approached very carefully by giving special attention to legumes and regarding them as a separate functional group, as they have been found to have disproportionate effects on ecosystem processes (Spehn *et al.*, 2002). Whereas in previous biodiversity experiments, usually three functional groups were included (grasses, herbs and legumes), it was decided in this experiment to further divide herbs into small and tall herbs. At the same time, the testing of species-richness effects was given special attention in the design of the experiment, allowing us to disentangle the effects of SR, functional-group richness (FGR) and the presence of individual functional groups as much as possible.

## 4.2 Material and Methods

### 4.2.1 Experimental design

This study was conducted on the Jena Experiment field site, located in the floodplain of the River Saale at the northern edge of Jena (Thuringia, Germany, 50°55N, 11°35E, 130 m a.s.l.). Mean annual air temperature in the Jena area is 9.3°C with an annual precipitation of 587 mm (Kluge *et al.*, 2000). The site had originally been grassland and was converted into arable land around 1960. Soil conditions resemble a Eutric Fluvisol (FAO, 1994) and soil texture changes from silty clay to sandy loam with increasing distance to the river.

In May 2002, a system representing an extensive Central European mesophilic grassland (Arrhenatherion community; Ellenberg, 1996) was established at the site. Sixty species typical for this type of grassland were used to create a gradient in plant SR (1, 2, 4, 8, 16 and 60) and in FGR (1, 2, 3 and 4). Functional groups were defined according to the morphological, phenological and physiological traits of the plant species as grasses ($n = 16$), small herbs ($n = 12$), tall herbs ($n = 20$) and legumes ($n = 12$) (for detailed list of plant species see Table A.1). Eighty-two plots ($20 \times 20$m) were established in four blocks (Figure A.2), the blocks accounting for the differences in soil texture. Sixteen possible combinations of SR and FGR were realized and replicated over the four blocks (Figure 4.1). The location of the mixtures within each block was fully randomized. The management of the site was typical of that for extensive grassland under the actual site conditions, with two cuts per year (late May and late August) and no application of fertilizer. Plots were weeded twice a year to maintain the original species composition. The experimental set-up is described in full detail by Roscher *et al.* (2004).
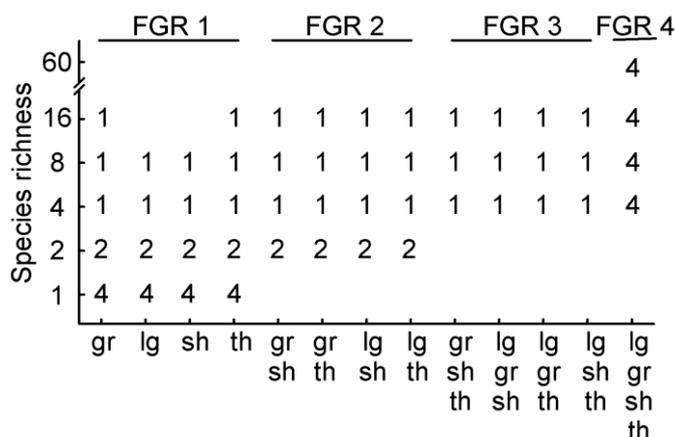
**Figure 4.1** Combinations of functional groups and their replications (numbers) according to species-richness level. Each replication represents one of the plots used in this study; the total sum therefore is 82. The x-axis gives the different functional groups compositions (gr = grasses, lg = legumes, th = tall herbs and sh = small herbs). Functional-group richness (FGR) is shown on top.

### 4.2.2 Biomass yield and ash

Aboveground biomass was harvested twice in the years 2008 and 2009, immediately before the first and the second cut. Three randomly placed samples of 20 × 50 cm were harvested 3 cm above soil surface. Biomass samples were separated into target species, dead plant material and weeds, dried (70°C, 48 h) and weighed. Mean total biomass in tonnes of dry matter (DM) per hectare (t DM ha$^{-1}$) was derived from the three samples. Annual biomass yield was calculated as the sum of biomass from first and second cut. Aboveground biomass was also analysed for crude ash according to standard methods (Naumann and Bassler, 2004).

### 4.2.3 Evenness

The relative abundance $p_i$ of a species in a mixture, measured as its relative contribution to the biomass, was used to calculate the Shannon-Wiener index: H' $= \sum p_i \times \ln (p_i)$ and put in relation to the total number of species $S$ as the Shannon evenness $E = $ H'/ln($S$). Thus, $E$ can have values between zero and one with $E = 0$ being a mixture entirely dominated by one species and with $E = 1$ being a mixture with equal proportions for all occurring species. $E$ was not calculated for monoculture plots; thus, the number of plots with values of E was $n = 66$ in the first cut and $n = 63$ in the second (owing to three plots with SR = 2 that only had one target species present).

### 4.2.4 Energy content and lignin

From the aboveground biomass, 300 g was harvested 3 cm above soil surface within the core area (3 × 3 m) of each plot, dried (70°C, 48 h) and weighed. C, H and N concentrations (g kg$^{-1}$ DM) of the biomass were analysed using an elemental analyser (vario MAX CHN, Elementar Analysensysteme GmbH, Hanau, Germany). The energy content or HHV is the amount of heat released during combustion and takes into account the latent heat of vaporization of water in the combustion product. HHV was calculated based on the concentrations of C, H and N with the empiric equation for biofuels from Friedl *et al.* (2005):

$$HHV = 0.0355 \times C^2 - 23.2 \times C - 223 \times H$$

$$+ \, 0.512 \times C \times H + 13.1 \times N + 20600 \; (kJ \; kg^{-1} \; DM)$$

Gross energy yield was calculated by multiplying HHV with the biomass yield.

Acid detergent lignin (ADL) was analysed, according to standard methods (Naumann and Bassler, 2004), as a chemical component rich in C and thereby potentially affecting HHV. For ADL 100 of the 164 samples were analysed in the laboratory and then used for near-infrared-calibration. With a near-infrared-spectroscope (XDS Rapid Content Analyser, FOSS NIRSystems Inc., Laurel, USA), the 164 samples were predicted after cross-validation (standard error of cross-validation, SECV = 1.32 g kg$^{-1}$ DM; $R^2$ = 0.74; residual predictive deviation, RPD = 2.35).

### 4.2.5 Constituents relevant for corrosion and emissions

From the harvested aboveground biomass (see description above), S, Cl, K, Ca and Mg (g kg$^{-1}$ DM) were analysed by X-ray fluorescence analysis. To estimate the potential concentration of K, Ca and Mg in the ash, their DM concentration was put in relation to their ash concentration of the biomass sample. Thereby, it is assumed that the elements will all go entirely into the ash even though, in the case of K, this is highly dependent on the temperature (Knudsen *et al.*, 2004).

### 4.2.6 Statistical analysis

The Jena experiment was designed to vary SR, FGR and functional-group composition (FGC) as orthogonally as possible. However, a fully balanced design is not possible, as for example, the lowest SR cannot be combined with highest FGR. This is not an unusual situation in biodiversity experimentation and is best solved by analysing the dependent variable in an

analysis of variance (ANOVA) with sequential sum of squares (Schmid *et al.*, 2007). In this type of analysis, variables that are fitted before others take up all the variation they can explain, ignoring the possibility that the later variables might also explain some of this variation (McCullagh and Nelder, 1999; Hector *et al.* 2010). These characteristics of the analysis can be used to identify effects that are independent of what was fitted before.

To account for the gradient in soil conditions, block-wise weeding, mowing and sampling, block effects were fitted first. It can therefore be assumed that all variances that are explained by variables fitted after the block effect are independent of these.

As the main focus of this study is on the effects of increasing SR, this variable was fitted first after block. To test the effects of SR the log linear contrast of 1 to 16 species was used. The 60 species plots were used as a point of reference for highest possible diversity, but were not included in the statistical analysis. Two plots had to be omitted from the data set (one small herb monoculture plot and one tall herb monoculture plot) due to difficulties with their establishment. Thus the total number of plots in the statistical analysis was n = 76. All values used in the ANOVA were mean values of both years.

To test for the effects of FGR and FGC, those variables were fitted after SR in a first model (Table 4.2A, Table 4.4A). The first model was performed as a repeated measure ANOVA to test for the effects of the two harvest dates. In the following two models, one for each cut (Table 4.2B, C and Table 4.4B, C for first cut and second cut), the presence/absence of functional groups were fitted after SR to test for their individual effects.

Multiple regression analysis was conducted (on all plots including SR = 60) to estimate the influence of legume abundance and possible other functional group abundances on GE by selecting the terms for inclusion in the model depending on standard statistical model selection methods (Draper and Smith, 1998). This implies that effect terms with $P < 0.05$ were included according to the rules of hierarchy and marginality (Nelder, 1994; Nelder and Lane, 1995). The marginality principle (Nelder and Lane, 1995) implies that if a term appears as part of a more complex element in the model, then, in general, the term itself is not tested for significance, because the meaning of such terms is open to misinterpretation (Connolly and Wachendorf, 2001).

The initial model contained all functional-group abundance terms (lg, gr, sh, th), including their quadratic terms and all possible pairwise interactions. Functional group abundances were tested for co-linearity (with *cor (x, y, method="pearson")* in *R*) prior to the analysis. The

possible correlations between functional group abundances were all within $-0.21 > r < -0.44$ and, on average, at $r = -0.33$ in both cuts.

All statistical analyses were done in R 2.12.2 (R Core Team, 2012).

## 4.3 Results

### 4.3.1 Energy content and relevant chemical constituents for its estimation

Amongst the 80 species mixtures, representing 13 different FGC, HHV varied between 16.3 and 19.2 MJ kg$^{-1}$ DM with an overall mean of 18.13 MJ kg$^{-1}$ DM. Amongst the functional-group monocultures (plots with FGR = 1), legumes had the highest HHV in both cuts, whereas grasses, tall herbs and small herbs were lower (Table 4.1). Diversity parameters (SR, FGR and FGC) and the presence of individual functional groups had the following effects: HHV was not affected by SR (Figure 4.2) or FGR while FGC showed a substantial effect on HHV in the repeated measure model (Table 4.2A). As the cut affected HHV (Table 4.2A), with mean values being higher in the first than in the second cut (Table 4.1), the two cuts were analysed separately. Here, FGC had no effect, indicating that most information of FGCs is contained in the presence/absence of the functional groups. While SR had no effects on HHV in the separate analysis of the two cuts, the presence of legumes increased HHV (Table 4.2, Figure 4.2). Other functional-group effects on HHV were less consistent, i.e., the presence of grasses in the first cut and the presence of small herbs in the second cut both decreased HHV (Table 4.2).

Across all mixtures, C content ranged from 396 to 477 g kg$^{-1}$ DM with an overall mean of 454 g kg$^{-1}$ DM. In the functional-group monocultures, legumes had the highest C values in both cuts while all other functional groups were equally low (Table 4.1). C was affected by diversity parameters in the same way as HHV (Table 4.2) and values were slightly higher in the first than in the second cut (Table 4.1). Analysing the two cuts in separate models the increase of C through the presence of legumes was most pronounced in both cuts (Table 4.2).

H content ranged from 46 to 58 g kg$^{-1}$ DM with an overall mean of 55 g kg$^{-1}$ DM. In the functional-group monocultures, both legumes and grasses were slightly higher than tall and small herbs (Table 4.1). The only diversity parameter affecting H in the repeated measure model was FGC (Table 4.2A). Cut was affecting H resulting in higher H values in the first than in the second cut (Table 4.1). Regarding functional-group effects, the presence of tall and

small herbs had a decreasing effect on H content in both cuts, while grasses had an increasing effect in the second cut (Table 4.2).
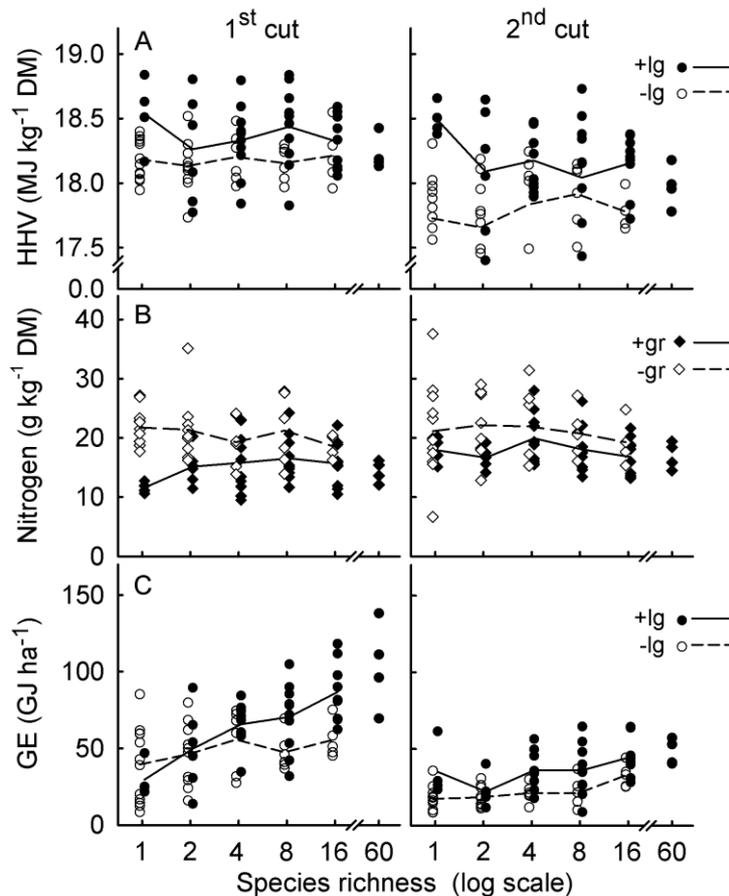


**Figure 4.2** (A) Higher heating value (HHV), (B) nitrogen content and (C) gross energy yield (GE) in the first (late May) and second cut (late August) at different levels of species richness. Mean values of species-richness levels are indicated by a solid line for plots with legumes present (+lg) or grasses present (+gr); mean values of plots with legumes absent (–lg) or grasses absent (–gr) are indicated by a dashed line. Values are means of both years.

N content ranged from 8.9 to 38.4 g kg$^{-1}$ DM with an overall mean of 18.7 g kg$^{-1}$ DM. Highest N values amongst the functional-group monocultures were found in the legume plots and lowest values occurred in the grass plots (Table 4.1). N was affected by SR and FGC in the repeated measure model (Table 4.2A). Being affected by the cut, N values were lower during the early growth period than during the late one (Table 4.1). N content was also affected by SR in both cuts with a decline from 19.4 to 16.4 to 14.3 g kg$^{-1}$ DM (from SR = 1 to 16 to 60) in the first cut and in the second cut from 21.3 to 17.5 to 16.8 g kg$^{-1}$ DM, respectively. In both cuts the presence of legumes resulted in an increase of N, whereas the presence of grasses reduced it (Figure 4.2, Table 4.2).

Lignin ranged from 14 to 136 g kg$^{-1}$ DM across both years with an overall mean of 74 g kg$^{-1}$ DM. In the functional-group monocultures, lignin was highest in the small-herb plots

followed by legumes, tall herbs and grasses (Table 4.1). Lignin was affected by cut (Table 4.2) and lignin contents were lower in the first than in the second cut (Table 4.1). Lignin content was not affected by SR but increased because of the presence of legumes in both cuts. By contrast, the presences of grasses had a decreasing effect on lignin in the first cut and an increasing effect in the second cut. Small herbs increased lignin content in the second cut but had no effect in the first cut (Table 4.2).

**Table 4.1** Energy content (HHV), carbon, hydrogen, nitrogen, lignin and ash content as means of the functional-group (FG) monocultures of both years. Values are shown separately for both cuts, and variance is indicated by standard deviation (SD).

| | Grasses | | Legumes | | Small herbs | | Tall herbs | | All FG mixtures | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| HHV (MJ kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 18.09 | ± 0.16 | 18.64 | ± 0.21 | 18.29 | ± 0.16 | 18.23 | ± 0.13 | 18.24 | ± 0.24 |
| 2nd cut | 17.92 | ± 0.20 | 18.51 | ± 0.15 | 17.74 | ± 0.17 | 17.79 | ± 0.34 | 17.99 | ± 0.36 |
| Carbon (g kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 455.0 | ± 3.5 | 463.6 | ± 5.0 | 456.4 | ± 4.3 | 455.5 | ± 3.0 | 457.6 | ± 4.9 |
| 2nd cut | 449.3 | ± 6.2 | 461.0 | ± 4.4 | 444.0 | ± 6.8 | 445.5 | ± 11.1 | 450.9 | ± 9.7 |
| Hydrogen (g kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 55.44 | ± 0.53 | 56.13 | ± 0.83 | 54.85 | ± 0.69 | 54.50 | ± 1.07 | 55.25 | ± 0.58 |
| 2nd cut | 55.78 | ± 0.83 | 56.00 | ± 0.76 | 52.86 | ± 0.69 | 52.63 | ± 1.41 | 54.06 | ± 1.73 |
| Nitrogen (g kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 11.4 | ± 1.1 | 25.9 | ± 4.5 | 21.6 | ± 2.1 | 18.5 | ± 2.9 | 16.6 | ± 3.6 |
| 2nd cut | 16.4 | ± 2.1 | 28.8 | ± 4.2 | 17.4 | ± 3.5 | 17.8 | ± 1.8 | 18.4 | ± 3.6 |
| Lignin (g kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 49.2 | ± 11.7 | 74.9 | ± 12.7 | 80.3 | ± 15.2 | 72.1 | ± 11.9 | 66.6 | ± 10.7 |
| 2nd cut | 45.1 | ± 8.1 | 86.9 | ± 18.1 | 101.2 | ± 13.8 | 82.2 | ± 11.5 | 80.7 | ± 15.4 |
| Ash (g kg$^{-1}$ DM) | | | | | | | | | | |
| 1st cut | 68.0 | ± 4.5 | 73.0 | ± 10.7 | 86.6 | ± 8.6 | 86.1 | ± 6.4 | 76.9 | ± 9.0 |
| 2nd cut | 103.6 | ± 10.1 | 91.3 | ± 11.3 | 120.5 | ± 13.2 | 124.3 | ± 20.7 | 105.0 | ± 19.3 |

## 4.3.2  Biomass and gross energy yield

Gross energy yield was calculated as the product of HHV and biomass yield and was therefore affected by their respective patterns. The correlation of GE and biomass yield was at $R^2 = 0.99$ in both cuts, highlighting the importance of biomass yield for GE. Across the species mixtures, biomass yield ranged from 0.2 to 8.1 t DM ha$^{-1}$, with an overall mean of 2.4 t DM ha$^{-1}$, and the GE of the single cuts, with an overall mean of 43 GJ ha$^{-1}$, ranged from 3 to 150 GJ ha$^{-1}$. Biomass yield was almost twice as high in the first cut (3.1 t DM ha$^{-1}$) as in the second cut (1.6 t DM ha$^{-1}$) as was GE (57 GJ ha$^{-1}$ and 29 GJ ha$^{-1}$ respectively). In the separate analysis of the two cuts, GE and biomass were significantly affected by SR (all $P < 0.001$). However, the magnitude of increase in GE with increasing SR in the first cut was from 40 GJ ha$^{-1}$ (SR = 1) to 104 GJ ha$^{-1}$ (SR = 60), while it was only from 26 to 48 GJ ha$^{-1}$ in

the second cut (Figure 4.2). Legumes were the only functional group whose presence had an effect on GE and biomass in both cuts (1st cut: both $P = 0.004$; 2nd cut: both $P < 0.001$). In terms of functional-group abundance, legumes again were the only functional group with a noticeable effect (Table 4.3). According to the models established through multiple regression analysis for predicting GE ($R^2 = 0.33$ in the first and $R^2 = 0.38$ in the second cut), GE increased with increasing legume abundance up to 47 % of DM in the first cut and up to 58 % DM in the second (Figure 4.3A).

**Table 4.2** Analysis of variance of higher heating value (HHV) (MJ kg$^{-1}$ DM), carbon, hydrogen, nitrogen and lignin content (all in g kg$^{-1}$ DM) as functions of diversity parameters (species richness (SR), functional-group richness (FGR), functional group composition (FGC) and with presence/absence of individual functional groups. All values are means of both years. (A) The overall effects of diversity and the significance of cut were tested. The effects of individual functional groups were tested separately for (B) first cut and (C) second cut. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| Factor | DF | HHV | | Carbon | | Hydrogen | | Nitrogen | | Lignin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P | F | P | F | P |
| (A) | | | | | | | | | | | |
| Block | 3 | 4.2 | 0.010 | 4 | 0.010 | 8 | <0.001 | <1 | 0.781 | 7 | 0.001 |
| Log (SR) | 1 | 0.9 | 0.340 | 3 | 0.083 | <1 | 0.957 | 14 | <0.001↓ | <1 | 0.879 |
| FGR | 1 | 0.2 | 0.633 | <1 | 0.710 | <1 | 0.617 | <1 | 0.679 | <1 | 0.853 |
| FGC | 11 | 5.0 | <0.001 | 3 | 0.002 | 6 | <0.001 | 15 | <0.001 | 9 | <0.001 |
| Residuals | 59 | | | | | | | | | | |
| Repeated measures | | | | | | | | | | | |
| Cut | 1 | 56 | <0.001 | 48 | <0.001 | 35 | <0.001 | 11 | 0.002 | 42 | <0.001 |
| Cut x Log (SR) | 1 | <1 | 0.877 | <1 | 0.877 | <1 | 0.613 | <1 | 0.535 | 1 | 0.293 |
| Cut x FGR | 1 | 2 | 0.212 | 1 | 0.383 | <1 | 0.806 | 3 | 0.109 | 4 | 0.066 |
| Cut x FGC | 11 | 2 | 0.154 | 1 | 0.315 | 2 | 0.016 | 3 | 0.003 | 2 | 0.094 |
| Residuals | 62 | | | | | | | | | | |
| (B) | | | | | | | | | | | |
| Block | 3 | 6 | 0.002 | 5 | 0.002 | 8 | <0.001 | 2 | 0.073 | 4 | 0.012 |
| Log (SR) | 1 | 1 | 0.243 | 4 | 0.047 | <1 | 0.611 | 5 | 0.037↓ | <1 | 0.483 |
| Legumes | 1 | 16 | <0.001↑ | 12 | 0.001↑ | 4 | 0.052 | 20 | <0.001↑ | 15 | <0.001↑ |
| Grasses | 1 | 13 | 0.001↓ | 7 | 0.010↓ | 2 | 0.147 | 36 | <0.001↓ | 39 | <0.001↓ |
| Tall herbs | 1 | 3 | 0.079 | 3 | 0.113 | 9 | 0.003↓ | 1 | 0.277 | <1 | 0.828 |
| Small herbs | 1 | 2 | 0.186 | 3 | 0.110 | 5 | 0.023↓ | 1 | 0.366 | 4 | 0.057 |
| FGC | 8 | 2 | 0.123 | 1 | 0.267 | 1 | 0.397 | 5 | <0.001 | 1 | 0.664 |
| Residuals | 59 | | | | | | | | | | |
| (C) | | | | | | | | | | | |
| Block | 3 | 2 | 0.152 | 2 | 0.095 | 4 | 0.014 | 2 | 0.108 | 6 | 0.001 |
| Log (SR) | 1 | <1 | 0.595 | 1 | 0.248 | <1 | 0.841 | 13 | 0.001↓ | <1 | 0.728 |
| Legumes | 1 | 22 | <0.001↑ | 12 | 0.001↑ | 3 | 0.099 | 92 | <0.001↑ | 17 | <0.001↑ |
| Grasses | 1 | <1 | 0.489 | <1 | 0.747 | 7 | 0.011↑ | 9 | 0.005↓ | 31 | <0.001↑ |
| Tall herbs | 1 | 2 | 0.217 | 1 | 0.309 | 12 | 0.001↓ | 2 | 0.213 | <1 | 0.983 |
| Small herbs | 1 | 6 | 0.021↓ | 4 | 0.051 | 10 | 0.003↓ | 11 | 0.001↓ | 24 | <0.001↓ |
| FGC | 8 | 1 | 0.341 | 1 | 0.437 | 2 | 0.022 | 2 | 0.033 | 3 | 0.009 |
| Residuals | 59 | | | | | | | | | | |
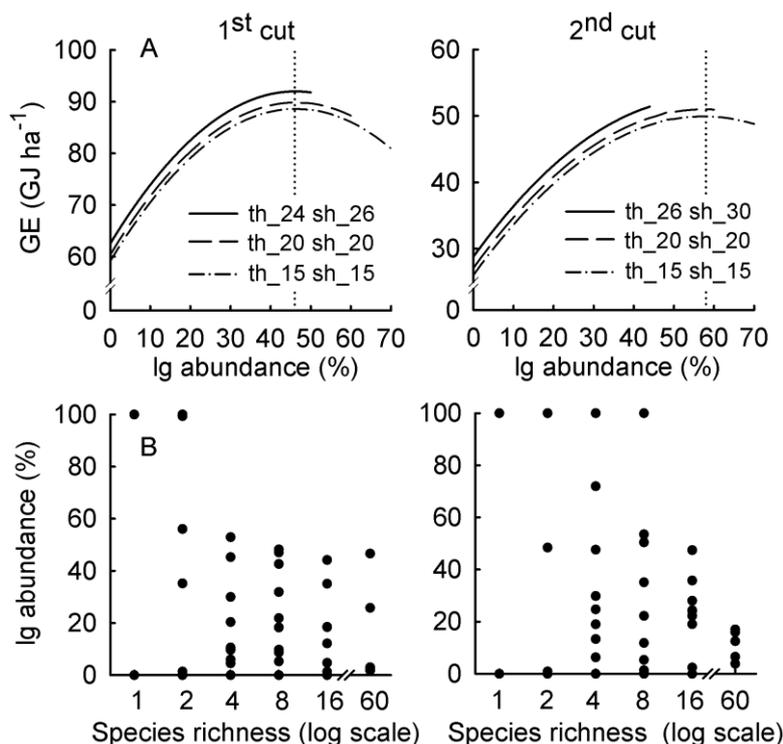
**Figure 4.3** (A) Predictions of gross energy yield (GE) in the first (late May) and second cut (late August) according to legume (lg) abundance (Table 4.3). The abundances of the other functional groups relevant for the multiple regression model [small herbs (sh) and tall herbs (th)] were varied (solid line indicates sh and th at their mean abundance; numbers indicate abundance in %). Dotted lines indicate the point of highest GE according to the model. (B) Legume abundance in the biomass at different levels of species richness.

**Table 4.3** Parameter estimates for the multiple regression analysis of gross energy yield. Abundances for legumes, grasses, tall and small herbs are given as DM yield percentage.

|         | Factor            | Estimate  | SE     | *t* value | *P*     |
|---------|-------------------|-----------|--------|-----------|---------|
| 1st cut | INTERCEPT         | 61.03887  | 5.3854 | 11.334    | <0.001  |
|         | Legumes (lg)      | 1.27363   | 0.2869 | 4.439     | <0.001  |
|         | lg$^2$            | -0.01375  | 0.0030 | -4.558    | <0.001  |
|         | Tall herbs (th)   | -0.13372  | 0.0878 | -1.523    | 0.132   |
|         | Small herbs (sh)  | -0.27605  | 0.0901 | -3.064    | 0.003   |
|         | th × sh           | 0.01874   | 0.0064 | 2.913     | 0.005   |
|         |                   |           |        |           |         |
| 2nd cut | INTERCEPT         | 25.7760   | 3.4485 | 7.475     | <0.001  |
|         | lg                | 0.83347   | 0.1698 | 4.909     | <0.001  |
|         | lg$^2$            | -0.00727  | 0.0018 | -4.104    | <0.001  |
|         | th                | -0.06183  | 0.0514 | -1.202    | 0.233   |
|         | sh                | -0.06966  | 0.0499 | -1.395    | 0.167   |
|         | th × sh           | 0.00985   | 0.0033 | 2.969     | 0.004   |

To better understand underlying diversity mechanisms, possible effects of SR on functional-group abundance were investigated and the dominance structure was quantified. As intended by the experimental design, legume abundance was not affected by SR (1st cut: *P* = 0.14; 2nd cut: *P* = 0.22; Figure 4.3B), and none of the interactions between abundance of a functional group and SR showed any effect. The same was true for the abundance of grasses (*P* = 0.46 and *P* = 0.94, respectively), small herbs (*P* = 0.86 and *P* = 0.54) and tall herbs (*P* = 0.51 and

$P = 0.46$). The Shannon evenness (E) as an indicator for the dominance structure of mixtures increased with increasing SR in both cuts (with $E = 0.54$; 0.48, 0.60, 0.64 and 0.67 in the first cut and $E = 0.53$, 0.58, 0.63 and 0.67 in the second cut, for SR = 2 (only in the first cut), 4, 8, 16 and 60, respectively).

The total annual GE, the sum of GE from both cuts, varied from 19 to 265 GJ ha$^{-1}$ year$^{-1}$, with a mean of 86 GJ ha$^{-1}$ year$^{-1}$. Total annual GE was affected by SR and increased consistently with increasing SR ($59 \pm 27$, $116 \pm 33$, $152 \pm 36$ GJ ha$^{-1}$ year$^{-1}$ at SR = 1, 16, 60, respectively; Figure 4.4). The presence of legumes increased total annual GE at all levels of SR (Figure 4.4). However, the increase of total annual GE owing to legumes was more pronounced at higher diversity levels (43 GJ ha$^{-1}$ year$^{-1}$ at SR = 16) as compared to only 12 GJ ha$^{-1}$ year$^{-1}$ at SR = 1.
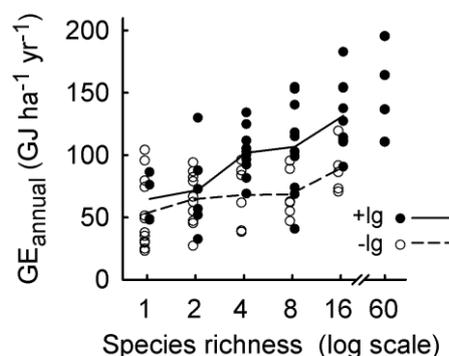


**Figure 4.4** Annual gross energy yield (GE$_{annual}$) at different levels of species richness. Mean values of species-richness levels are indicated by solid line for plots with legumes present (+lg) and by dashed line for means with legumes absent (–lg). Values are means of both years.

### 4.3.3 Ash and ash-forming elements

The ash content amongst the species mixtures varied between 53 and 220 g kg$^{-1}$ DM, with an overall mean of 94 g kg$^{-1}$ DM. In the functional-group monocultures both legumes and grasses had the lowest mean values, while contents were higher in tall and small herbs (Table 4.1). Ash was negatively affected by SR and affected by FGC in the repeated measure model (Table 4.A). Cut had an effect on ash (Table 4.4A), and there was lower ash content in the first cut than in the second (Table 4.1). Analysing ash in two separate models, there were no effects of SR (Table 4.4) while FGC had an effect in the first cut. Most functional-group effects were not consistent between the cuts: ash content was lower with grasses in the first cut and with legumes in the second, and small herbs increased ash in the first cut. The only consistent functional-group effect was tall herbs, increasing ash in both cuts (Table 4.4).

Across all mixtures, K ranged from 7.6 to 34.1 g kg$^{-1}$ DM, with a mean of 17.7 g kg$^{-1}$ DM. The K content was affected by the presence of legumes, which reduced the K concentrations in both cuts, whereas small herbs increased it (Table 4.4). K in the ash was lowest in legume monocultures, with 183 g kg$^{-1}$ ash in the first cut and 120 g kg$^{-1}$ ash in the second cut (Figure 4.5). Mean K concentrations in the ash were equally high in the other three functional-group monocultures and the all-functional-group mixtures (FGR = 4), with 242-255 g kg$^{-1}$ ash in the first cut and 149-168 g kg$^{-1}$ ash in the second cut (Figure 4.5).

**Table 4.4** Analysis of variance of ash, potassium, calcium, magnesium, sulphur and chlorine content (all in g kg$^{-1}$ DM) as function of diversity parameters (species richness (SR), functional groups richness (FGR), functional group composition (FGC) and with presence/absence of individual functional groups. All values are means of both years. (A) The overall effects of diversity and the significance of cut were tested. The effects of individual functional groups were tested separately for (B) first cut and (C) second cut. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| Factor | DF | Ash F | Ash P | Potassium F | Potassium P | Calcium F | Calcium P | Magnesium F | Magnesium P | Sulphur F | Sulphur P | Chlorine F | Chlorine P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (A) | | | | | | | | | | | | | |
| Block | 3 | 6 | 0.001 | 10 | <0.001 | 7 | <0.001 | 5 | 0.004 | 2 | 0.085 | 1 | 0.507 |
| Log (SR) | 1 | 6 | 0.020↓ | 1 | 0.261 | <1 | 0.773 | 12 | 0.001↓ | 42 | <0.001↓ | <1 | 0.650 |
| FGR | 1 | <1 | 0.872 | <1 | 0.909 | <1 | 0.503 | <1 | 0.935 | 3 | 0.081 | <1 | 0.690 |
| FGC | 11 | 4 | <0.001 | 5 | <0.001 | 13 | <0.001 | 10 | <0.001 | 3 | 0.011 | 2 | 0.129 |
| Residuals | 59 | | | | | | | | | | | | |
| Repeated measures | | | | | | | | | | | | | |
| Cut | 1 | 236 | <0.001 | 80 | <0.001 | 462 | <0.001 | 150 | <0.001 | 409 | <0.001 | 35 | <0.001 |
| Cut x Log (SR) | 1 | 1 | 0.404 | 1 | 0.404 | 4 | 0.060 | 1 | 0.307 | 2 | 0.148 | <1 | 0.710 |
| Cut x FGR | 1 | <1 | 0.688 | <1 | 0.644 | 2 | 0.140 | 1 | 0.350 | 2 | 0.221 | <1 | 0.598 |
| Cut x FGC | 11 | 1 | 0.239 | 1 | 0.177 | 2 | 0.084 | 1 | 0.346 | 4 | <0.001 | 1 | 0.370 |
| Residuals | 62 | | | | | | | | | | | | |
| (B) | | | | | | | | | | | | | |
| Block | 3 | 3 | 0.022 | 8 | <0.001 | 7 | <0.001 | 4 | 0.013 | 7 | <0.001 | 1 | 0.600 |
| Log (SR) | 1 | 4 | 0.059 | 3 | 0.106 | 1 | 0.409 | 10 | 0.003↓ | 35 | <0.001↓ | 1 | 0.341 |
| Legumes | 1 | <1 | 0.997 | 8 | 0.007↓ | 30 | <0.001↑ | 68 | <0.001↑ | 3 | 0.105 | <1 | 0.578 |
| Grasses | 1 | 15 | <0.001↓ | 6 | 0.016↓ | 69 | <0.001↓ | 74 | <0.001↓ | 21 | <0.001↓ | 2 | 0.164 |
| Tall herbs | 1 | 9 | 0.004↑ | 3 | 0.092 | 6 | 0.015↑ | <1 | 0.498 | <1 | 0.817 | 2 | 0.194 |
| Small herbs | 1 | 16 | <0.001↑ | 20 | <0.001↑ | 4 | 0.041↑ | <1 | 0.919 | 7 | 0.009↓ | 4 | 0.062 |
| FGC | 8 | 2 | 0.024 | 4 | <0.001 | 3 | 0.006 | 5 | <0.001 | 4 | 0.001 | 1 | 0.416 |
| Residuals | 59 | | | | | | | | | | | | |
| (C) | | | | | | | | | | | | | |
| Block | 3 | 5 | 0.005 | 8 | <0.001 | 7 | 0.001 | 5 | 0.003 | 2 | 0.146 | 1 | 0.250 |
| Log (SR) | 1 | 4 | 0.062 | <1 | 0.587 | 1 | 0.273 | 8 | 0.006↓ | 23 | <0.001↓ | <1 | 0.896 |
| Legumes | 1 | 7 | 0.013↓ | 9 | 0.005↓ | 23 | <0.001↑ | 18 | <0.001↑ | 19 | <0.001↓ | <1 | 0.828 |
| Grasses | 1 | 1 | 0.324 | 2 | 0.205 | 31 | <0.001↓ | 13 | 0.001↓ | <1 | 0.741 | <1 | 0.581 |
| Tall herbs | 1 | 6 | 0.020↑ | 2 | 0.157 | 22 | <0.001↑ | 1 | 0.416 | <1 | 0.998 | 5 | 0.032↓ |
| Small herbs | 1 | 3 | 0.092 | 16 | <0.001↑ | 1 | 0.317 | <1 | 0.578 | <1 | 0.691 | 5 | 0.031↑ |
| FGC | 8 | 1 | 0.331 | 1 | 0.343 | 2 | 0.062 | 2 | 0.037 | 2 | 0.176 | 1 | 0.562 |
| Residuals | 59 | | | | | | | | | | | | |

Ca concentrations in the biomass ranged from 2.9 to 37.1 g kg$^{-1}$ DM, with a mean of 15.2 g kg$^{-1}$ DM and were lower when grasses were present (Table 4.). There was a strong correlation between Ca and the abundance of grasses (1st cut: r = –0.73; 2nd cut: r = –0.81). The presence of legumes increased Ca concentrations irrespective of the cut (Table 4.4). Mean Ca concentrations in the ash were lowest in grass monocultures (1st cut = 64 g kg$^{-1}$ ash;

2nd cut = 110 g kg$^{-1}$ ash) and highest in legume monocultures (1st cut = 209 g kg$^{-1}$ ash; 2nd cut = 229 g kg$^{-1}$ ash) (Figure 4.5). Mean Ca concentrations in the ash of all-functional-group mixtures were at 140 g kg$^{-1}$ ash in the first cut and 193 g kg$^{-1}$ ash in the second (Figure 4.5).
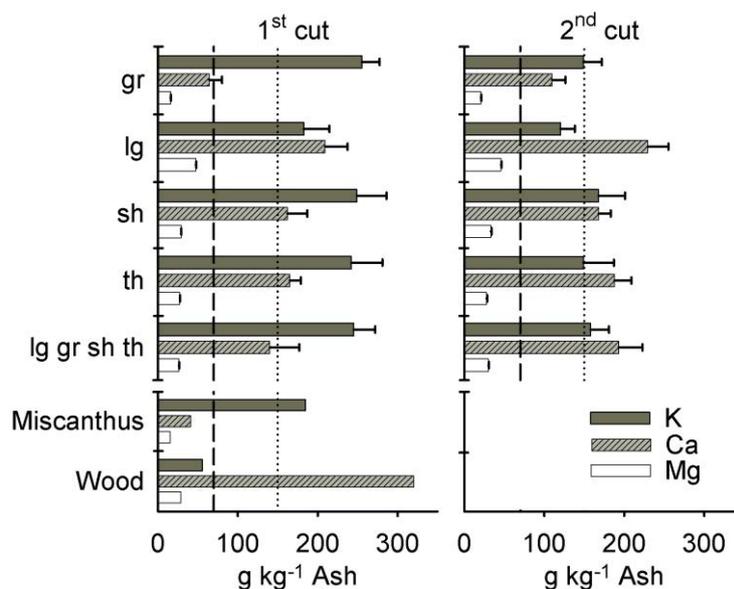


**Figure 4.5** Concentrations of ash constituents in the ash of functional-group monocultures (gr = grasses, lg = legumes, sh = small herbs, th = tall herbs) and the all-functional-group mixtures, displayed separately for both cuts. Values are means of both years. Standard deviation is indicated by error bars. Ca, K and Mg are minerals relevant for ash-melting behaviour whereby the concentrations of Ca in the ash should be above 150 g kg$^{-1}$ ash (dotted line) and K concentrations should be lower than 70 g kg$^{-1}$ ash (dashed line), according to Obernberger *et al.* (2006). Values for wood (spruce as wood chips from Obernberger *et al.*, 2006) and miscanthus (calculated from Hartmann, 2009) were included for comparison.

Mg ranged from 0.8 to 7.2 g kg$^{-1}$ DM, with a mean of 2.7 g kg$^{-1}$ DM and, similar to Ca, were lower when grasses were present (Table 4.4). The correlation between Mg concentration and the abundance of grasses was r = -0.81 in the first cut and r = -0.59 in the second. The presence of legumes had a positive effect on the concentration of Mg, irrespective of the cut (Table 4.4). Mg was the only major ash-forming element that was affected by SR (Table 4.4, Figure 4.6). With increasing SR from 1 to 60, the mean values of Mg concentration in the biomass decreased by 30 % and 22 % in the first and second cut (Figure 4.6). Mean Mg concentrations in the ash were lowest in grass monocultures (1st cut = 16.0 g kg$^{-1}$ ash; 2nd cut = 20.7 g kg$^{-1}$ ash) and highest in legume monocultures (1st cut = 47.5 g kg$^{-1}$ ash; 2nd cut = 45.9 g kg$^{-1}$ ash) (Figure 4.5). Mean Mg concentrations in the ash of all-functional-group mixtures were at 26.5 g kg$^{-1}$ ash in the first cut and 30.1 g kg$^{-1}$ ash in the second (Figure 4.5).
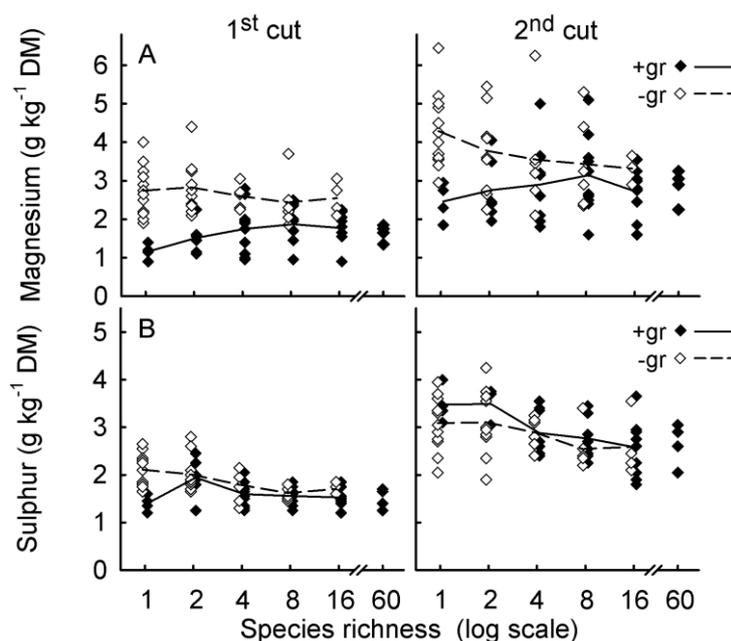
**Figure 4.6** (A) Magnesium and (B) Sulphur in the first (late May) and second cut (late August) at different levels of species richness. Mean values of species-richness levels are indicated by solid line for plots with grasses present (+gr) and indicated by dashed line for plots with grasses absent (−gr). Values are means of both years.

### 4.3.4 Cl and S concentrations

The Cl concentrations ranged from 0.9 to 9.2 g kg$^{-1}$ DM, with a mean of 3.0 g kg$^{-1}$ DM. The only functional-group effects on Cl concentration were weak and indicating a decline because of tall herbs, as well as an increase because of small herbs in the second cut (Table 4.4). Mean Cl concentrations did not vary much between the functional-group monocultures and were between 2.3-2.7 g kg$^{-1}$ DM in the first cut and 2.6-3.3 g kg$^{-1}$ DM in the second (Figure 4.7). The all-functional-group mixtures were in the same range.

The S concentrations ranged from 1.1 to 4.9 g kg$^{-1}$ DM with a mean of 2.3 g kg$^{-1}$ DM. S concentrations were significantly affected by SR (Table 4.4) and declined from 1- to 60-species plots by 23 % in the first cut and by 16 % in the second cut (Figure 4.6). Comparing the two cuts regarding functional group presence, no consistent effects could be found. In the first cut the presence of grasses and the presence of small herbs both increased S, while in the second cut the presence of legumes decreased it (Table 4.4). Therefore, S concentrations were lowest in the grass monocultures, with 1.5 g kg$^{-1}$ DM in the first cut, and in the legume monocultures, with 2.6 g kg$^{-1}$ DM in the second cut. The all-functional-group mixtures were as low as the lowest functional-group monocultures in the respective cut (Figure 4.7).
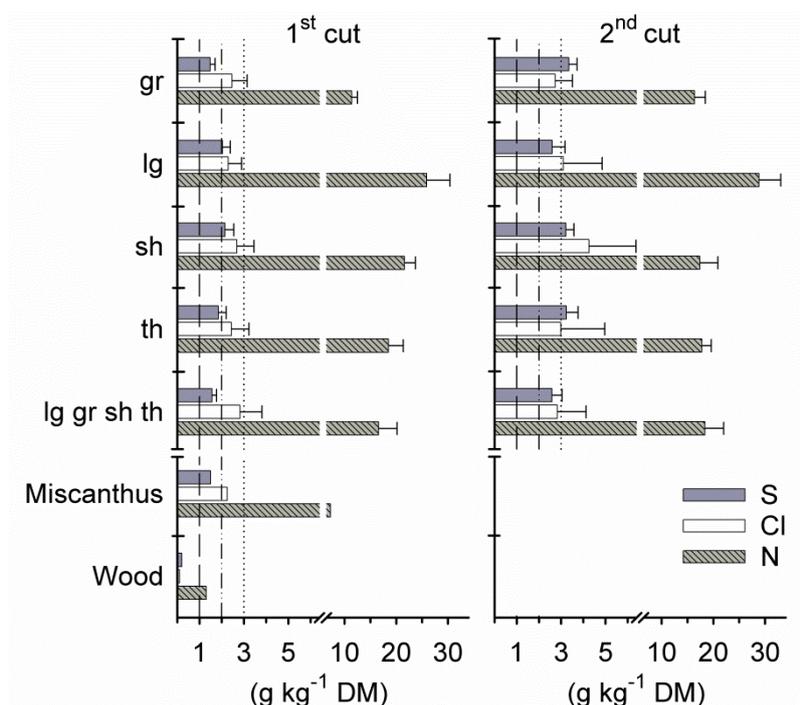
**Figure 4.7** Concentrations of S, Cl and N in the biomass of the functional-group monocultures (gr = grasses, lg = legumes, sh = small herbs, th = tall herbs) and the all-functional-group mixtures, displayed separately for both cuts. Values are means of both years. Standard deviation is indicated by error bars. The concentrations of Cl should be below 1 g kg$^{-1}$ DM (dashed line) to prevent corrosion and HCl emissions and below 3 g kg$^{-1}$ DM (dotted line) to prevent emissions of polychlorinated dibenzodioxines and dibenzofuranes (PCDD/F). S concentrations should be below 1 g kg$^{-1}$ DM (dashed line) to prevent corrosion and below 2 g kg$^{-1}$ DM (dashed dotted line) to prevent SOx emissions. N concentrations should be lower than 6 g kg$^{-1}$ DM to prevent emission and corrosion (all thresholds according to van Loo and Koppejan, 2008). Comparison values included for wood (spruce, as wood chips) and miscanthus (van Loo and Koppejan, 2008).

## 4.4   Discussion

### 4.4.1   Patterns of energy content and chemical composition

The energy content in the biomass of the Jena grassland communities measured as HHV was well in the range of values reported in other grassland studies (Table 4.5). However, in contrast to the approach of the Jena experiment, where emphasis was given to proper statistical methods leading to sound conclusions on the relationships between diversity parameters and energy content, their focus was on investigating energy content along a spatial gradient across multiple sites. The results presented here clearly suggest that changes in SR do not affect the energy content of extensive grassland, as represented by the investigated assembled species communities investigated here, while the presence of legumes has a significantly positive effect on HHV (Figure 4.2A). A possible explanation for legumes having a positive effect on HHV may be their higher lignin content. Lignin is known to have a

carbon content of 640 g kg$^{-1}$ lignin, as compared to cellulose with a carbon content of 420 g kg$^{-1}$ cellulose (Lewandowski, 2009), and therefore, a higher lignin content should result in a higher HHV. A study by Jung *et al.* (1997) observed similar patterns in lignin concentrations for legumes (77 g kg$^{-1}$ DM) and grasses (33 g kg$^{-1}$ DM) as found in this study. Looking at the relation between lignin content in the ash-free biomass and ash-free HHV, a positive correlation could be found in the first cut ($R^2 = 0.34$, $P < 0.001$), while it was weak in the second ($R^2 = 0.08$, $P = 0.01$). Better correlations might be found if lignin is entirely estimated through standard methods rather than by near-infrared spectroscopy. Andrés *et al.* (2005) discussed the difficulties with predicting ADL through near-infrared spectroscopy.

According to the formula that was used to calculate HHV, C is the most important element determining HHV, while N and H play a minor role in the equation. The range of C found in this study is lower than that found by Florine et al. (2006), which was between 484 and 537 g kg$^{-1}$ DM. Of all C concentrations found here, 75 % were at or above 450 g kg$^{-1}$ DM, and the overall mean with 454 g kg$^{-1}$ DM is similar to data for grassland, which were reported by Hartmann (2009). The C content was negatively correlated to the ash content ($R^2 = 0.65$, $P < 0.001$).

**Table 4.5** Mean, minimum (min) and maximum (max) values of energy content and combustion-relevant parameters from three different grassland experiments compared with values from the present study. Included are values from miscanthus and spruce wood as two types of biomass frequently used for combustion.

| Source and experimental setup | | MJ kg$^{-1}$ DM | g kg$^{-1}$ DM | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | HHV | N | Ash | K | Ca | Mg | Cl | S |
| Jena Experiment (2008 – 2009), | Mean | 18.1 | 18.7 | 93 | 17.7 | 15.2 | 2.7 | 3.0 | 2.3 |
| extensive grassland, east | Min | 16.3 | 8.9 | 53 | 7.6 | 2.9 | 0.8 | 0.9 | 1.1 |
| Germany, one site, two harvests | Max | 19.2 | 38.4 | 220 | 34.1 | 37.1 | 7.2 | 9.2 | 4.9 |
| Tonn *et al.* (2010), | Mean | - | 13.4 | 84 | 15.0 | 10.8 | 2.3 | 4.1 | 2.2 |
| extensive grassland, southwest | Min | 18.2 | 10.3 | 61 | 8.4 | 6.7 | 1.1 | 1.7 | 1.5 |
| Germany, six sites, different harvest dates | Max | 19.1 | 17.4 | 99 | 24.2 | 14.9 | 3.7 | 11.2 | 3.5 |
| Florine *et al.* (2006), | Mean | 18.5 | 12.0 | 79 | - | - | - | 2.7 | 1.6 |
| perennial pastures, south Iowa, | Min | 17.7 | 7.0 | 59 | - | - | - | 0.8 | 0.7 |
| USA, ten sites, one harvest | Max | 19.5 | 22.0 | 118 | - | - | - | 7.6 | 3.4 |
| Richter *et al.* (2010) | Mean | 17.8 | 13.7 | - | 12.0 | 10.0 | 3.2 | 4.8 | 1.6 |
| extensive grassland, Germany | Min | - | - | - | - | - | - | - | - |
| five sites, one harvest | Max | - | - | - | - | - | - | - | - |
| Hartmann (2009) | | | | | | | | | |
| Miscanthus | Mean | 19.1 | 7.3 | 39 | 7.2 | 1.6 | 0.6 | 2.2 | 1.5 |
| Wood (spruce) | Mean | 20.2 | 1.3 | 6 | 1.3 | 7.0 | 0.8 | 0.1 | 0.2 |

## 4.4.2   Patterns and drivers of biomass and GE yield

Both biomass yield and GE showed relatively low minima compared with other studies. The lowest biomass presented here was 0.2 t DM ha$^{-1}$, which is largely because of the species

monocultures and the functional-group monoculture plots. The lowest GE measured was 3 GJ ha$^{-1}$, which is about 30 GJ ha$^{-1}$ lower than the lowest value observed by Tonn *et al.* (2010). Nevertheless, the experimental set-up used in this study allows a comparison with other diversity studies such as the study by Tilman *et al.* (2006), representing a perennial grassland (with one removal of aboveground biomass per year through fire), where a range of 20 GJ ha$^{-1}$ year$^{-1}$ at SR = 1 up to 68 GJ ha$^{-1}$ year$^{-1}$ at SR = 16 was found. This is comparable to the GE values measured here in the first cut. However, GE of the monocultures had a mean of 40 GJ ha$^{-1}$ year$^{-1}$, which is double as high as those observed by Tilman. This is largely due to the variation in biomass from the plant species grown in monoculture, because the assumed HHV of 18.5 MJ kg$^{-1}$ by Tilman is almost identical with the mean HHV found in our study. The increase in annual GE from a one species plot to a high diversity 16 species plot was on average 108% and even 172% from a one to a 60 species plot, while Tilman *et al.* (2006) reported a 157% and a 238% increase respectively.

The experimental approach chosen here makes it possible to further investigate the underlying mechanisms driving the diversity-productivity relation, such as (i) 'sampling effect', describing the greater chance of including more productive species with increasing SR, (ii) 'facilitation', the presence of a species increases the growth or survival of another species or (iii) 'niche-separation', the plant community can utilize more of the total nutrient pool and habitat space. The last two effects are often referred to as complementarity so that the question is whether diversity effects are driven by sampling or complementarity.

If E declines (from $E = 1$ toward $E = 0$), a plant community is increasingly dominated by one high-yielding species. In this study, E increased slightly with SR, which indicates that high-diversity plots had a more balanced dominance structure than low-species plots. This is a weak indication for increasing importance of complementarity between the species at high species richness. A more conservative and sound test for complementarity is by proving transgressive overyielding, as by definition, in case of transgressive overyielding the productivity of a mixture is higher than the productivity of the most productive monoculture of the species contained in the mixture. Marquard *et al.* (2009) analysed five years (2003-2007) of biomass date from the Jena-Experiment and found strong evidence for transgressive overyielding in a replacement series analysis on functional group level. Their results also showed that the complementarity effect, according to the method of additive partitioning by Loreau and Hector (2001), gained importance for the overall diversity effect over the years, while the 'selection effect' (species with higher than average monoculture yields dominate the

mixtures, Loreau and Hector, 2001) declined over time. A further indication for complementarity being the main mechanism ruling diversity effects is when no functional groups interact with SR. This was tested here by fitting the presence of all four functional groups in the model before SR which did not eliminate the significance of SR (1st cut: $P < 0.001$; 2nd cut: $P = 0.008$). In other words, SR affects biomass, independent of the presence of any functional group (including the legumes), which confirms similar findings in a biodiversity study by Tilman *et al.* (2001).

Looking at the complementarity effect more closely, the legume effect on biomass and GE is of utmost importance. Legumes had a significant positive effect on biomass and, as they were fitted after SR in the model, this effect was independent of SR. The evenness was not affected by the presence of legumes, indicating that legume presence did not enhance total biomass through high legume species biomass. Marquard *et al.* (2009) did not find any significant effect of legumes on the "selection effect", which could explain the increase in biomass by a disproportionate contribution of legume biomass to the total biomass. Thus, it can be concluded that legumes affected biomass through facilitating the growth of other plants or through promoting complementary resource use and doing so in a similar pattern on all levels of SR. The facilitation mechanism of legumes is related to their ability to fix atmospheric N via symbiotic root bacteria. $N_2$-fixing plants often accumulate high concentrations of N in their tissue, which is released into the soil during their decomposition (Tomm *et al.*, 1995). Non N-fixing plants (grasses and herbs) can directly benefit from these processes by taking up the plant available N released into the soil (Haynes, 1980; Spehn *et al.*, 2002). As N was found to be primarily limiting biomass production in the Jena-Experiment (Oelmann *et al.*, 2007), it can be assumed that legumes have provided plant available N for the system which promoted productivity and thereby GE. The highest productivity was observed at legume abundance of 47-58 %, which is similar to findings by Nyfeler *et al.* (2011) from a grassland study with moderate levels of fertilizer application (50 and 150 kg N ha$^{-1}$ year$^{-1}$). Marquard *et al.* (2009) found the highest productivity after dividing legume abundance in five classes (0%, 0-33 %, 33-66 %, 66-100 % and 100 %) at an abundance between 66-100 % in four of the five years.

### 4.4.3   Patterns in ash, ash forming elements and ash-softening temperature

Ash contents are detrimental for combustion of biomass as they correlate negatively with the energy content (in this study, the correlation was $R^2 = 0.54$; for rice straw correlation was

found to be $R^2 = 0.46$ in Jenkins *et al.* 1995) and thus reduce energy output. Mean values of ash content found here were slightly higher than those found in other grassland studies (Table 4.5). While maximum values in the latter studies only went up to around 100 g kg$^{-1}$ DM, maximum values presented here were as high as 220 g kg$^{-1}$ DM, similar to 231 g kg$^{-1}$ ash reported for biomass from grass verge (Hartmann, 2009). These high ash contents may be partly explained by the characteristics of the low diversity plots, as the data set of this experiment (plots: $n = 76$) contained many plots with only one functional group ($n = 34$) of which several plots were species monocultures ($n = 16$). The biomass yield of these plots was often low, especially in the second cut, and had a large percentage of uncovered soil. Heavy rainfall on bare ground may have caused the pollution of plants with soil particles and thereby increased the ash content substantially. The higher ash contents in the second cut thus led to lower HHVs than observed in the first cut (see Table 4.1 and Figure 4.2A).

Another explanation for differences in ash contents may be found in the nutrient uptake mechanisms of the plants or their morphology. The fact that functional group monocultures of herbaceous species were higher in ash content than grasses and legumes (Table 4.1) is similar to the results observed by Tonn *et al.* (2010). Such patterns may occur because dicotyledonous herbaceous plants, owing to a high cation exchange capacity of their roots, which often have a higher uptake of nutrients that are available as divalent ions than monocotyledons grass species (Asher and Ozanne, 1961; Drake *et al.*, 1951). For grass species, silicon is of great importance (Marschner, 2011) and it was found to largely contribute to the ash contents of grass-like species (65 % of the total ash in switch grass, Jenkins *et al.* 1998). The strong increase of ash content in the grass functional-group monocultures from the first to the second cut may therefore be related to differences in silicon concentrations.

Morphological traits of the plants have been found to affect ash content, as higher nutrient concentrations are expected in leaves than in the stems (Pahkala and Pihala, 2000). Furthermore, plants with relatively small growth, such as small herbs, might be much more prone to pollution with soil particles after heavy rainfall events. Highest ash contents can therefore be expected in biomasses from grassland vegetation dominated by dicotyledonous, small growing plants with a high leaf to stem ratio. Furthermore, high ash contents are to be expected in grass-dominated vegetation at late stages of maturity when silicon concentrations are increased (Tyler, 1971)

Besides discussing ash content itself, ash composition needs to be investigated as certain components that are negative for the combustion process and harmful to parts of the combustion chamber are contained in the ash. Common problems with ashes from grassland biomass are, for example, slagging owing to low melting points of the ashes and corrosion of metal parts, which can reduce the plants lifetime (Obernberger *et al.*, 2006). Of the major ash forming elements of biofuels, concentrations of K, Ca and Mg in the DM were analysed as they are known to affect AST. K-rich salts in combination with phosphates and silica were found to be responsible for low AST (Steenari *et al.*, 2009), and the ratio between K, on the one hand, and Ca and Mg on the other hand, indicate whether low or high AST can be expected. Furthermore, Hartmann (2009) suggested that K, Ca and Mg concentrations can be used to calculate AST.

The positive effect of legume presence on Ca and Mg, and the negative correlation of grass abundance with Ca and Mg observed in this study, are in line with the results from Tonn *et al.* (2010) and can partly be explained by the difference in cation exchange capacity, as discussed for ash content. However, the low K concentrations in the legume functional-group monocultures cannot be explained by this.

Whether the decline in Mg concentration with increasing SR was a true diversity effect and not merely a coincidental with other variables was tested by fitting each functional-group term before SR in a sequence of analysis (results not shown). In both cuts, fitting grasses before SR removed the significance (Figure 4.6) while fitting any of the other functional groups before SR had no effect on the significance of SR. This means that the negative diversity effect regarding Mg could not be separated from the effect of grasses being present. This corresponds with the observation of different slopes in Mg concentration with increasing SR for grasses being present (+gr) or absent (−gr) (Figure 4.6). It is likely that this phenomenon was linked to the characteristics of +gr and −gr: the difference in Mg concentrations was most pronounced at SR = 1 where mono- and dicotyledonous species were fully separated (ratio of plots with monocotyledonous plants to plots with dicotyledonous plants to plots with a mixture of both was 1:3:0, see Figure 4.1), whereas at SR > 1, +gr and −gr did not segregate mono- and dicotyledonous plants any longer. While −gr only included dicotyledonous plants, +gr now consisted of either pure monocotyledonous plants (functional-group monocultures with grasses) or of mixtures of both mono- and dicotyledonous plants (the ratio for SR = 2 was 1:5:2). At higher levels of SR, the number of mixed plots further increased (plot ratio at SR = 4 and 8 is 1:6:6 and 1:4:6 at SR = 16). Thus, at higher SR, +gr

was very much affected by the presence of dicotyledonous plants, which results in a higher Mg content than a pure grass plot would have.

Comparing the concentrations of K, Ca and Mg in the ash of the functional-group monocultures with the recommended thresholds by Obernberger *et al.* (2006), the following trends were observed in both cuts (Figure 4.5): (i) all functional-group monocultures, except for grasses, have a Ca concentration above the recommended 150 g kg$^{-1}$ ash, which is acceptable with regard to AST, (ii) highest Ca concentrations were found in the legume monocultures, (iii) K concentrations generally exceeded the recommended value of 70 g kg$^{-1}$ ash, therefore contributing to a lower AST, with lowest concentrations in the legume monocultures. Regarding the four functional-group mixtures, the same patterns with sufficient Ca and excessive K concentrations were found. Ca concentrations in the monocultures were higher than in miscanthus but lower than in wood (Obernberger *et al.*, 2006). With regard to the K concentrations, it must be considered that the temperature of the combustion plays a major role in the accumulation of K in the ash. Knudsen *et al.* (2004) found little to no K release into the gas phase at temperatures below 700°C, while 50-80 % were released at temperature above 1100°C, depending on the substrate. Thus at temperature < 700°C, K would mainly contribute to ash softening whereas at temperatures > 1100°C, it could become a major emission and fouling issue.

The equation suggested by Hartmann (2009) to estimate AST originates from biomass ashes (woody and herbaceous materials, *n* = 67) and yields relatively accurate results ($R^2$ = 0.60; SE = 88°C; Hartmann *et al.* 2000). In accordance with the above discussed effects of legumes and grasses on K, Ca and Mg concentrations it can be assumed that the presence of legumes would increase AST while grasses would decreased it. However, this equation is rather simple regarding the number of variables included and may thus underestimate the complexity of chemical interactions leading to ash softening behaviour as shown in other studies (Bryers, 1996; Seggiani, 1999).

### 4.4.4   Patterns in N, Cl and S content and their impacts on corrosion and emission

N contained in the vegetation plays a major role during combustion as it is the source of undesired $NO_x$ emissions. The measured range of N concentrations was wider, and the mean N concentrations were slightly higher, than in other studies on extensive grasslands (Table 4.5). The decline of N concentrations with increasing species richness was not independent of the presence of grasses as revealed by fitting other functional group terms prior to SR in the

model (data not shown) and by the non- parallel slopes of N concentration with increasing SR for +gr and –gr (Figure 4.2). Again, the characteristics of +gr and -gr may be a possible explanation for this effect, as it was discussed for Mg.

N concentrations were above the critical level of 6 g kg$^{-1}$ DM as suggested by Obernberger *et al.* (2006) and were more than double as high as those of miscanthus (7.3 g kg$^{-1}$ DM) and out of the range of values for wood from spruce (1.3 g kg$^{-1}$ DM, van Loo and Koppejan, 2008) (Figure 4.7).

The range of Cl concentrations measured here was comparable to values found in other studies (Table 4.5). According to van Loo and Koppejan (2008), a critical value for Cl is 1 g kg$^{-1}$ DM if corrosion and HCl emissions are to be prevented, while the Cl concentrations have to be below 3 g kg$^{-1}$ DM to prevent PCDD/F emissions. All functional-group monocultures and mixtures exceed the 1 g kg$^{-1}$ DM, and the values in the second cut even exceeded the 3 g kg$^{-1}$ DM threshold (Figure 4.7).

Sulphur contained in the biomass forms gaseous SO$_x$ and alkali, as well as earth-alkali sulphates. If a sufficient amount of alkali and earth alkali metals, especially Ca, are present, more than 50% of the S can be fixed in the ash (e.g. as K$_2$SO$_4$ or CaSO$_4$) (Obernberger *et al.*, 2006). The S not fixed in the ash will either contribute to corrosion by releasing and replacing the Cl in alkali chlorides or cause SO$_x$ emission. The S concentrations measured here were similar to the findings by Tonn *et al.* (2010) (Table 4.5). Critical values for S in biomasses are 1 g kg$^{-1}$ DM to prevent corrosion and 2 g kg$^{-1}$ DM to prevent SO$_x$ emission (van Loo and Koppejan, 2008). None of the functional-group monocultures and mixtures was below 1 g kg$^{-1}$ DM, while biomass from the first cut (except for small herb monocultures) and from all monocultures and mixtures of the second cut (Figure 4.7), exceeded 2 g kg$^{-1}$ DM. Testing whether the SR effect on S was intermingled with any other functional group by changing the order of terms entering the model confirmed that S was affected by SR independent of any other variable.

### 4.4.5 Tackling fuel related problems and the advantage of high diversity

For grassland biomass as solid fuel, it has been suggested there should be an extensive grassland management with one late cut and no application of fertilizers (Prochnow *et al.*, 2009a). Regarding the biomass investigated in this study, it clearly shows that mineral contents (e.g. K, S, Cl) from an early cut are less favourable for combustion than the ones from a late cut. However, there are several ways to deal with fuel-quality deficiencies of

grassland biomasses: (i) the combustion plant can be designed in a way that the amount of problematic flue gases is reduced through a staged combustion, while dust precipitation units (most relevant flue gas-cleaning step for solid fuels) further reduce emissions (Obernberger *et al.*, 2006), (ii) the substrate can be pre-treated prior to combustion through hydro-thermal conditioning and mechanical dewatering of nutrients to reduce ash concentration (Richter *et al.*, 2010, 2011), (iii) delayed harvest can improve fuel quality [in the case of extensive grassland harvested in February (Tonn *et al.*, 2007); and in the case of reed canary grass harvested in March (Christian *et al.*, 2002 and Xiong *et al.*, 2008) ] mainly because of natural leaching through precipitation and loss of mineral-rich matter such as leaf biomass.

By hydro-thermal conditioning prior to mechanical dewatering through a screw press ash constituents of extensive grasslands were reduced by 74-84 % for K, by 79-89 % for Cl, by 39-57 % for S and by 19-43 % for N, depending on the substrate and the conditioning temperature applied (Richter *et al.*, 2010, 2011). Reducing mineral concentrations prior to combustion through delayed harvest will result in similar fuel quality improvements, but may cause large biomass losses in the field of 30-70% owing to particle breakdown, depending on the substrate and climatic conditions (Christian *et al.*, 2002; Tonn *et al.*, 2007). The effect of precipitation intensity as part of natural leaching has been studied by analysing samples after natural rain events (for straw by Jenkins *et al.*, 1996) and after simulated rain (for permanent grassland by Tonn *et al.*, 2011).

Considering that ash-related problems can partly be addressed by pre-treatment of the biomass and by adjusting the combustion plant, the most important and positive effect of high-diversity over low-diversity extensive grassland is the increase in energy yield. Looking closely at the diversity steps relevant for practice, the annual GE in this study increased by only 4% from 4 to 8 species, while it showed an increase of 26% from 8 to 16 species and increased by 65% from 8 to 60 species (Figure 4.4). It is evident that an increased energy yield of between 26 and 65% will have a direct impact on the profitability of using extensive grassland biomass for energy production.

### 4.4.6    Experimental approaches and their relevance for extensive grassland farming

Diversity effects in grassland communities have been widely studied in artificially assembled communities of plant species. However, there has been a debate whether the results of such studies can be applied to natural communities (Huston 1997; Loreau *et al,*. 2001; Diaz *et al.*,

2003; Pfisterer *et al,.* 2004). Especially when interpreted in the framework of agricultural management the relevancy of these studies has to be assessed.

The clear advantage of studies with artificial communities is that SR or FGR can be precisely maintained, and the variance in abiotic conditions and community structure between replicates is kept small to yield sound statistic results. Regarding grassland management, their results may apply directly to, for example, the practise of ley farming in which crop growing is alternated by grassland for several years to improve soil fertility. However, Hopkins and Holz (2006) argued that further experiments should be more geared towards agricultural practises since many of the diversity-productivity experiments were conducted without fertilizer and other agricultural inputs and were conducted on prepared sites.

While Weigelt *et al.* (2009) studied diversity-productivity interactions in artificially assembled communities along a management and a fertilization gradient, others have suggested that removal experiments are best suitable for understanding the role of functional groups under natural conditions (Diaz *et al.*, 2003; McLaren and Turkington 2010). However, Diaz *et al.* (2003) concluded from a comparison of removal experiments and experiments with artificially assembled communities that both experimental approaches should be used to complement each other, rather than one approach being favoured over the other.

The results from the presented study have relevance for extensive, non-fertilized grassland, but the presented effects should be further examined with other methodologies that will shed light from different angles on relationships that cannot be assessed through experiments with artificial communities.

## 4.5 Conclusions

Using artificial grassland communities to identify diversity effects on fuel quality was successful and supports the following conclusions: (i) SR does not affect the energy potential of extensive grassland by modifying the energy content directly; rather, it is the increase in biomass that makes high-diversity grassland more valuable for bioenergy production, (ii) legumes increase GE yield mainly by increasing biomass owing to facilitation and complementary resource use and, to a smaller extend by increasing energy content due to higher carbon contents, (iii) identifying one functional group as being solely beneficial or disadvantageous for fuel quality is impossible, as they exhibit contrary effects with regard to different parameters, for example, legumes increase Ca concentrations (increasing AST) and, at the same time, increase N concentrations (resulting in higher $NO_x$ emissions), whilst

grasses have lower N concentrations (lower $NO_x$ emissions) but the highest K concentrations (decreasing AST), (iv) several positive aspects of high SR regarding ash properties exist, such as lower N and S concentrations, even though they could not always be fully disentangled from functional-group effects.

It was further concluded that in order to facilitate a high energy output in extensive grassland systems, the defoliation and nutrient management should be focused on the maintenance of highly diverse, legume-based grassland communities. Given the high concentrations of corrosion and emission-related constituents, a treatment of the fuel through nutrient leaching as well as technical adaptation of the combustion plants is recommended for grassland biomass at all levels of diversity.

# 5 Effects of species richness and functional groups on chemical constituents relevant for methane yields from anaerobic digestion: results from a grassland diversity experiment

**Abstract** Changes in livestock production systems have led to land-use changes and abandonment, especially of semi-natural grassland in agriculturally less favoured regions. The generation of energy from biomass of extensive, high-diversity grasslands can be an alternative to their abandonment, and anaerobic digestion is one possible method for converting grassland biomass into energy. However, little is known about the effects of species richness (SR) and functional groups on chemical constituents relevant for anaerobic digestion and the resulting energy potential. In this study, changes in the herbage chemical constituents that are relevant for forage quality were studied along a well-defined diversity gradient (one to sixty species) and across different combinations of functional groups (legumes, small herbs, tall herbs and grasses). Substrate-specific methane yield ($CH4_{sub}$) was estimated through the concentrations of forage quality parameters such as crude fibre (CF), crude protein (CP), crude lipid, nitrogen-free extract and their documented digestibility values, as well as the respective methane yields. Results show that with increasing SR, the CF increased and CP decreased, even though these effects could not be fully disentangled from the presence of grasses. These trends led to a negative effect of SR on $CH4_{sub}$, while the area-specific methane yield ($CH4_{area} = CH4_{sub} \times$ biomass yield) increased due to a strong increase in biomass with increasing SR. The $CH4_{sub}$ was increased when legumes were present, and it declined with the presence of grasses. Generally, $CH4_{sub}$ and $CH4_{area}$ varied between functional-group monocultures and all functional group mixtures.

## 5.1 Introduction

Semi-natural grasslands, originally used to supply forage for livestock production, also provide many ecosystem functions and are often important hotspots of biodiversity in rural areas. However, their species diversity often declines when the land-use changes, whether as a result of intensification or abandonment (Mitchley, 2001). In countries that have developed productive systems of livestock agriculture, the feed quality of forage from semi-natural grassland is generally inadequate to meet the nutritional requirements for high levels of milk output or rapid liveweight gain, and this has resulted in many grassland areas being abandoned by farmers (Isselstein *et al.*, 2005). For some regions in Germany, it has been estimated that up to one quarter of the permanent grassland area is likely to be abandoned in the near future (Rösch *et al.*, 2007). This is leading to a surplus of permanent grassland that requires regular management through grazing and/or cutting if its species richness (SR) is to be protected (Mitchley, 2001).

Grassland biomass that is no longer required to supply forage for animal production can be used for energy production without necessarily creating competition for food production and possible environmental drawbacks. Anaerobic digestion of extensive grassland biomass for biogas production is one conversion technique that can be used in the management of grassland. In providing a use for surplus grassland, it can contribute to maintaining biodiversity and, at the same time, create an economic incentive for farmers. Overall, in developed countries, the production of biogas from surplus grassland has a high potential for bioenergy (Prochnow *et al.*, 2009). On a European scale, 10.9 million tons of oil equivalents (Mtoe) were produced as biogas in the year 2010, which was a 31.3% increase compared with the previous year (EurObserv'ER, 2011). Among the substrates used for biogas production in Germany, grass silage is one of the most commonly used co-substrates (used in 40% of the biogas plants in Germany) after maize (which is used in 90% of all plants; Weiland, 2006; FNR, 2009). However, the percentage of extensive grassland contributing to 'grass silage' remains unspecified.

In Prochnow *et al.* (2009), more than fifteen European studies on biogas and methane yields from different types of grassland, grown under different abiotic conditions and with different management strategies, were summarized and compared. However, the subject of how SR or functional-group composition in extensive grassland may be linked directly with biogas potential has so far not been addressed. For farmers managing extensive grasslands within the framework of nature conservation programmes (e.g. Natura 2000), it can be important to

understand how changes in botanical diversity may affect the potential methane yield. This determines the associated economic value of the site beyond that provided by environmental support payments for nature conservation.

In a multi-site study with managed grassland, White *et al.* (2004) showed the relationship between measures of vegetation quality [such as crude protein (CP) and fibre content (measured as neutral detergent fibre)] and SR. In a summer and autumn cut, CP concentrations were lower in high-SR sites than in low-SR sites, while the fibre tended to be higher only in herbage at the second cut of high-SR sites. However, the results presented by White *et al.* (2004) show differences in biomass quality with different levels of SR as a result of site-dependent factors such as resource availability and therefore do not indicate SR effects per se. Yet, combining the observed relations between SR and concentrations of crude fibre (CF) and CP with what is known from research on methane yields of carbohydrates (including CF), CP and crude lipid (CL; Baserga, 1998), and from a study indicating a decline in plant-available N in the soil solution with increasing SR (Oelmann *et al.*, 2007), it can be expected that the substrate-specific methane yield ($CH_{4\ sub}$) would decline along a gradient of increasing SR. The $CH_{4\ sub}$ expresses the potential energy contained in the biomass that can be realized through anaerobic digestion, and it can be used as a measure for comparing the biogas potentials of different substrates. In addition to the potential effects of SR on $CH_{4\ sub}$, there is a lack of knowledge on the variation in $CH_{4\ sub}$ of grassland biomass caused by the differences in plant species composition (Prochnow et al., 2009).

If $CH_{4\ sub}$ declines with SR, the question arises of how the area-specific methane yield ($CH4_{area}$) will be affected by the negative SR–$CH_{4\ sub}$ relationship? In general, area-specific energy yield ($E_{area}$) is calculated as the product of biomass yield and substrate-specific energy yield ($E_{sub}$) and is important for assessing the potential value of a grassland site for bioenergy production. In a study on solid-fuel quality of grassland biomass for combustion and the effect of SR on $E_{area}$, it was shown that $E_{area}$ increased with SR due to a biomass yield increase, while $E_{sub}$ remained constant (Khalsa *et al.*, 2012). Thus, the aim of this study was to approach systematically the relationships of functional groups (presence/absence or abundance), as representatives of plant species with similar functional traits, and SR with (i) chemical constituents relevant for methane yield from anaerobic digestion, (ii) $CH_{4\ sub}$ and (iii) $CH_{4\ area}$ and annual $CH_{4\ area}$. The herbage biomass investigated in this study came from a large-scale biodiversity grassland experiment with artificial species mixtures set up along two diversity gradients: SR and functional group richness (FGR; Roscher *et al.*, 2004). Testing of

SR effects was given special attention in the design of the experiment, to disentangle as much as possible the effects of SR, FGR and the presence of individual functional groups. To address criticisms provoked by previous diversity studies, the choice of functional groups was also considered very carefully by giving special attention to forbs and legume species. Legumes were therefore regarded as a separate functional group, as they have been found to have disproportionate effects on ecosystem processes (Spehn *et al.*, 2002). Whereas previous biodiversity experiments have usually included three functional groups (grasses, herbs and legumes), it was decided in this experiment to further divide the herbs group into small herbs and tall herbs.

## 5.2 Material and Methods

### 5.2.1 Experimental design

In May 2002, an experimental site with semi-natural mesophilic grassland was established on the floodplain of the River Saale (near Jena, Thuringia, Germany, 50°55N, 11°35E, 130m a.s.l.). Mean annual air temperature in the Jena area is 9.3°C with an annual precipitation of 587 mm (Kluge *et. al.*, 2000). The site had originally been grassland and was converted into arable land around 1960. Soil conditions resemble a Eutric Fluvisol (FAO, 1994) and soil texture changes from silty clay to sandy loam with increasing distance from the river.

Sixty plant species were used to create a gradient in plant SR (1, 2, 4, 8, 16, and 60) and in FGR (1, 2, 3, and 4). Functional groups were defined, according to the morphological, phenological and physiological traits of the plant species, as grasses ($n = 16$), small herbs ($n = 12$), tall herbs ($n = 20$) and legumes ($n = 12$) (for a detailed list of plant species see Table A.1). Eighty-two plots (20 × 20m) were established in four blocks (Figure A.2), the blocks accounting for the differences in soil texture. Sixteen possible combinations of SR and FGR were realized and replicated over the four blocks (Figure 5.1). The location of the mixtures within each block was fully randomized. Management of the site was, as typical for semi-natural grassland under the actual site conditions, two cuts per year (late May and late August) and no application of fertilizer. Plots were weeded twice a year to maintain the original species composition. A full description of the experimental setup was published in Roscher *et al.* (2004).

```
           FGR 1            FGR 2           FGR 3    FGR 4

      60                                                    4
   16      1          1  1  1  1  1  1  1  1  1  4
    8      1  1  1  1  1  1  1  1  1  1  1  1  4
    4      1  1  1  1  1  1  1  1  1  1  1  1  4
    2      2  2  2  2  2  2  2  2
    1      4  4  4  4

          gr lg sh th  gr gr lg lg  gr lg lg lg lg
                       sh th sh th  sh gr gr sh gr
                                    th sh th th sh
                                                 th
```
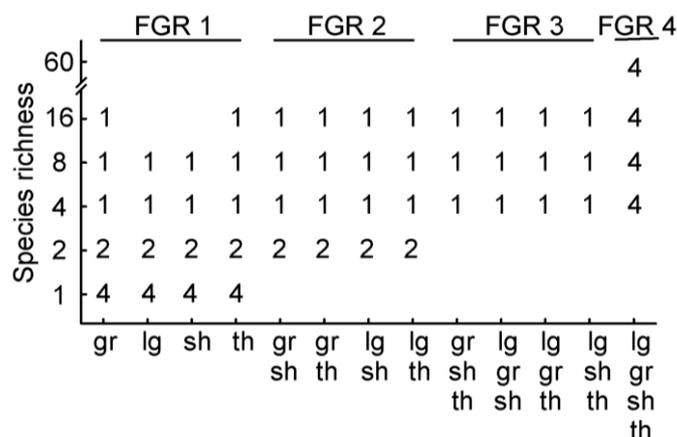
Species richness

**Figure 5.1** Combinations of functional groups and their replications (numbers) according to species-richness level. Each replication represents one of the plots used in this study; the total sum therefore is 82. The x-axis gives the different functional groups compositions (gr = grasses, lg = legumes, th = tall herbs and sh = small herbs). Functional-group richness (FGR) is shown on top.

### 5.2.2 Biomass sampling

Above-ground biomass was harvested twice in 2008 and 2009, in late May and late August (immediately before cutting). Three randomly placed samples of $20 \times 50$ cm were harvested 3 cm above soil surface. Biomass samples were separated into target species, dead plant material and weeds, dried (70°C, 48 h) and weighed. Mean total biomass (t DM ha$^{-1}$) was derived from the three samples. Annual biomass was calculated as the sum of biomass from the first and second cut. Furthermore 300g aboveground biomass was sampled from each plot ($n = 82$) for both years and both cuts (total $n = 164$) for forage quality analysis.

### 5.2.3 Forage-quality parameters

From each of the 164 biomass samples, the following were determined: crude ash (CA), by loss on ignition at 550°C, and CP, calculated from the nitrogen (N) content (CP = 6.25 × N) analysed with an elemental analyser (vario MAX CHN, Elementar Analysensysteme GmbH, Hanau, Germany).

For CF and CL, 100 of the 164 samples were analysed in the laboratory according to standard methods (Naumann and Bassler, 2004) and then used for near-infrared-calibration. With a near-infrared-spectroscope (XDS Rapid Content Analyser, FOSS NIRSystems Inc., Laurel, USA) all values for the 164 samples were predicted after cross-validation (CF: $R^2 = 0.96$, residual prediction deviation (RPD) = 3.68; CL: $R^2 = 0.74$, RPD = 1.70).

Nitrogen-free extract (NfE) was calculated as: NfE (% DM) = 100 – CA (% DM) – CF (% DM) – CL (% DM) – CP (% DM) and the sum of NfE and CF was assumed to represent the fraction of carbohydrates.

### 5.2.4 Estimating substrate-specific methane yield

To calculate $CH_{4\ sub}$ for this heterogeneous dataset, the guidelines of VDI 4630 (VDI, 2006) were used and modified to also account for the effects of botanical composition and date of harvest in the calculations. The method was based on the concentrations of the constituents CF, CL and CP (g $kg^{-1}$ DM), a digestibility coefficient ($D$) for each constituent and the potential biogas yield ($B$) of carbohydrates, CL and CP. $B$ values (which were estimated through stoichiometric equations) and the average methane content of the biogas *(M)* produced by the different constituents were taken from VDI 4630 (VDI, 2006) (Table 5.1). $CH_{4\ sub}$ is expressed in norm litres per kg volatile solids (VS) which refer to norm conditions of 273.2 K and 101.3 kPa:

$$CH_{4\ sub}\ (l_n\ kg^{-1}\ VS) = \ (CF \times D_{(CF)} + NfE \times D_{(NFE)}) \times B_{(CF+NfE)} \times M_{(CF+NfE)}$$

$$+ CL \times D_{(CL)} \times B_{(CL)} \times M_{(CL)} + CP \times D_{(CP)} \times B_{(CP)} \times M_{(CP)}$$

In the present study $D$ was calculated for each plot individually with respect to its dominant functional group, date of cut and the CF content of the total biomass. This was accomplished by linear regressions based on a dataset by University of Hohenheim (2007) (Table 5.2), which contains mean values derived from investigation ranging between 84 to 557 samples. The linear regressions were based on $n = 7$ values for the grass dominated plots and $n = 7$ values for the legume and herb dominated plots.

**Table 5.1** Maximum biogas production ($B$) of different chemical constituents (crude protein (CP), crude lipid (CL), carbohydrates) and average methane content ($M$) according to VDI 4630 (2004).

| Constituent | $B$ (l $kg^{-1}$ VS) | $M$ (%/100) |
|---|---|---|
| Carbohydrates (CF + NfE) | 746 | 0.50 |
| CL | 1390 | 0.72 |
| CP | 800 | 0.70 |

**Table 5.2** Linear regressions for grassland biomass to estimate digestibility values ($D$, in g kg$^{-1}$ DM) for chemical constituents [crude protein (CP), crude lipid (CL), crude fibre (CF) and nitrogen-free extract (NfE)] according to functional-group dominance (FGD), CF content (g kg$^{-1}$ DM) and cut.

| FGD | Constituent | Linear Regression | $R^2$ | RSE |
|---|---|---|---|---|
| Grass (> 70% of biomass), 1st cut | CF | $D_{(CF)} = -0.98 \times CF + 1023.4$ | 0.88 | 2.11 |
| | NfE | $D_{(NfE)} = -1.27 \times CF + 1078.2$ | 0.94 | 1.89 |
| | CL | $D_{(CL)} = 0.26 \times CF + 577.1$ | 0.80 | 0.78 |
| | CP | $D_{(CP)} = -1.07 \times CF + 964.8$ | 0.95 | 1.48 |
| Grass (> 70% of biomass), 2nd and further cuts | CF | $D_{(CF)} = -0.54 \times CF + 858.6$ | 0.99 | 0.18 |
| | NfE | $D_{(NfE)} = -0.52 \times CF + 830.2$ | 0.69 | 1.81 |
| | CL | $D_{(CL)} = -0.99 \times CF + 863.7$ | 0.70 | 3.36 |
| | CP | $D_{(CP)} = -0.85 \times CF + 894.5$ | 0.69 | 3.00 |
| Legumes + herbs (> 30% of biomass), 1st cut | CF | $D_{(CF)} = -1.75 \times CF + 1236.7$ | 0.95 | 2.03 |
| | NfE | $D_{(NfE)} = -2.42 \times CF + 1361.6$ | 0.98 | 1.80 |
| | CL | $D_{(CL)} = -0.14 \times CF + 619.1$ | 0.01 | 6.47 |
| | CP | $D_{(CP)} = -0.87 \times CF + 938.3$ | 0.91 | 1.33 |
| Legumes + herbs (> 30% of biomass), 2nd cut | CF | $D_{(CF)} = -1.21 \times CF + 1010.1$ | 0.99 | 0.30 |
| | NfE | $D_{(NfE)} = -1.67 \times CF + 1133.3$ | 0.99 | 0.01 |
| | CL | $D_{(CL)} = -1.02 \times CF + 734.0$ | 0.59 | 3.93 |
| | CP | $D_{(CP)} = -0.92 \times CF + 936.5$ | 0.98 | 0.59 |

## 5.2.5   Statistical analysis

The Jena Experiment was designed to vary SR, FGR and functional-group composition (FGC) as orthogonally as possible (Figure 5.1). However, a fully balanced design is not possible; for instance, the lowest SR cannot be combined with highest FGR. This is not an unusual situation in biodiversity experiments and can be approached by analysing the dependent variable in an analysis of variance (ANOVA) with sequential sum of squares (Schmid *et al.*, 2007). In this type of analysis variables that are fitted before others take up all the variation they can explain, ignoring the possibility that the later variables might also explain some of this variation (McCullagh and Nelder, 1999; Hector *et al.*, 2010). The characteristics of this type of analysis can then be used to identify effects that are independent of the variables fitted before.

To account for the gradient in soil conditions, block-wise weeding and mowing, as well as sampling, block effects were fitted first. It can therefore be assumed that all variance that is explained by variables fitted after the block effect is independent of it.

As the main focus of this study was on the effects of increasing SR this variable was fitted first after block. To test the effects of SR the log linear contrast of 1 to 16 species was used. The 60 species plots were used as a point of reference for highest possible diversity but were not included in the statistical analysis. Two plots had to be omitted from the data set

(one small herb monoculture plot and one tall herb monoculture plot) due to difficulties with their establishment.

To test for the effects of FGR and FGC, those two variables were fitted after SR in a first model. This initial model was performed as a repeated measure ANOVA to test for the effects of the two harvest dates. In the following steps, one for each cut, the presence/absence of functional groups was fitted after SR to test for their individual effects. All values used in the ANOVA were mean values of both years.

Multiple regression analysis was conducted (on all plots including SR = 60) to estimate the influence of functional-group abundance on forage quality and $CH_{4\,sub}$ by selecting the terms for inclusion in the model depending on standard statistical model selection methods (Draper and Smith, 1998). This implies that effect terms with $P < 0.05$ were included according to the rules of hierarchy and marginality (Nelder, 1994; Nelder and Lane, 1995). The marginality principle (Nelder and Lane, 1995) requires a term that appears as part of a more complex element in the model not to be tested for significance of its main effect, because the meaning of such terms is open to misinterpretation (Connolly and Wachendorf, 2001).

The initial model contained all functional-group abundance terms (lg, gr, sh, th) including their quadratic terms and all possible pairwise interactions. Functional-group abundances were tested for co-linearity (with *cor (x, y, method='pearson')* in R) prior to the analysis. The correlations between functional-group abundances were all within $-0.21 > r < -0.44$ and on average at $r = -0.33$ in both cuts.

All statistical analyses were done in R 2.14.1 (R Core Team, 2012).

## 5.3  Results

### 5.3.1  Forage quality with changing diversity

Crude fibre ranged from 157 to 369 g kg$^{-1}$ DM across all plant species compositions and the two harvest dates, with an overall mean of 251 g kg$^{-1}$ DM. Differences between the two cutting dates were observed, with CF content being higher in the first than in the second cut. Statistical analysis revealed that CF content was positively affected by SR in both cuts (Table 5.3), and increased in the first cut from 1 to 60 species by 65 g kg$^{-1}$ DM, and in the second cut by 34 g kg$^{-1}$ DM (Figure 5.2a, b). Furthermore, grasses had a strong increasing effect on CF in both cuts while legumes had a decreasing effect (Table 5.3). This is also reflected in the CF mean values of the functional-group monocultures (FGR = 1) which were highest for grasses

(Table 5.4). Predicting CF content from the functional-group abundance based on multiple regression analysis revealed that, in the first cut, few parameters (grass and small herb abundance) were necessary to achieve a model accuracy of $R^2 = 0.55$ (Table 5.5), whereas in the second cut, even with the full information of legume, grass and tall herb abundance, accuracy was much lower ($R^2 = 0.35$). Furthermore, multiple regression analysis revealed an interaction between grasses and small-herb abundance in the first cut (Figure 5.3a) while no interactions occurred in the second cut (Figure 5.3b).
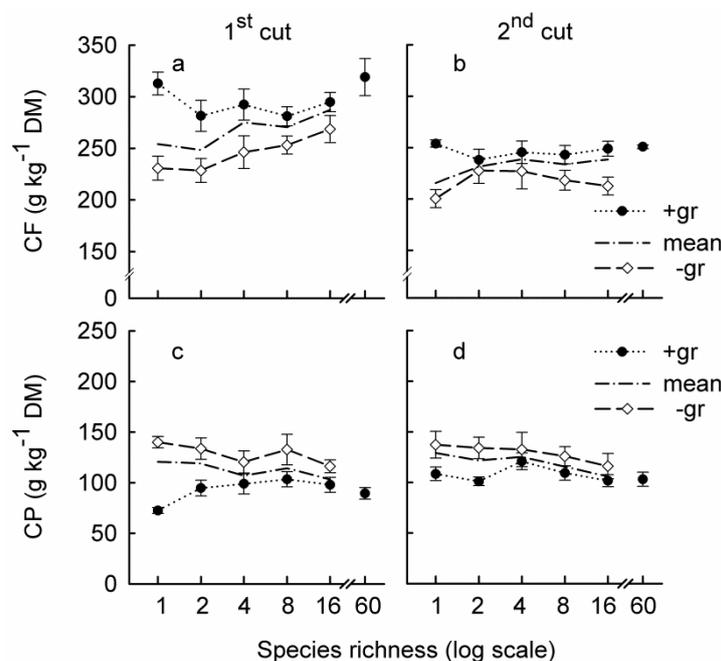


**Figure 5.2** (a, b) Crude fibre (CF) and (c, d) crude protein (CP) content with increasing species richness plotted separately for each cut. Data for grasses present (+gr: solid circles and dotted line) and grasses absent (−gr: open diamonds and dashed line) were plotted individually. Overall trend of mean values with increasing species richness was indicated by dash-dotted line. Values are means of both years. Error bars indicate standard error.

Crude protein, which was included as it is a major determinant of the CH4 sub (even though it was previously published as nitrogen, N = CP/6.25 in Khalsa *et al.* (2012)), ranged from 60 to 228 g kg$^{-1}$ DM with a mean of 115 g kg$^{-1}$ DM. Differences between the two cuts were small, so that similar effects were found in the statistical analysis (Table 5.3). The content of CP was negatively affected by SR and declined from 121 to 90 g kg$^{-1}$ DM in the first cut along the gradient of one to sixty species and from 129 to 103 g kg$^{-1}$ DM in the second cut respectively (Figure 5.2c, d). Legumes increased CP content, while grasses reduced it, which was again reflected in the mean values of the functional-group monocultures being highest for legumes and lowest for grasses (Table 5.4). The CP content could be accurately predicted ($R^2 = 0.60$) by legume, tall herb and small herb abundance in the first cut (Table 5.5)

including an interaction between legume and tall-herb abundance (Figure 5.3c). In the second cut legume abundance was sufficient to predict CP content with a $R^2 = 0.64$ (Figure 5.3d).
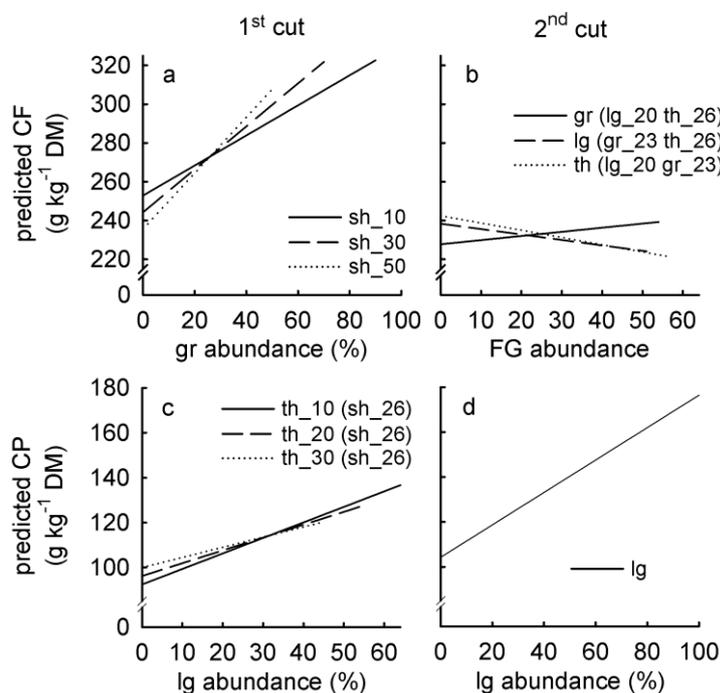


**Figure 5.3** Predictions of crude fibre (CF) and crude protein (CP) according to the multiple regression models in Table 5.5. (a) Interaction between small herbs (sh) and grasses (gr) affecting predicted CF in the first cut was plotted for three levels of sh abundance (10, 30 and 50 %). (b) The three functional groups affecting CF in the second cut were plotted individually along the respective functional group (FG) abundance while the other two functional groups were kept constant at their mean value. (c) Interaction between tall herbs (th) and legumes (lg) affecting predicted CP in the first cut was plotted for three levels of th abundance (10, 30 and 50 %) while sh was kept constant at its mean value. (d) lg abundance affecting CP in the second cut.

The values of CL were much lower than those of CF and CP and ranged between 11 to 36 g kg$^{-1}$ DM, with a mean of 21 g kg$^{-1}$ DM. The CL content was higher in the second cut than in the first, while statistical analysis revealed only few but non-consistent functional-group effects. Legumes, for example, were found to have an increasing effect in the first cut and a decreasing in the second, while grasses only had an increasing effect in the first cut (Table 5.3). The mean CL values in the functional-group monocultures in the first cut were relatively low in grasses compared with the other monocultures. In the second cut, grasses had the highest CL content relatively to the other functional-group monocultures (Table 5.4).

NfE ranged from 431 to 599 g kg$^{-1}$ DM with a mean of 519 g kg$^{-1}$ DM. The NfE means were not significantly different between the two cuts and, as with CL, statistical analysis revealed only few but non-consistent functional-group effects between the cuts. Small herbs increased NfE in the first cut, while in the second cut legumes decreased and tall herbs increased it (Table 5.3). Regarding the functional-group monocultures, in both cuts, legumes had the

lowest NfE values, whereas small herbs were highest in the first cut and tall herbs were highest in the second cut (Table 5.4).

**Table 5.3** Analysis of Variance of crude fiber (CF), crude lipid (CL), crude protein (CP) and nitrogen free extract (NfE) content (all in g kg$^{-1}$ DM) as function of diversity parameters (species richness (SR), functional group richness (FGR), functional group composition (FGC) and presence/absence of individual functional groups. (A) The overall effects of diversity and the significance of cut were tested. The effects of individual functional groups were tested separately for (B) first cut and (C) second cut. Arrows indicate increase (↑) or decrease (↓) with presence of respective functional group or with increasing SR.

| Factor | DF | CF | | CL | | CP | | NfE | |
|---|---|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P | F | P |
| (A) | | | | | | | | | |
| Block | 3 | 1 | 0.244 | 3 | 0.044 | <1 | 0.803 | 1 | 0.325 |
| Log (SR) | 1 | 17 | <0.001↑ | 3 | 0.093 | 14 | <0.001↓ | <1 | 0.983 |
| FGR | 1 | <1 | 0.852 | 1 | 0.233 | <1 | 0.653 | <1 | 0.822 |
| FGC | 11 | 9 | <0.001 | <1 | 0.997 | 15 | <0.001 | 2 | 0.152 |
| Residuals | 59 | | | | | | | | |
| Repeated measure | | | | | | | | | |
| Cut | 1 | 55 | <0.001 | 66 | <0.001 | 4 | 0.041 | 3 | 0.119 |
| Cut x Log (SR) | 1 | 2 | 0.225 | 1 | 0.413 | <1 | 0.582 | 6 | 0.015 |
| Cut x FGR | 1 | <1 | 0.677 | 1 | 0.422 | 3 | 0.112 | 2 | 0.132 |
| Cut x FGC | 11 | 2 | 0.018 | 6 | <0.001 | 3 | 0.003 | 1 | 0.384 |
| Residuals | 62 | | | | | | | | |
| (B) | | | | | | | | | |
| Block | 3 | 3 | 0.027 | 1 | 0.258 | 2 | 0.074 | 1 | 0.274 |
| Log (SR) | 1 | 12 | 0.001↑ | 1 | 0.407 | 5 | 0.037↓ | 4 | 0.050 |
| Legumes | 1 | 10 | 0.003↓ | 9 | 0.004↑ | 20 | <0.001↑ | <1 | 0.728 |
| Grasses | 1 | 30 | <0.001↑ | 21 | <0.001↓ | 35 | <0.001↓ | <1 | 0.964 |
| t. Herbs | 1 | <1 | 0.512 | 1 | 0.403 | 1 | 0.270 | 1 | 0.386 |
| s. Herbs | 1 | 11 | 0.001↓ | 1 | 0.459 | 1 | 0.370 | 6 | 0.022↑ |
| FGC | 8 | 3 | 0.009 | 1 | 0.239 | 5 | <0.001 | 1 | 0.562 |
| Residuals | 59 | | | | 0.258 | | | | |
| (C) | | | | | | | | | |
| Block | 3 | 3 | 0.032 | 7 | <0.001 | 2 | 0.100 | 1 | 0.298 |
| Log (SR) | 1 | 6 | 0.022↑ | 3 | 0.098 | 13 | 0.001↓ | 2 | 0.143 |
| Legumes | 1 | 8 | 0.007↓ | 6 | 0.022↓ | 90 | <0.001↑ | 3 | 0.094↓ |
| Grasses | 1 | 17 | <0.001↑ | 4 | 0.066 | 8 | 0.006↓ | 3 | 0.119 |
| t. Herbs | 1 | 9 | 0.004↓ | <1 | 0.511 | 2 | 0.189 | 5 | 0.023↑ |
| s. Herbs | 1 | 1 | 0.312 | 3 | 0.067 | 11 | 0.001↓ | <1 | 0.657 |
| FGC | 8 | 1 | 0.220 | 1 | 0.715 | 2 | 0.036 | 1 | 0.804 |
| Residuals | 59 | | | | | | | | |

**Table 5.4** Mean values (all in g kg$^{-1}$ DM) including standard deviation (SD) of crude fibre (CF), crude protein (CP), crude lipid (CL) and nitrogen free extract (NfE) in functional group (FG) monocultures as well as the all functional group mixtures. Values are means of both years.

| Parameter | | Grasses | | Legumes | | Small herbs | | Tall herbs | | All FG mixtures | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| CF | 1st cut | 321 | ± 18.2 | 235 | ± 37.2 | 218 | ± 24.7 | 258 | ± 35.7 | 283 | ± 36.2 |
| | 2nd cut | 262 | ± 9.3 | 208 | ± 23.7 | 229 | ± 34.7 | 200 | ± 22.7 | 237 | ± 21.7 |
| CP | 1st cut | 71.7 | ± 6.11 | 162 | ± 28.5 | 135 | ± 12.9 | 115 | ± 17.3 | 104 | ± 22.4 |
| | 2nd cut | 99.4 | ± 13.3 | 175 | ± 24.9 | 106 | ± 21.9 | 108 | ± 9.81 | 112 | ± 21.3 |
| CL | 1st cut | 16.4 | ± 2.63 | 20.8 | ± 3.00 | 21.2 | ± 1.39 | 20.2 | ± 2.32 | 19.1 | ± 2.23 |
| | 2nd cut | 29.3 | ± 3.70 | 23.0 | ± 3.90 | 22.5 | ± 6.02 | 22.8 | ± 2.71 | 22.6 | ± 3.91 |
| NfE | 1st cut | 523 | ± 17.9 | 509 | ± 18.7 | 539 | ± 24.0 | 521 | ± 33.6 | 517 | ± 18.6 |
| | 2nd cut | 506 | ± 16.6 | 503 | ± 29.1 | 522 | ± 19.0 | 545 | ± 32.0 | 524 | ± 16.2 |

**Table 5.5** Parameter estimates for the multiple regression analysis of crude fibre (CF), crude protein (CP) and substrate specific methane yield (CH$_{4 \text{ sub}}$). Abundances for legumes (lg), grasses (gr), tall (th) and small herbs (sh) are given as DM yield percentage.

| CF (g kg$^{-1}$ DM) | | | CP (g kg$^{-1}$ DM) | | | CH$_{4 \text{ sub}}$ (l$_n$ kg$^{-1}$ VS) | | |
|---|---|---|---|---|---|---|---|---|
| Effect | Estimate | *P* | Effect | Estimate | *P* | Effect | Estimate | *P* |
| 1st cut | | | | | | | | |
| INTERCEPT | 257.167 | <0.001 | INTERCEPT | 75.998 | <0.001 | INTERCEPT | 293.674 | <0.001 |
| gr | 0.609 | <0.001 | lg | 0.810 | <0.001 | lg | 0.222 | 0.021 |
| sh | -0.432 | <0.001 | th | 0.375 | <0.001 | gr | -0.225 | 0.012 |
| gr × sh | 0.017 | 0.005 | sh | 0.493 | <0.001 | sh | 0.437 | <0.001 |
| | | | lg × th | -0.012 | 0.016 | gr × sh | -0.017 | <0.001 |
| $R^2 = 0.55$ | RSE = 30.01 | | $R^2 = 0.60$ | RSE = 19.90 | | $R^2 = 0.53$ | RSE = 22.21 | |
| 2nd cut | | | | | | | | |
| INTERCEPT | 243.110 | <0.001 | INTERCEPT | 104.249 | <0.001 | INTERCEPT | 283.790 | <0.001 |
| lg | -0.280 | 0.011 | lg | 0.722 | <0.001 | lg | 0.333 | <0.001 |
| gr | 0.213 | 0.040 | | | | th | 0.336 | <0.001 |
| th | -0.373 | <0.001 | | | | sh | 0.110 | 0.078 |
| | | | | | | th × sh | -0.012 | 0.003 |
| $R^2 = 0.35$ | RSE = 26.54 | | $R^2 = 0.64$ | RSE = 17.96 | | $R^2 = 0.40$ | RSE = 16.01 | |

### 5.3.2 Methane yields with changing diversity

CH$_{4 \text{ sub}}$ yields of 234 to 387 l$_n$ kg$^{-1}$ VS, with an overall mean of 299 l$_n$ kg$^{-1}$ VS, could be estimated across all plant species compositions and the two harvest dates. Statistical analysis revealed no significant difference between the two cuts and showed similar functional-group effects (Table 5.6). In both cuts, SR had a decreasing effect on CH$_{4 \text{ sub}}$, which was more pronounced in the first than in the second cut (decrease from one to sixty species by 55 l$_n$ kg$^{-1}$ VS and by 26 l$_n$ kg$^{-1}$ VS respectively; Figure 5.4). Presence of legumes in both cuts, as well as small herbs in the first, and tall herbs in the second cut, increased CH$_{4 \text{ sub}}$. Presence of grasses, on the other hand, had a strong decreasing effect on CH$_{4 \text{ sub}}$, irrespective of the cut. This is

also reflected in the functional-group monocultures, where grasses had the lowest mean values in both cuts, while legumes had the highest (Table 5.7). $CH_{4\,sub}$ could be predicted by legume, small-herb and grass abundance in the first cut ($R^2 = 0.53$), whereas in the second cut, the model consisted of legumes, small herbs and tall herbs ($R^2 = 0.40$) (Table 5.5). The multiple regression models included an interaction between grasses and small herbs in the first cut (Figure 5.5a) and between tall herbs and small herbs in the second cut (Figure 5.5b).
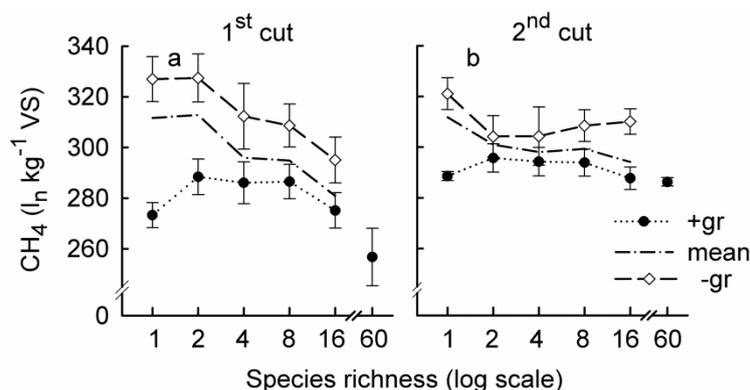


**Figure 5.4** $CH_{4\,sub}$ yields plotted with increasing species richness and separate for both cuts. Data was plotted separately for grasses present (+gr) and absent (–gr). Overall trend of mean values with increasing species richness was indicated by a dash-dotted line. Values are means of both years. Error bars indicate standard error.

$CH_{4\,area}$ had a mean value of 656 m$^3$ ha$^{-1}$ with a wide range from 131 to 1654 m$^3$ ha$^{-1}$, which was largely due to the variance in biomass yield of 0.5 to 7.6 t DM ha$^{-1}$. As the biomass yield between the two cuts was significantly different, with 3.2 t DM ha$^{-1}$ in the first cut and 1.4 t DM ha$^{-1}$ in the second cut, $CH_{4\,area}$ was 867 m$^3$ ha$^{-1}$ and 445 m$^3$ ha$^{-1}$ respectively.
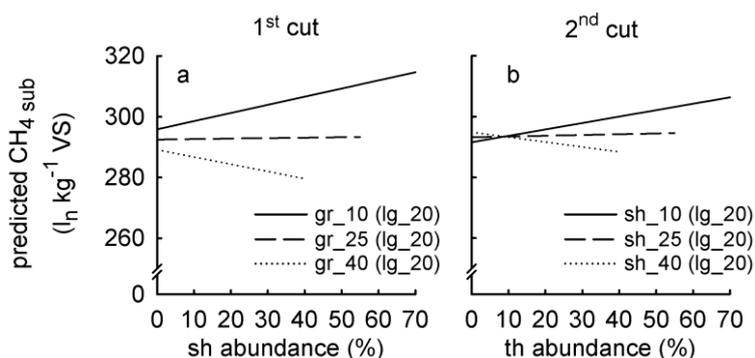


**Figure 5.5** Predictions of substrate specific methane yields ($CH_{4\,sub}$) according to the multiple regression models in Table 5.5. (a) Interaction between small herbs (sh) and grasses (gr) affecting predicted $CH_{4\,sub}$ in the first cut plotted for three levels of gr abundance (10, 25 and 40 %) while legumes (lg) were kept constant at their mean. (b) Interaction between tall herbs (th) and sh affecting $CH_{4\,sub}$ in the second cut plotted for three levels of sh abundance (10, 25 and 40 %) while lg was kept constant at its mean.

Due to the close relationship between biomass yield and $CH_{4\,area}$ ($R^2 = 0.97$), the effects found in the statistical analysis were very similar (Table 5.6). There was a positive effect of SR in both cuts, with $CH_{4\,area}$ increasing from one to sixty species by 752 m$^3$ ha$^{-1}$ and by 296 m$^3$ ha$^{-1}$, in the first and second cut respectively (Figure 5.6). Legume presence increased $CH_{4\,area}$

in both cuts, and this was also reflected in the mean values of the functional-group monocultures, where legumes had the highest $CH_4$ $_{area}$ in the second cut and the second-highest value in the first cut (Table 5.7).

The annual $CH_4$ $_{area}$ also had a wide range (378 to 2595 $m^3$ $ha^{-1}$ $year^{-1}$) with a mean of 1292 $m^3$ $ha^{-1}$ $year^{-1}$. It was significantly affected by SR ($P < 0.001$) from one to sixty species, it increased by more than 100% from 979 to 2027 $m^3$ $ha^{-1}$ $year^{-1}$ (Figure 5.6). The presence of legumes had a strong effect ($P < 0.001$) on annual $CH_4$ $_{area}$, which was in line with the patterns found in the functional-group monocultures where legumes had the highest annual $CH_4$ $_{area}$ (Table 5.7).

**Table 5.6** Analysis of Variance of substrate specific methane yield ($CH_4$ $_{sub}$), biomass yield and area specific methane yield ($CH_4$ $_{area}$) as function of diversity parameters (species richness (SR), functional groups richness (FGR), functional group composition (FGC) and presence/absence of individual functional groups. (A) The overall effects of diversity and the significance of cut were tested. The effects of individual functional groups were tested separately for (B) first cut and (C) second cut. Arrows indicate increase (↑) or decrease (↓) with presence of respective functional group or with increasing SR.

| Factor | DF | $CH_4$ $_{sub}$ ($ln$ $kg^{-1}$ VS) | | Biomass yield (t $ha^{-1}$) | | $CH_4$ $_{area}$ ($m^3$ $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| (A) | | | | | | | |
| Block | 3 | 1 | 0.458 | 3 | 0.065 | 2 | 0.069 |
| Log (SR) | 1 | 31 | <0.001↓ | 31 | <0.001↑ | 26 | <0.001↑ |
| FGR | 1 | 1 | 0.332 | 2 | 0.124 | 2 | 0.143 |
| FGC | 11 | 9 | <0.001 | 2 | 0.014 | 3 | 0.006 |
| Residuals | 59 | | | | | | |
| Repeated measure | | | | | | | |
| Cut | 1 | <1 | 0.680 | 192 | <0.001 | 188 | <0.001 |
| Cut x  Log (SR) | 1 | 3 | 0.079 | 10 | 0.002 | 7 | 0.010 |
| Cut x FGR | 1 | <1 | 0.943 | <1 | 0.613 | <1 | 0.690 |
| Cut x FGC | 11 | 3 | 0.010 | 2 | 0.045 | 2 | 0.120 |
| Residuals | 62 | | | | | | |
| (B) | | | | | | | |
| Block | 3 | 3 | 0.037 | 3 | 0.048 | 3 | 0.040 |
| Log (SR) | 1 | 19 | <0.001↓ | 30 | <0.001↑ | 24 | <0.001↑ |
| Legumes | 1 | 6 | 0.016↑ | 9 | 0.004↑ | 13 | 0.001↑ |
| Grasses | 1 | 29 | <0.001↓ | 1 | 0.378 | <1 | 0.989 |
| t. Herbs | 1 | <1 | 0.789 | <1 | 0.957 | <1 | 0.959 |
| s. Herbs | 1 | 9 | 0.004↑ | 2 | 0.129 | 2 | 0.218 |
| FGC | 8 | 2 | 0.021 | 2 | 0.091 | 2 | 0.164 |
| Residuals | 59 | | | | | | |
| (C) | | | | | | | |
| Block | 3 | 4 | 0.010 | 4 | 0.015 | 3 | 0.027 |
| Log (SR) | 1 | 9 | 0.004↓ | 16 | <0.001↑ | 14 | 0.001↑ |
| Legumes | 1 | 12 | 0.001↑ | 17 | <0.001↑ | 21 | <0.001↑ |
| Grasses | 1 | 17 | <0.001↓ | <1 | 0.826 | <1 | 0.872 |
| t. Herbs | 1 | 6 | 0.021↑ | 1 | 0.471 | <1 | 0.567 |
| s. Herbs | 1 | 3 | 0.088 | 1 | 0.389 | 1 | 0.297 |
| FGC | 8 | 1 | 0.311 | 1 | 0.668 | 1 | 0.679 |
| Residuals | 59 | | | | | | |
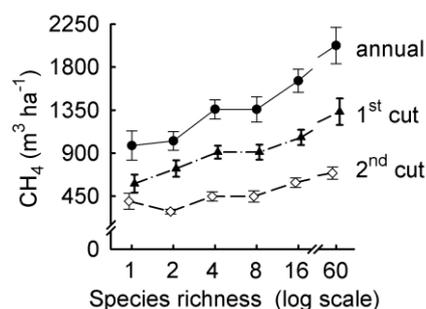
**Figure 5.6** CH$_4$ $_{area}$ yield, separate for both cuts, and annual CH$_4$ $_{area}$ yield plotted with increasing species richness. Values are means of both years. Error bars indicate standard error.

**Table 5.7** Substrate specific (CH$_4$ $_{sub}$), area specific (CH$_4$ $_{area}$) and annual CH$_4$ $_{sub}$ yield of the different functional-group (FG) monocultures and the all-functional-group mixtures. Values are means of both years.

| Methane yield | Grasses | | Legumes | | Small herbs | | Tall herbs | | All FG | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| CH$_4$ $_{sub}$ (l$_n$ kg$^{-1}$ VS) | | | | | | | | | | |
| 1st cut | 270 | ± 8.4 | 325 | ± 30.7 | 336 | ± 19.1 | 304 | ± 27.7 | 284 | ± 27.5 |
| 2nd cut | 289 | ± 4.1 | 321 | ± 16.2 | 300 | ± 24.5 | 317 | ± 14.4 | 296 | ± 14.7 |
| CH$_4$ $_{area}$ ( m$^3$ ha$^{-1}$) | | | | | | | | | | |
| 1st cut | 876 | ± 180 | 753 | ± 394 | 608 | ± 394 | 623 | ± 348 | 1113 | ± 316 |
| 2nd cut | 384 | ± 140 | 565 | ± 372 | 257 | ± 64 | 268 | ± 84 | 562 | ± 207 |
| annual | 1260 | ± 221 | 1317 | ± 696 | 865 | ± 413 | 892 | ± 340 | 1674 | ± 487 |

## 5.4  Discussion

### 5.4.1   Chemical constituents affecting anaerobic digestion and CH$_4$ $_{sub}$

Biogas production is influenced by many factors such as temperature in the digestion plant, pH value of the digestate, diversity of microorganisms and concentration of trace elements (Weiland, 2010). Furthermore, the actual methane yield is influenced by the retention time, type of digestion system and substrate quality of the feed. An ideal substrate for biogas production should be rich in degradable carbohydrates (e.g. sugars), lipids and proteins and at the same time be poor in hemicelluloses and lignin (El Bassam, 1998).

In general, high fibre contents limit energy availability from forages (Buxton and Redfearn, 1997) and, in particular, high lignin contents are not suitable for biogas production (Zubr, 1986). Chopping of the harvested biomass, as for instance in the preparation of material for ensiling, helps to break down the lingo-cellulose structures in the fermenter and may lead to a better digestibility of the fibre fraction (Prochnow *et al.*, 2009b).

High protein concentrations, on the other hand, may lead to process failure due to an accumulation of ammonia, whereas a balanced C/N ratio of 25 is expected to gain the highest methane yields (Zubr, 1986). Calculated from the data presented in Khalsa *et al.* (2012a), the

C/N ratio of the all-functional-group mixtures (first cut: 27.6; second cut: 24.5) was near the optimum.

A consideration of forage quality in terms of its quality requirements for ruminant nutrition may provide a first simple approach to estimate whether a substrate is valuable for energy production via anaerobic digestion. For dairy production, the forage quality of grassland biomass is regarded as low at CF values of 350-400 g kg$^{-1}$ DM and CP values of approximately 63 g kg$^{-1}$ DM, while CF between 150-200 g kg$^{-1}$ DM and CP of 180-220 g kg$^{-1}$ DM would indicate high quality (Peeters, 2009). However, anaerobic digestion in the digestion tract of ruminants may differ from anaerobic digestion in a biogas plant. Biogas plants can degrade cellulose to a much greater extent than ruminants, partly because the digestion time in biogas plants is longer and the population of microorganisms is different (Amon *et al.*, 2007).

### 5.4.2 Crude fibre

In this study, herbage from all the functional-group monocultures would, based on their mean CF values, fall within an intermediate forage-quality category, according to Peeters (2009) and were in the range of values found by Richter *et al.* (2009) across five semi-natural grasslands typical for European mountainous regions. The results also showed that grass monocultures had higher fibre contents than, for example, legume species, which confirms the findings on fibre content of different plant species reported by Buxton and Redfearn (1997). Furthermore, the presence of grasses had an effect of increasing the CF across all mixtures, while that of legumes had a decreasing effect (Table 5.3).

In previous work, NDF (closely correlated with CF) was found to be higher in high SR plots than in low SR plots in the autumn cut of a multi-site grassland experiment, whereas no significant effect was found in the summer cut (White *et al.*, 2004). The slope for CF with increasing SR found in this study was positive in both cuts. White *et al.* (2004) attributed both the variation in biomass quality and the variation in SR to differences in nutrient availability in the soil. In the Jena Experiment, the initial variance in soil properties across the experimental site was accounted for in the statistical analysis and therefore, the positive slope of the SR-CF relation can be attributed to species diversity and its effects on, for instance, soil nutrient availability.

It is often argued that SR effects are due to the so called sampling effect (e.g. the probability of including more highe-yielding species with increasing SR, which then leads to higher

biomass). If this were true, it would be reflected, amongst other factors, in the dominance structure of the plots; however, Khalsa *et al.* (2012a) showed no notable dominance structure with increasing SR. Furthermore, the independence of SR from functional-group presence/absence could be proven by fitting each functional-group term individually before SR in the hierarchy of the statistical model. It turned out that SR was not affected by any functional-group term except for grasses, which cancelled the significance of SR (Figure 5.2). Thus, it appears that the existing effect of SR on CF is dependent on the presence of grasses in the grassland mixture. However, this might be partly due to the characteristics of the presence/absence approach for grasses in the Jena Experiment. When the SR relation was plotted for grasses present (+gr) and grasses absent (–gr), it revealed, especially at low SR levels, an undesired bias (Figure 5.2). While the mean value over all the one-species plots of –gr contains small-herb, tall-herb and legume monocultures, +gr is solely a mean value of the different grass species sown. It is apparent that the mean of –gr will be lower in CF than the +gr mean, as it contains all the non-grass species which are known to have lower CF contents than grasses (Buxton and Redfearn, 1997). In the +gr plots with SR = 2, grasses are no longer in monoculture but are in combination with either of the other functional groups. Thus, differences between +gr and –gr were less pronounced at this SR level and, consequently, they declined further with increasing SR. Thus, it can be assumed that the SR-CF relation at SR >2 is no longer intermingled with the presence of grasses. In the second cut, however, the 2- and 4-species plots of +gr and –gr have very similar mean values and start to diverge again with increasing SR.

The prediction of CF content from functional group abundance, which was generated through multiple regression analysis, included an interaction between grass and small-herb abundance in the first cut (Table 5.5). Plotting this interaction and its effect on predicted CF ($CF_{pred}$) content revealed that increasing small-herb contents led to a steeper slope for $CF_{pred}$ with increasing grass abundance (Figure 5.3a). This may reflect stress due to competition between plants of different functional groups but also within a functional group. Competition could occur in the form of small growing species being forced to grow taller, in order to intercept more light in the presence of more tall growing species. This in turn would increase fibre contents, as it is known that fibre and especially lignin plays an important role for the strength and the rigidity of plant walls (Buxton and Redfearn, 1997). In the second cut, however, no interactions between functional groups could be observed and, thus, the three functional group abundances affected $CF_{pred}$ independently (Figure 5.3b).

Regarding multiple regression analysis on ecological data, Graham (2003) suggested that co-linearity between factors can cause problems with the interpretation of the results if $r > 0.28$. As $r$ was on average 0.33 between the functional-group abundances, the results of the multiple regression analysis may not be entirely free of bias. However, the presented regression models help to interpret possible mechanisms driving biomass quality that are reasonable from a biological point of view.

### 5.4.3   Crude protein

According to Peeters (2009), the functional-group monocultures of grasses would be considered to be of low quality, whereas legume monocultures almost reach high-quality standards. The higher CP content of herbage from legume monocultures relative to grass monocultures is widely known (Paulson *et al.*, 2008; Posler *et al.*, 1993) and this was also supported by the significant functional-group effects (Table 5.3).

For biogas production, CP is an important constituent, as it allows relatively high specific methane yields (800 $l_n$ $kg^{-1}$ VS) and methane contents in the biogas (70%), as well as high decomposition rates (VDI, 2006). The overall mean CP value in this study was in the range of values found by Richter *et al.* (2009).

Similar to the results of an earlier grassland study with multiple sites (White *et al.*, 2004), CP was lower when SR was high. However, in the study by White et al. (2004), nutrient availability was found to be the driver for biomass quality and SR. In other studies, high nutrient inputs led to low SR, with only a few species dominating the vegetation, and low nutrient inputs gave room for the coexistence of a larger pool of species (Mitchley, 2001; Vermeer and Berendse, 1983). In the Jena Experiment, SR was part of the experimental design, and as no fertilizers were applied, the differences in nutrient availability could be considered to have occurred in response to the SR level. In an earlier study on the Jena Biomass, it was found that the plant-available N in the soil solution decreased with increasing SR (Oelmann *et al.*, 2007). In other words, nutrient availability was a result of the competition of a certain number of species for the available nutrients. Thus it was not surprising to find that nitrogen (N = CP/6.25) availability was lower in high-species plots than in plots with a low number of species.

To better understand the SR impact on CP, it was tested for any dependency on functional-group presence/absence. When legumes were fitted in the model before SR, no changes in the significance of the SR effect were observed. In contrast, fitting grass presence/absence first

cancelled its significance (Figure 5.2). Thus, it appears that the existing SR effect on CP is dependent on the presence of grasses in the grassland mixture. However, this might again be due to the characteristics of the presence/absence of grasses as discussed for CF. Here, the mean value of all the one-species –gr plots is much higher in CP than the +gr plots. This was due to the higher CP concentrations in the herb and legume monocultures as compared to the grass monocultures (Table 5.4). The legume species may have played an important role, as they can supply the system with additional N through atmospheric-N fixation, and high concentrations of N in their tissue are released into the soil during their decomposition (Tomm *et al.*, 1995). In both cuts the 4-, 8- and 16- species plots of +gr and –gr are clearly different in magnitude but now follow the same pattern. Thus, it can be concluded that the negative SR-CP relation is independent of the presence of grasses at SR >2.

A significant interaction between tall herbs and legumes occurred for predicted CP ($CP_{pred}$) in the first cut (Table 5.5). To visualize this interaction, $CP_{pred}$ was plotted over a range of legume abundances at different levels of tall-herb abundance, while small-herb abundance was kept constant at its mean (Figure 5.3c). With increasing proportion of tall herbs, the slope of $CP_{pred}$ was lower, which means that the increasing effect of legumes on CP was weakened. This may indicate a lower N-fixation by legumes due to the higher abundance of tall-growing herbs. Regarding the growth of N-fixing species, it is known that shading may limit their growth in relation to non-fixing species, and thus, N-limitation in an ecosystem may be stronger (Vitousek and Field, 1999). This is further supported by the findings that N-fixing plants have difficulties growing through communities with dense canopy structure (Bormann and Gordon, 1984). However, N-fixation by legumes was not measured in this study and cannot be identified as the driver of the legume – tall herb interaction.

### 5.4.4 Crude fat and nitrogen-free extract

Regarding anaerobic digestion, CL has the highest biogas yield of all chemical constituents [1390 $l_n$ $kg^{-1}$ VS, according to VDI 4630 (VDI, 2006)] but the slowest conversion rates due to low bioavailability (Weiland, 2010). Richter *et al.* (2009) found a mean CL value that was slightly lower than found here, yet the grass monoculture value in the first cut was similar to theirs. In general, there was no large variance between the other functional-group monocultures and the two cuts except for grasses, which had almost double the amount of CL in the second than in the first cut. This may be due to the rather inaccurate prediction of CL by NIRS ($R^2$ = 0.74, RPD = 1.70). However, estimated $CH_4$ $_{sub}$ according to VDI 4630 (VDI,

2006) was barely affected by the CL content, especially in the second cut ($R^2 = 0.03$), which is not surprising, as CL contributed on average to only 2.1% DM of the total biomass.

NfE, even though it contributes on average to 51.9% DM of the total biomass, affects the biogas production as part of the carbohydrate fraction together with CF. The variance between the functional-group monocultures was small, and there was no significant trend with SR.

### 5.4.5 Substrate-specific methane yields

$CH_{4\ sub}$ was higher than $CH_{4\ sub}$ as estimated by Richter *et al.* (2009) across five different extensive grassland sites, probably due to lower CP values, while CF was similar to the values found here. Gerstl (2008)(also cited in Prochnow *et al.*, 2009) measured $CH_{4\ sub}$ in a batch experiment on extensive grassland silage of 290 $l_n$ $kg^{-1}$ VS in the first cut (June) and 299 $l_n$ $kg^{-1}$ VS in the second cut (October), which is very similar to the here presented data.

Richter *et al.* (2009) compared values from batch experiments with values calculated according to VDI 4630 (VDI, 2006) and found a close relation ($R^2 = 0.99$). However, their experiment consisted of five different plant communities, whereas the data in the study presented here were obtained from a total of 82 different communities. The digestibility data of University of Hohenheim (2007) are limited to two types of grassland communities (grass-rich and grass-poor) and several stages of maturity. Further digestibility values had to be extrapolated to account for all the occurring mixtures in the Jena Experiment (Table 5.2). This is a potential source of error that would have to be specified by digestibility experiments on the specific plant communities. Nevertheless, the reasonable values obtained from this approach, as compared to values from batch experiments, support the method.

The increasing effect of legume presence on $CH_{4\ sub}$ (Table 5.6) corresponds to reduced CF and increased CP contents (Table 5.3). The opposite is true for grasses, which increased CF and decreased CP and thereby affected $CH_{4\ sub}$ negatively.

The decrease in $CH_{4\ sub}$ with increasing SR is in line with the patterns of CF and CP values changing along the SR gradient and confirms the hypothesis stated in the Introduction.

To test whether the relationship of $CH_{4\ sub}$ and SR was dependent on any functional-group presence/absence, the sequence of model fitting was changed. No functional group fitted before SR in the first cut had any effect on its significance, while fitting grasses before SR in the second cut cancelled the significance of the SR effect. This can be explained following the same line of argumentation as for CF and CP. The slope of +gr and −gr in the first cut of the

high SR plots (>2) is very similar and the SR-CH$_4$ $_{sub}$ relation can be considered a true SR effect at SR > 2 (Figure 5.4). In the second cut, the slopes at high SR kept diverging, and the SR effect was dependent on the presence/absence of grasses over the entire range of SR (Figure 5.4). Taking a closer look at the second cut, it appears that, in the absence of grasses, increasing SR has a slight increasing effect on CH$_4$ $_{sub}$ (at SR >1). This is contrary to the first cut and may result from the slight decline in CF with increasing SR in the −gr plots of the second cut (Figure 5.2b).

Multiple regression analysis revealed interactions between functional-group abundances that may further shed light on the relationships between species composition and CH$_4$ $_{sub}$ (Figure 5.5). In the first cut, the strong increasing effect of small herbs on predicted CH$_4$ $_{sub}$ was only noticeable when grass abundance was low. It is possible that the imposed competition on small herbs with increasing grass abundance caused a decline in CH$_4$ $_{sub}$. Thus, as soon as there were more than 10% grasses present, the small herbs had to grow taller in order to intercept more light and thereby strengthen their vertical growth through fibre and possibly lignin structures (Buxton and Redfearn, 1997). In the second cut, a similar interaction could be observed between small and tall herbs. According to the multiple regression analysis, tall herbs had a highly significant and increasing effect on predicted CH$_4$ $_{sub}$, while small herbs, as main effect, had no significant effect. Yet, with increasing abundance of small herbs, the increasing effect of tall herbs ceased, and the reason for this may be due to the same competition mechanisms as described above.

### 5.4.6 Area-specific methane yields

The wide range of values amongst the biomass yield and CH$_4$ $_{area}$ of all plots was due to the fact that especially the low species-number communities were kept off equilibrium through weeding, and thus, they had a low biomass, while the sixty-species plots could utilize most of the available ecological niches for biomass production. The advantages and disadvantages of such an approach were discussed in Khalsa *et al.* (2012a).

The differences in CH$_4$ $_{area}$ between the two cuts were comparable with the results presented by Amon *et al.* (2007) for a two cut hill site where the first cut contributed about 65% to the annual CH$_4$ $_{area}$, while the second cut only contributed 35%. However, in their study, the annual biomass yield was higher (6.4 t DM year$^{-1}$) while CH$_4$ $_{sub}$ was considerably lower (first cut: 171 l$_n$ kg$^{-1}$ VS; second cut: 128 l$_n$ kg$^{-1}$ VS) than the yields in this study.

As revealed by the statistical analysis (Table 5.6), legumes had a strong effect on $CH_{4\ area}$. This was partly due to legumes increasing $CH_{4\ sub}$ but much more so due to legumes increasing biomass yield. It can be assumed that this was not simply a sampling effect but rather a combination of facilitating plant growth of other species and promoting complementary resource use amongst species, as discussed in Khalsa *et al.* (2012a). Estimated mean annual $CH_{4\ area}$ was similar to data found by Gerstl (2008), who measured biomass from a two-cut Alpine hill site. The strong positive slope of the SR-annual $CH_{4\ area}$ relationship was mainly driven by the SR-biomass relationship. The first cut especially showed a strong SR-$CH_{4\ area}$ relationship, whereas it was less pronounced in the second cut (Figure 5.4). The negative SR-$CH_{4\ sub}$ slope was thus compensated by the positive SR-biomass slope.

Focusing on the most relevant SR levels in extensive grasslands (8 to 16 to 60 species), annual $CH_{4\ area}$ still increased by 669 $m^3$ $ha^{-1}$ $year^{-1}$ from an eight- to sixty- species plot and by 370 $m^3$ $ha^{-1}$ $year^{-1}$ from a sixteen- to a sixty-species plot. The range found in this study from an eight-species plot (1358 $m^3$ $ha^{-1}$ $year^{-1}$) to a sixty-species plot (2027 $m^3$ $ha^{-1}$ $year^{-1}$) is comparable with values obtained by Gerstl (2008), measured for a two-cut hill site (1580 $m^3$ $ha^{-1}$ $year^{-1}$) and a four-cut valley site (2359 $m^3$ $ha^{-1}$ $year^{-1}$).

## 5.5 Conclusions

Chemical constituents most relevant for methane production from anaerobic digestion were successfully investigated along experimental diversity gradients. The results provide a novel insight into plant diversity-bioenergy relations. Methane yields could be derived from the concentrations of chemical constituents and reported DM digestibility, and these yield measures could be used to express the bioenergy potential of this experimental grassland biomass, which is a type that could occur in extensive grassland managed for nature conservation purposes.

Several effects of SR on digestion-relevant parameters could be found: (i) CP decreased with increasing SR, while CF increased; however, these two effects were not fully disentangled from the presence of grasses, (ii) increasing SR resulted in lower $CH_{4\ sub}$ and (iii) increasing SR increased $CH_{4\ area}$. Functional groups, especially legumes and grasses, played key roles in the variance of chemical constituents and $CH_{4\ sub}$ respectively.

The results of this study suggest that for a two-cut extensive management, it is important to maintain legume abundance, as legumes affects $CH_{4\ sub}$ directly and affect $CH_{4\ area}$ through positive feedback on biomass yield.

# 6 Solid fuel production from extensive grassland biomass through hydrothermal conditioning and mechanical dewatering: effects of species richness and functional group composition

**Abstract**      The potential of extensive grassland biomass for energy production through combustion has been investigated in a multitude of studies. It has been proposed that their solid fuel quality needs improvement for a risk free utilization in commercial combustion plants. The 'Integrated generation of solid Fuel and Biogas from Biomass' (IFBB) improves solid fuel quality through hydro-thermal conditioning followed by mechanical dewatering of the biomass prior to combustion. As extensive grasslands widely vary in plant species composition and richness their solid fuel quality does too. To assess possible effects of species richness (SR) and functional group composition on the solid fuel quality after applying the IFBB procedure, we analysed the press-cake (PC) of different experimental grassland communities that were established along two diversity gradients, SR (1 to 60 species) and functional group presence (grasses, legumes, small herbs and tall herbs), regarding ash, N, Cl, S, K, Ca and Mg content, as well as ash softening temperature (AST), higher heating value (HHV) and gross energy yield (GE).

Besides reducing the concentrations of chemical constituents and bringing them below critical levels (except for N), the most important findings were: (i) SR had no effect on concentrations of the chemical constituents in the PC, (ii) functional group composition should be well balanced between grass and non-grass species for a reduced risk of emissions and corrosion and a high AST, and (iii) regarding the energy output, SR was highly relevant and GE doubled from 1 to 60 species.

## 6.1 Introduction

Extensive grassland cut has an energy content of approximately 17-19 MJ kg$^{-1}$ DM which can be utilized through combustion. Depending on the management strategy, one cut per year can accumulate biomasses of 0.6 to 11 t DM ha$^{-1}$ year$^{-1}$ (as found by a study on three different regions in Europe; Hensgen *et al.*, 2012) and a two cut system with varying species diversity can range between 0.2 and 8.1 t DM ha$^{-1}$ year$^{-1}$ (as observed in an experimental grassland study on one site; Khalsa *et al.*, 2012a). Especially the high yielding sites may provide enough biomass in order to make a conversion into energy feasible for farmers, while the fate of the low yielding sites depends on nature conversation schemes and governmental subsidies.

However, biomass yield and energy content are not the only factors determining how efficient a conversion of their biomass to utilizable energy will be. The ash content together with the content of emission and corrosion related constituents are further relevant factors affecting solid fuel quality and thus affecting the longevity of the combustion plant and deciding what type of plant and filters are required.

From an ecological point of view the value of extensive grassland is high as it provides ecosystem services, i.e. nature and species conservation, groundwater collection, prevention of erosion and soil degradation (Wilkins and Vidrih, 2000). Beyond that extensive grasslands are valuable to society due to their aesthetics and recreational functions.

In regard of the solid fuel quality, however, biomass from extensive grassland is less than ideal, having high ash and mineral concentrations and high concentrations of unfavourable constituents such as N, S and K. Because of that, efforts have been made to improve solid fuel quality of such biomasses by (i) pre-treating the substrate prior to combustion through hydro-thermal conditioning and mechanical dewatering (Richter *et al.*, 2010, 2011), which is part of a procedure known as the Integrated generation of solid Fuel and Biogas from Biomass – IFBB, or (ii) simply by delaying harvest (e.g. harvesting biomass in February; Tonn *et al.*, 2007), which causes natural leaching through precipitation and loss of mineral-rich matter such as leaf biomass. To reduce mineral concentrations through delayed harvest will result in similar fuel quality improvements as by applying the IFBB procedure, but may cause large biomass losses in the field of 30-70% due to particle breakdown, depending on the substrate and climatic condition (Christian *et al.*, 2002; Tonn *et al.*, 2007). Furthermore, harvesting the material in late winter or early spring may be difficult due to lodging, snow fall and difficult hay drying conditions.

The IFBB-procedure (Wachendorf *et al.*, 2009) may therefore be a preferable approach to enhance solid fuel quality of extensive grassland biomass and has so far been studied for suitability of different substrates (Hensgen *et al.*, 2011; Bühle *et al.*, 2012a) most efficient process temperature (Wachendorf *et al.*, 2009), harvest date (Richter *et al.*, 2011), economic feasibility (Blumenstein *et al.*, 2012) and environmental performance (Bühle *et al.*, 2012b).

In a study by Khalsa *et al.* (2012a) the variation in concentrations of constituents relevant for combustion, as driven by plant species richness (SR) and functional group presence, has been outlined. Yet, the diversity effects were all observed in a range of concentrations that would not be suitable for commercial biomass combustion. Only through an improvement of the fuel quality these biomasses could be considered for combustion in modern plants. Thus, the question arises, how will a procedure such as IFBB affect the biomass from different plant communities and will diversity effects still be relevant after its application?

The biomass we used to approach these questions came from a large-scale biodiversity experiment, known as the Jena-Experiment, which consists of artificially assembled plant mixtures that represents extensive grassland communities (Roscher *et al.*, 2004). In the design of the experiment the choice of functional groups was approached very carefully by dividing the non-grass species into legumes, small herbs and tall herbs. At the same time, the testing of SR effects was given special attention, allowing us to disentangle the effects of SR, functional-group richness (FGR) and the presence of individual functional groups as much as possible.

Thus, we investigated (i) if the solid fuel improvement (reduction in chemical constituents) through IFBB was significant, (ii) if the mass-flows of different combustion related constituents from the biomass of the Jena-Experiment into its press-cake (which is one product of the IFBB procedure and can later be pelletized and used in e.g. commercial heating plants) varied along the diversity gradients, and (iii) if the concentrations of the most relevant constituents in the press-cake varied along the diversity gradients.

## 6.2 Materials and methods

### 6.2.1 Experimental design

In May 2002 an experimental site with semi-natural mesophilic grassland was established in the floodplain of the River Saale (near Jena, Thuringia, Germany, 50°55N, 11°35E, 130m a.s.l.). Mean annual air temperature in the Jena area is 9.3°C with an annual precipitation of

587 mm (Kluge *et al.*, 2000). The site had originally been grassland and was converted into arable land around 1960. Soil conditions resemble a Eutric Fluvisol (FAO, 1994) and soil texture changes from silty clay to sandy loam with increasing distance to the river.

60 plant species were used to create a gradient in plant SR (1, 2, 4, 8, 16 and 60) and in FGR (1, 2, 3 and 4). Functional groups were defined, according to the morphological, phenological and physiological traits of the plant species, as grasses ($n = 16$), small herbs ($n = 12$), tall herbs ($n = 20$) and legumes ($n = 12$) (for detailed list of plant species see Table A.1). Eighty-two plots (20 × 20m) were established in four blocks (Figure A.2), the blocks accounting for the differences in soil texture. Sixteen possible combinations of SR and FGR were realized and replicated over the four blocks (Figure 6.1). The location of the mixtures within each block is fully randomized. Management of the site was, as typical for semi-natural grassland under the actual site conditions, two cuts per year (late May and late August) and no application of fertilizer. Plots were weeded twice a year to maintain the original species composition. The experimental setup is described in full detail in Roscher *et al.* (2004).



**Figure 6.1** Combinations of functional groups and their replications (numbers) according to species-richness level. Each replication represents one of the plots used in this study; the total sum is 73 (9 out of the 82 original plots could not be used). The x-axis gives the different functional groups compositions (gr = grasses, lg = legumes, th = tall herbs and sh = small herbs). Functional-group richness (FGR) is shown on top.

### 6.2.2 Biomass and silage

Aboveground biomass was harvested in late May of the years 2008 and 2009. Three randomly placed samples of 20 × 50 cm were harvested 3 cm above soil surface. Biomass samples were separated into target species, dead plant material and weeds, dried (70°C, 48 h) and weighed. Mean total biomass (t DM ha$^{-1}$) was derived from the three samples as well as the abundance of functional groups. The rest of the biomass on the 3 × 3m core area of each plot was harvested (3cm above soil surface), chopped at a mean length of 5cm and ensiled (>90 days) in 50l polyethylene barrels. The low pH and the bacterial activity during ensiling enhance the

disintegration of the plant material and thereby promote the mass-flow (MF) of minerals and nutrients from the parent-material (PM) into the press-fluid (PF).

### 6.2.3 Chemical composition of the parent material

On 300g biomass of the biomass harvested within the core area, Carbon (C), hydrogen (H) and nitrogen (N) concentrations (g kg$^{-1}$ DM) were analysed using an elemental analyser (vario MAX CHN, Elementar Analysensysteme GmbH, Hanau, Germany). Potassium (K), magnesium (Mg), calcium (Ca), chlorine (Cl) and sulphur (S) (g kg$^{-1}$ DM) were analysed by X-ray fluorescence analysis. To estimate the potential concentration of K, Ca and Mg in the ash their DM concentration was put in relation to their ash concentration of the biomass sample. Thereby it is assumed that the elements will all go entirely into the ash, even though in the case of K this is highly dependent on the temperature (Knudsen *et al.*, 2004). Aboveground biomass was analysed for ash according to standard methods (Naumann and Basler, 2004).

### 6.2.4 Hydrothermal conditioning and mechanical dehydration

Each sample went through a hydrothermal conditioning process at the end of the ensiling period. The hydrothermal conditioning was conducted in a modified concrete mixer, which contained a mixture (also referred to as mash) of PM and water in a proportion of 1:4. The material was heated by gas burners and kept constant at a temperature of 60°C while being continuously stirred for 15 minutes. In a last step the mash was separated into a liquid phase (the PF) and a solid phase (press-cake, PC) by mechanical dehydration using a screw-press (Type Av, Anhydro Ltd., Kassel, Germany). The conical screw had a pitch of 1:6 and a rotational speed of 6 revolutions min$^{-1}$. The cylindrical screen encapsulating the screw had a perforation of 1.5 mm.

Samples of PM before and after conditioning, PF and PC were analysed for DM content after 48 h drying at 105°C.

### 6.2.5 Chemical composition of the press-cake and mass-flow calculation

The concentrations of K, Mg, Ca, Cl and S in PC were predicted with a near-infrared-spectroscope (XDS Rapid Content Analyser, FOSS NIRSystems Inc., Laurel, USA) based on a calibration set of 641 to 752 samples for each parameter (Table 6.1).

While near-infrared-spectroscopy made the assessment of chemical composition of the PC handy, the MF of chemical components from the PM into the PC had to be calculated in a series of steps also involving calculating the press fluid's chemical composition.

In a first step the concentration of any specific component (Z) in the PF was calculated from Z in the PM, Z in the PC and the DM contents of PC, PF and the mash (PMC) according to:

$$Z_{PF} = \frac{DM_{PMC} \times Z_{PM} - Y \times DM_{PC} \times Z_{PC}}{X \times DM_{PF}}$$

where X describes PF as a proportion of the mash and Y describes PC as a proportion of the mash:

$$X = \frac{DM_{PC} - DM_{PMC}}{DM_{PC} - DM_{PF}} \qquad Y = 1 - X$$

Thereafter the MF of DM and of any other compound (Z) from the PM into the PF (equations 1 and 2), which is defined as the proportion of the substance's concentration in the PM that is transferred into the PF after mechanical dehydration, could be calculated as:

$$(1) \ MF\_DM_{PF} = \frac{X \times DM_{PF}}{DM_{PMC}} \qquad (2) \ MF\_Z_{PF} = \frac{X \times DM_{PF} \times Z_{PF}}{DM_{PMC} \times Z_{PMC}}$$

Finally the MF of DM and of any other compound (Z) from the PM into the PC (equations 3 and 4) could be calculated accordingly:

$$(3) \ MF\_DM_{PC} = 1 - MF\_DM_{PF} \qquad (4) \ MF\_Z_{PC} = 1 - MF\_Z_{PF}$$

The MF as calculate by the equations 1-4 is expressed in a dimensionless figure between 0 and 1.

### 6.2.6 Higher heating value

The energy content, or higher heating value (HHV), describes the amount of heat released during combustion and takes into account the latent heat of vaporization of water in the combustion product. HHV was calculated based on the concentrations of C, H and N with the empiric equation for biofuels from Friedl *et al.* (2005):

$$HHV = 0.0355 \times C^2 - 23.2 \times C - 223 \times H$$
$$+ 0.512 \times C \times H + 13.1 \times N + 20600 \ (kJ \ kg^{-1} \ DM)$$

Gross energy yield (GE) was calculated by multiplying HHV with the biomass yield.

**Table 6.1** Statistics of near-infrared-spectroscopic calibration and cross-validation of chemical constituents in the press-cakes.

| Parameter | *n* | (in g kg⁻¹ DM) | | Calibration | | Cross-Validation | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | SE | $R^2$ | SE | 1-VR |
| Ash | 757 | 75.7 | ± 2.74 | 7.59 | 0.92 | 8.13 | 0.91 |
| K | 649 | 11.4 | ± 7.30 | 1.70 | 0.95 | 1.88 | 0.93 |
| Mg | 647 | 2.04 | ± 1.07 | 0.27 | 0.93 | 0.30 | 0.92 |
| Ca | 656 | 9.77 | ± 6.44 | 1.21 | 0.96 | 1.34 | 0.96 |
| Cl | 649 | 2.32 | ± 1.64 | 0.60 | 0.87 | 0.66 | 0.84 |
| S | 641 | 1.64 | ± 0.76 | 0.25 | 0.90 | 0.26 | 0.88 |
| C | 715 | 465 | ± 17.7 | 5.13 | 0.92 | 5.43 | 0.91 |
| H | 711 | 55.1 | ± 2.19 | 0.87 | 0.84 | 0.91 | 0.83 |
| N | 752 | 15.3 | ± 4.61 | 0.79 | 0.97 | 0.83 | 0.97 |

Standard deviation (SD); Standard error (SE)

### 6.2.7 Calculation of ash softening temperature based on chemical constituents

Ash softening temperature (AST) of the biomass was calculated using an equation by Hartmann (2009) based on the K, Ca and Mg concentrations (in g kg⁻¹ DM) in the solid fuel:

AST (°C) = 1172 – 5.39 × K + 25.27 × Ca – 78.84 Mg

This equation has been approved for ashes from different biomass (woody and herbaceous materials, *n* = 67) and yields relatively accurate results ($R^2$ = 0.60; SE = 88°C) according to Hartmann *et al.* (2000). While this equation is rather simple regarding the number of variables included, other studies have found equations for estimating AST that better show the complexity of chemical interactions leading to ash softening behaviour (Bryers, 1996; Seggiani *et al.*, 1999).

### 6.2.8 Statistical analysis

The Jena experiment was designed to vary SR, FGR and FGC as orthogonally as possible (Figure 6.1). However, a fully balanced design is not possible as, for example, the lowest SR cannot be combined with highest FGR. This is not an unusual situation in biodiversity experiments and can be approached by analysing the dependent variable in an analysis of variance (ANOVA) with sequential sum of squares (Schmid *et al.*, 2007). In this type of analysis variables that are fitted before others take up all the variation they can explain, ignoring the possibility that the later variables might also explain some of this variation (Hector *et al.*, 2010). The characteristics of this type of analysis can then be used to identify effects that are independent of the variables fitted before.

To account for the gradient in soil conditions, block-wise weeding and mowing, as well as sampling, block effects were fitted first. It can therefore be assumed that all variance that is explained by variables fitted after the block effect is independent of it.

As the main focus of this study is on the effects of increasing SR, this variable was fitted first after block and then the presence/absence of functional groups was fitted to test for their individual effects. For testing the effects of SR the log linear contrast of 1 to 16 species was used. The 60 species plots were used as a point of reference for highest possible diversity but were not included in the statistical analysis. Nine plots had to be omitted from the data set (four one-species plots, four two-species plots and one sixteen-species plot) as the 3 × 3m core area did not contain enough biomass to fill a 50l barrel.

Multiple regression analysis was conducted (on all plots including SR = 60) to estimate the influence of functional-group abundance in the PM on concentrations of biomass constituents in the PC by selecting the terms for inclusion in the model depending on standard statistical model selection methods (Draper and Smith, 1998). This implies that effect terms with $P < 0.05$ were included according to the rules of hierarchy and marginality (Nelder, 1994; Nelder and Lane, 1995). The initial model contained all functional-group abundance terms including all possible pairwise interactions. Functional-group abundances were tested for co-linearity (with *cor (x, y, method='pearson')* in R) prior to the analysis. The correlations between functional-group abundances were all within $–0.21 > r < –0.44$ and on average at $r = –0.33$ in both cuts.

Differences between parameters in PM and PC were tested for significance with Welch t-test. All statistical analyses were done in R 2.15.1 (R Core Team, 2012).

## 6.3  Results

### 6.3.1  Mass-flow of dry matter, ash and chemical constituents into the press-cake

Along the entire experimental range of plant species compositions and different diversity levels, the mean MF of dry matter (DM) and ash into the PC were around 71% and 55. Neither the MF of DM nor the MF of ash showed any relation to SR (Figure 6.2). Regarding the effect of functional group presence on the MF of DM and ash, both legumes and grasses stood out (Table 6.2). Legumes decreased the MF of DM and ash into the PC while grasses increased it. Furthermore, tall herbs and small herbs both decreased the MF of ash.

The main ash building constituents investigated in this study did not all have the same magnitude of MF. While the MF of Ca and Mg were on average around 49% and 40%, K had a mean MF of only 11%. None of the MFs of one of the three constituents showed any relation to SR (Figure 6.2). The only noticeable effect of functional group presence on any of the MFs the presence of small herbs increasing MF of Ca and Mg and tall herbs increasing the MF of Ca (Table 6.3). To better understand the characteristics of the individual functional groups their monocultures (all plots that only contain species of one functional group, which can be the case for species richness level of 1 to 16 species, see Figure 6.1) were analysed and their performances compared to the all-functional group plots (FGR = 4). The highest MFs amongst the functional-group monocultures regarding Mg and Ca were observed for small and tall herbs, while grasses had the lowest MF. For K only small herbs stood out with the highest MF while all the others were at similar levels (Figure 6.3)

Regarding the constituents relevant for emissions and corrosion, N, Cl and S had a mean MF of 51%, 34% and 29%, with no consistent trend with increasing SR (Figure 6.2), except N, which was significantly increasing. The presence of small herbs increased the MF of N and S and legumes increased the MF of Cl (Table 6.4). Regarding the functional-group monocultures, grasses had the lowest MF while herbs were amongst the highest. Noticeable was the high MF of Cl in the legumes monocultures and the high MF of S in the small herb monocultures (Figure 6.3).

**Table 6.2** Analysis of variance of dry matter and ash mass-flow into the press-cake, as well as their concentrations in the press-cake (in g kg$^{-1}$ DM). Shown are the effects of species richness (SR) and the presence/absence of individual functional groups on the dependent variables. All values are means of both years. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| | | Mass-flow into the press-cake | | | | Concentration in the press-cake | | | |
| | | Dry matter | | Ash | | Dry matter | | Ash | |
| Factor | DF | F | P | F | P | F | P | F | P |
|---|---|---|---|---|---|---|---|---|---|
| Block | 3 | <1 | 0.692 | 2 | 0.220 | 18 | <0.001 | 6 | 0.001 |
| Log (SR) | 1 | 3 | 0.081 | <1 | 0.836 | 1 | 0.438 | <1 | 0.542 |
| Legumes | 1 | 10 | 0.002↓ | 14 | <0.001↓ | 3 | 0.105 | 6 | 0.014↓ |
| Grasses | 1 | 15 | <0.001↑ | 24 | <0.001↑ | 13 | 0.001↑ | 5 | 0.030↑ |
| Tall herbs | 1 | 3 | 0.088 | 5 | 0.036↓ | <1 | 0.983 | <1 | 0.807 |
| Small herbs | 1 | <1 | 0.695 | 6 | 0.016↓ | 1 | 0.241 | <1 | 0.522 |
| Residuals | 60 | | | | | | | | |
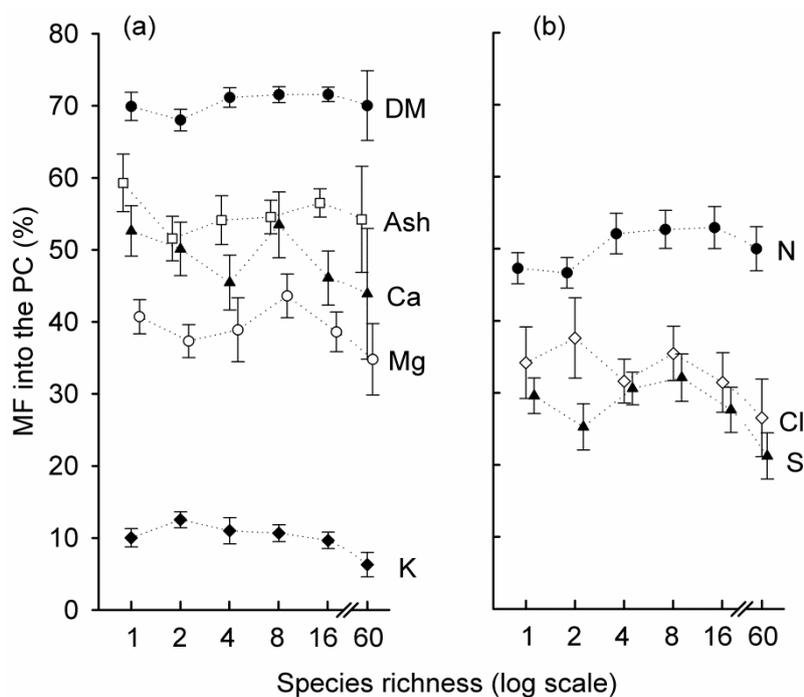
**Figure 6.2** Mass-flow (MF) into the press-cake (PC) of (a) DM, ash and biomass constituents that will accumulate in the ash of PC, and (b) biomass constituents relevant for corrosion and emission. All parameters are plotted as means of two years along the species richness gradient. Error bars indicate standard error.
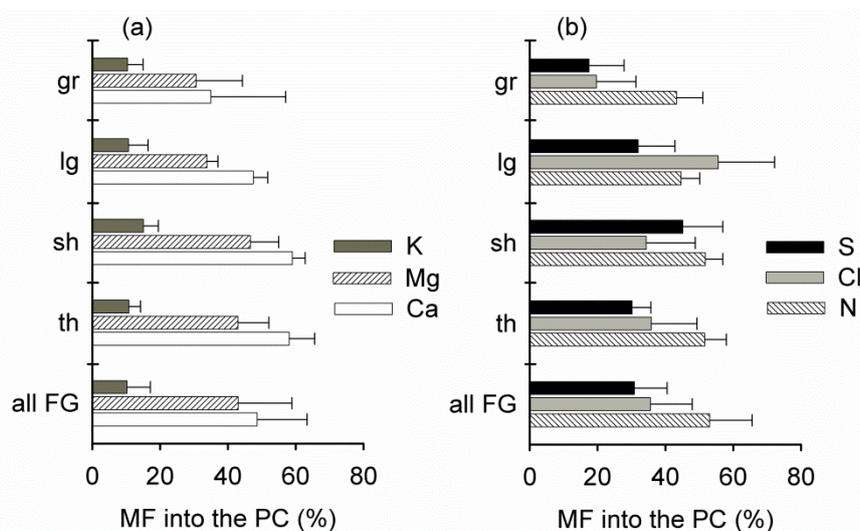


**Figure 6.3** Mass-flow (MF) into the press-cake (PC) of (a) biomass constituents relevant for ash softening, and (b) biomass constituents relevant for corrosion and emission. MF is shown for the different functional-group monocultures (gr = grasses, lg = legumes, sh = small herbs, th = tall herbs) and the all-functional-group mixtures (all FG). All bars are means of two years. Error bars indicate standard deviation.

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**Table 6.3** Analysis of variance of potassium, calcium and magnesium mass-flow into the press-cake, as well as their concentrations in the press-cake (in g kg$^{-1}$ DM). Shown are the effects of species richness (SR) and the presence/absence of individual functional groups on the dependent variables. All values are means of both years. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| | | Mass-flow into the press-cake | | | | | | Concentration in the press-cake | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Potassium | | Calcium | | Magnesium | | Potassium | | Calcium | | Magnesium | |
| Factor | DF | F | P | F | P | F | P | F | P | F | P | F | P |
| Block | 3 | 7 | <0.001 | 7 | 0.001 | 5 | 0.004 | 10 | <0.001 | 18 | <0.001 | 11 | <0.001 |
| Log (SR) | 1 | <1 | 0.775 | <1 | 0.609 | <1 | 0.621 | <1 | 0.828 | 1 | 0.393 | <1 | 0.568 |
| Legumes | 1 | 2 | 0.188 | <1 | 0.812 | <1 | 0.580 | 3 | 0.082 | 19 | <0.001↑ | 31 | <0.001↑ |
| Grasses | 1 | 1 | 0.402 | 2 | 0.193 | 1 | 0.460 | <1 | 0.750 | 97 | <0.001↓ | 30 | <0.001↓ |
| Tall herbs | 1 | <1 | 0.728 | 7 | 0.010↑ | 2 | 0.131 | 1 | 0.457 | 14 | <0.001↑ | <1 | 0.502 |
| Small herbs | 1 | 1 | 0.361 | 7 | 0.009↑ | 7 | 0.011↑ | 6 | 0.022↑ | 15 | <0.001↑ | 5 | 0.024↑ |
| Residuals | 60 | | | | | | | | | | | | |

**Table 6.4** Analysis of variance of nitrogen, chlorine and sulphur mass-flow into the press-cake as well as their concentrations in the press-cake (in g kg$^{-1}$ DM). Shown are the effects of species richness (SR) and the presence/absence of individual functional groups on the dependent variable. All values are means of both years. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| | | Mass-flow into the press-cake | | | | | | Concentration in the press-cake | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen | | Chlorine | | Sulphur | | Nitrogen | | Chlorine | | Sulphur | |
| Factor | DF | F | P | F | P | F | P | F | P | F | P | F | P |
| Block | 3 | 1 | 0.304 | 3 | 0.056 | 15 | <0.001 | 3 | 0.027 | 3 | 0.031 | 6 | 0.001 |
| Log (SR) | 1 | 5 | 0.028↑ | <1 | 0.606 | 1 | 0.399 | <1 | 0.696 | <1 | 0.899 | 3 | 0.107 |
| Legumes | 1 | 1 | 0.460 | 8 | 0.006↑ | 1 | 0.256 | 21 | <0.001↑ | 14 | <0.001↑ | 6 | 0.021↑ |
| Grasses | 1 | 1 | 0.351 | 4 | 0.061 | 3 | 0.094 | 38 | <0.001↓ | 3 | 0.098 | 13 | 0.001↓ |
| Tall herbs | 1 | 1 | 0.235 | <1 | 0.573 | 1 | 0.260 | <1 | 0.954 | 3 | 0.099 | 2 | 0.161 |
| Small herbs | 1 | 8 | 0.005↑ | <1 | 0.694 | 15 | <0.001↑ | 9 | 0.004↑ | 5 | 0.032↑ | 13 | 0.001↑ |
| Residuals | 60 | | | | | | | | | | | | |

## 6.3.2 Concentrations of ash and chemical constituents in the press-cake

While the MF indicates the percentage of a constituent's mass that is being transferred from the PM into the PC, the concentration describes the actual quantity of the constituent's mass in the PC dependent on the amount of DM that has been transferred into the PC.

Ash had a mean concentration of 59 g kg$^{-1}$ DM in the PC, which is a 23% reduction from the PM (Table 6.5). As with the MF, the concentration of ash in the PC was lower when legumes were present and higher when grasses were present (Table 6.2). The concentration of ash in the PC was not affected by SR (Figure 6.4).

K, Ca and Mg had a mean concentration of 2.8, 8.0 and 1.2 g kg$^{-1}$ DM. All three parameters were reduced through IFBB, with the reduction being most pronounced for K with 85%, while Mg was reduced by 43% and Ca by 27% (Table 6.5). While small herbs were the only functional group affecting the concentration of K in the PC, both Ca and Mg were affected by the presence of all functional groups except for tall herbs, which did not affect Mg concentrations (Table 6.3). The general trend was that grasses reduced concentrations of Ca

and Mg, while legumes and herbs increased them. Neither of the three constituents' concentration in the PC was affected by SR.
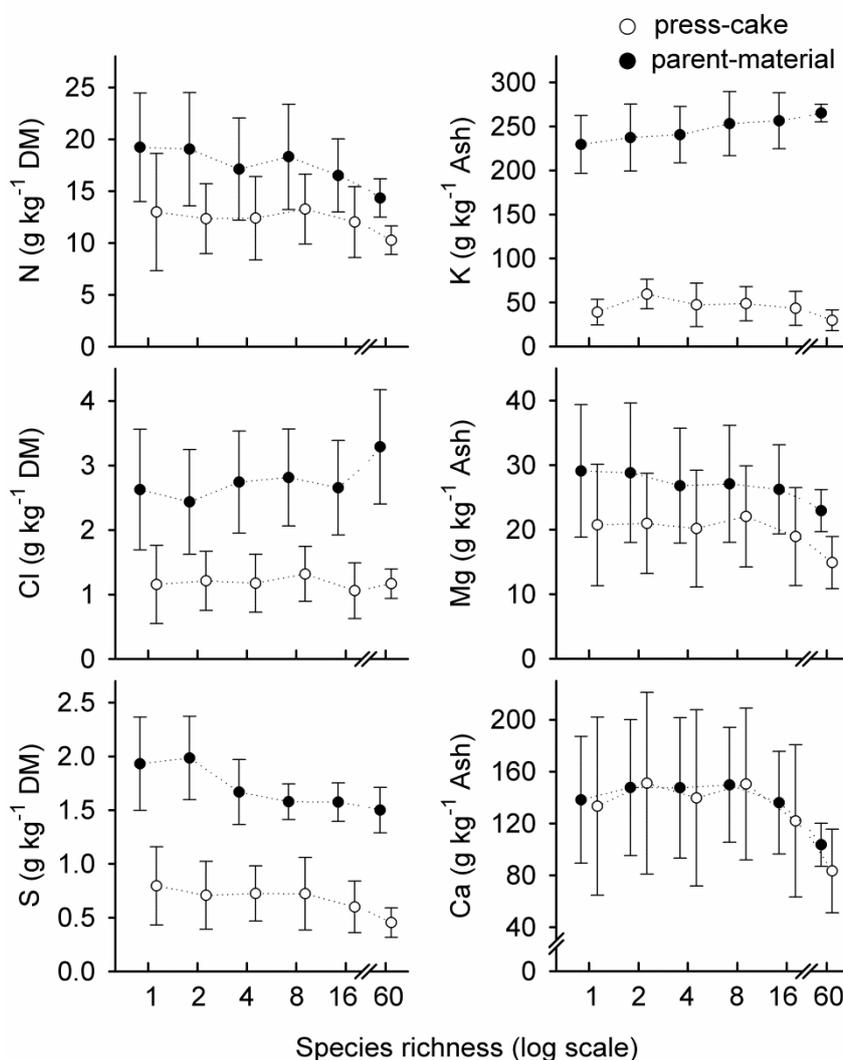


**Figure 6.4** Concentrations of N, Cl and S in the DM and K, Mg and Ca in the ash of the press-cake (open circles) and parent-material (solid circles). Concentrations are plotted along the species-richness gradient as means over both years. Error bars indicate standard deviation.

The concentrations of N, Cl and S were all three reduced through the IFBB procedure. N had a mean concentration of 12.5 g kg$^{-1}$ DM in the PC and was reduced by 28%, while Cl with a mean of 1.2 g kg$^{-1}$ DM and S with a mean of 0.7 g kg$^{-1}$ DM were reduced by 56% and 59% (Table 6.5). Regarding the effect of functional-group presence, the same general trend was observed as for K, Ca and Mg, where grasses reduced the concentrations while legumes and herbs increased the concentrations (Table 6.4). Most noticeable was that both the presence of legumes and small herbs affected all three parameters, while grasses only affected N and S, and tall herbs had no effect. Neither of the three parameters' concentrations in the PC was affected by SR (Figure 6.4). Comparing the concentrations in the functional-group

monocultures, N and Cl were highest and for S second highest (after small herbs) in the legume monocultures, while grasses always had the lowest concentrations (Figure 6.5a). Possible predictions of N, Cl and S concentrations (in g kg$^{-1}$ DM) in the PC from functional-group abundance (in % DM) in the PM were not very strong, except for N (N = –0.0065 × grass abundance + 0.003 × legume abundance + 1.39, $R^2$ = 0.61; Cl = –0.0004 × grass abundance – 0.0005 × legume abundance + 0.12, $R^2$ = 0.34; S = –0.0004 × grass abundance – 0.0003 × small herb abundance + 0.075, $R^2$ = 0.48). All three regressions models were highly significant (P<0.001).
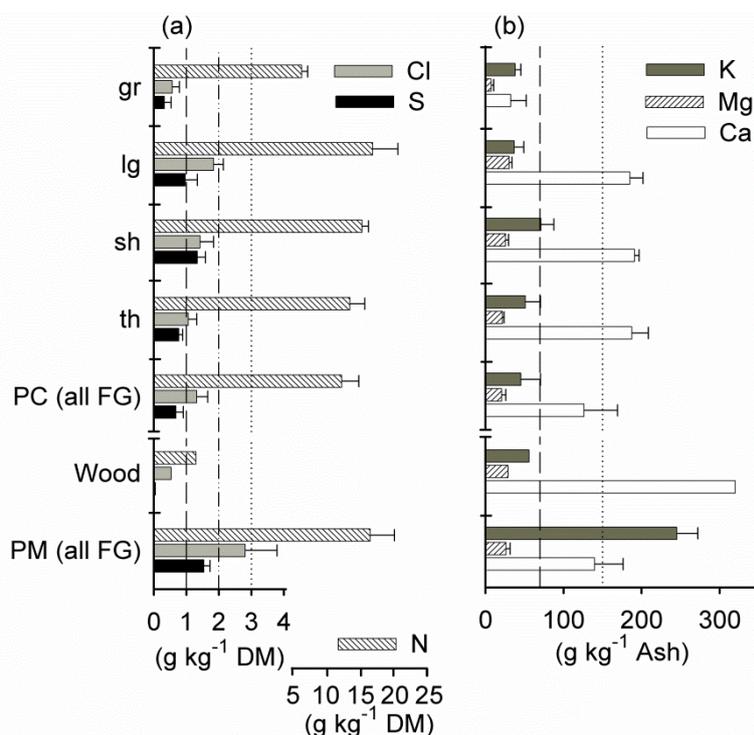


**Figure 6.5** (a) Concentrations of Cl, S and N in the DM of the press-cake (PC) of functional-group monocultures (gr = grasses, lg = legumes, sh = small herbs, th = tall herbs) and all-functional-group mixtures (all FG). The 1 g kg$^{-1}$ DM (dashed line), 2 g kg$^{-1}$ DM (dashed dotted line) and 3 g kg$^{-1}$ DM (dotted line) threshold for Cl, S and N concentrations, as proposed by Obernberger (2006), are indicated. (b) Concentrations of ash constituents in the ash of press cake (PC) from functional-group monocultures and all-functional-group mixtures (all FG). The 70 g kg$^{-1}$ ash (dashed line) threshold for K and the 150 g kg$^{-1}$ ash (dotted line) for Ca as proposed by Obernberger (2006) are indicated. All Values are means of both years and standard deviation is indicated by error bars. In both graphs values for wood (van Loo and Koppejan, 2008) and the parent-material (PM, Khalsa *et al.*, 2012a) of the press-cake (PC) are added for comparison.

The concentrations of K, Ca and Mg in the ash of the PC may have an indication for the ash melting behaviour and were thus calculated. K, assuming that it goes entirely into the ash during combustion, was reduced to a mean concentration of 47 g kg$^{-1}$ ash. The concentration of Mg was lowered to 20 g kg$^{-1}$ ash as compared to the ash of the PM, while the concentration of Ca remained stable at 137 g kg$^{-1}$ ash. All three constituents showed no effect with increasing SR (Figure 6.4) but the functional-group monocultures showed specific patterns.

Ca was about six times as high in the legumes, small herbs and tall herbs monocultures as in the grass monocultures (Figure 6.5b). For Mg the pattern was similar with the legumes and herbs being between three to four times as high as the grasses. Regarding the K concentration both legumes and grasses were equally low, while small herbs were double as high and tall herbs were in-between those two extremes. Interestingly, the concentration of Ca and Mg in the ash of the PC (in g kg$^{-1}$ ash) could be well predicted by grass abundance (in % DM) in the PM (Ca = $-1.52$ × grass abundance + 183.9, $R^2 = 0.82$; Mg = $-0.18$ × grass abundance + 25.9, $R^2 = 0.70$). K in the ash of the PC and the abundance of any functional group did not show strong correlations (all $R^2 < 0.06$ and $P>0.05$).

The calculated AST ranged from 1155°C to 1380°C with a mean of 1264°C and was, compared to the PM, increased by 6.8%. The K/Ca ratio in the ash of the PC had a mean of 0.57 and was lower than the K/Ca ratio of 2.09 in the ash of the PM.

**Table 6.5** Mean, minimum (min) and maximum (max) values of energy content (HHV) and combustion relevant parameters in the parent-material (PM) and the press-cake (PC) of the investigated biomass. Values are means of both years and significant differences between PM and PC are indicated. Included are values from miscanthus and spruce wood as two types of biomass frequently used for combustion (Hartmann, 2009)

| | | MJ kg$^{-1}$ DM | g kg$^{-1}$ DM | | | | | | | °C |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | HHV | Ash | K | Ca | Mg | Cl | S | N | AST |
| | Mean | 18.3 | 77 | 18.8 | 11.0 | 2.1 | 2.7 | 1.7 | 17.4 | 1183 |
| PM | Min | 17.8 | 60 | 9.9 | 3.5 | 0.9 | 1.3 | 1.2 | 9.5 | 1073 |
| | Max | 18.8 | 100 | 25.8 | 17.2 | 4.0 | 4.8 | 2.8 | 27.9 | 1328 |
| | Mean | 18.9 | 59 | 2.8 | 8.0 | 1.2 | 1.2 | 0.7 | 12.5 | 1264 |
| PC | Min | 18.1 | 44 | 0.3 | 0.7 | 0.1 | 0.3 | 0.1 | 5.6 | 1155 |
| | Max | 19.6 | 77 | 8.3 | 13.8 | 2.3 | 2.2 | 1.4 | 24.8 | 1380 |
| Δ PM$_{mean}$ − PC$_{mean}$ | | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Miscanthus | Mean | 19.1 | 39 | 7.2 | 1.6 | 0.6 | 2.2 | 1.5 | 7.3 | 973 |
| Wood (spruce) | Mean | 20.2 | 6 | 1.3 | 7.0 | 0.8 | 0.1 | 0.2 | 1.3 | 1426 |

n.s. ($P>0.05$), * ($P<0.05$), ** ($P<0.01$), *** ($P<0.001$)

### 6.3.3 Diversity and its effects on energy content and gross energy yield

The energy content in the PC, expressed as HHV, was on average 18.9 MJ kg-1 DM, being 3.4% higher in the PC than in the PM (Table 6.5). HHV, like ash content, showed no effects with increasing SR (Figure 6.6). However, functional-group presence showed an effect on HHV with legumes increasing (by 1.5%) and grasses decreasing HHV (by 1.7 %; Table 6.6)

Gross energy yield of the PC (GE$_{PC}$), with a mean of 46 GJ ha$^{-1}$, was reduced through conditioning and dewatering by about 27% compared to the gross energy yield of the PM (GE$_{PM}$). GE$_{PC}$ was clearly affected by SR and doubled from 36 GJ ha$^{-1}$ at SR = 1 to 73 GJ ha$^{-1}$ at SR = 60 (Figure 6.6).
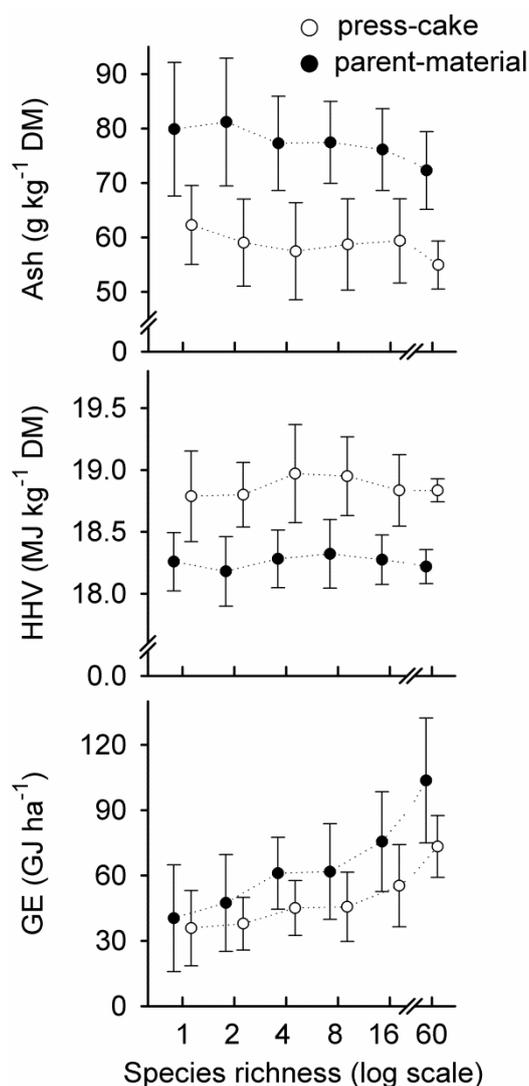
**Figure 6.6** Variation in ash concentration, energy content (HHV) and gross energy yield (GE) with increasing plant-species richness. All values are means of two years and error bars indicate standard deviation.

**Table 6.6** Analysis of variance of energy content (HHV, in MJ kg$^{-1}$ DM) and gross energy yield (GE, in GJ ha$^{-1}$). Shown are the effects of species richness (SR) and the presence/absence of individual functional groups on the dependent variable. All values are means of both years. Arrows indicate an increase (↑) or a decrease (↓) with presence of the respective functional group.

| Factor | DF | HHV | | GE | |
|---|---|---|---|---|---|
| | | *F* | *P* | *F* | *P* |
| Block | 3 | 3 | 0.036 | 2 | 0.075 |
| Log (SR) | 1 | 1 | 0.347 | 13 | 0.001↑ |
| Legumes | 1 | 26 | <0.001↑ | 4 | 0.054 |
| Grasses | 1 | 34 | <0.001↓ | 1 | 0.400 |
| Tall herbs | 1 | <1 | 0.642 | <1 | 0.999 |
| Small herbs | 1 | 6 | 0.018↑ | 1 | 0.248 |
| Residuals | 60 | | | | |

## 6.4 Discussion

### 6.4.1 Mass-flow of combustion related constituents into the press-cake

Along the SR gradient none of the MFs showed any consistent trend and compared to the MFs obtained by Wachendorf *et al.* (2009) many of them were in a similar range (DM, ash, Ca, Mg, K and N), even though 4 of the 5 sites investigated in their study were harvested much later (31st of August) than the Jena biomass. Richter *et al.* (2011) showed that, in general, harvest date has an inconsistent effect on MFs, with the MF of N into the PC being lower the younger the sward, while that of K is not affected by the maturity of the biomass at all. Yet the MFs of Cl and S into the PC were much different from what Wachendorf *et al.* (2009) reported, with the MF of Cl being almost three times as high when using the Jena biomass and the MF of S being 25% lower.

Regarding the functional-group effects, only small and tall herbs increased the MF of ash related constituents Ca and Mg, while no other functional group had any effect. On the contrary, both small and tall herbs and legumes decreased the overall MF of ash into the PC, while grass increased it. This indicates that grasses and non-grass species affect the MF of ash in different ways. While for grass species, silicon (Si) is of great importance (Marschner, 2001), and was found to contribute largely to the ash contents of grass-like species (65% of the total ash in switch grass; Jenkins *et al.*, 1998); dicotyledonous herbaceous plants, owing to a high cation exchange capacity of their roots, often have a higher uptake of nutrients available as divalent ions (such as Ca and Mg) than monocotyledons grass species (Drake *et al.*, 1951; Asher and Ozanne, 1961). Thus, the high MF of ash caused by grasses is most likely due to the MF of Si into the PC, which, in contrast, makes the other functional groups appear to be reducing the MF of ash. The fact that small herbs increased MF of the mostly cell wall-bound constituents N and S into the PC and legumes increased MF of Cl could not be explained.

### 6.4.2 Diversity and the chemical composition of the press-cake

The concentrations of the constituents in the PC result from the MFs and are most relevant for solid fuel quality and the layout of combustion facilities. As with the MF data, no significant trend with SR could be found, which is in contrast to the patterns found in the PM (data in Khalsa *et al.*, 2012a) regarding N, S and Mg (all significantly declining with increasing SR). Thus, SR effects vanished by applying the IFBB procedure, or in other words, the IFBB

procedure leads to equal levels of concentrations across all SR levels (Figure 6.4). The functional groups had more of an influence on the concentrations as they did on the MFs of the constituents. This was true for Ca, where all functional groups showed an effect, and for Mg, where three of them did (instead of just one). Even K was affected by one functional group, which was not affected by any, regarding the MF. N, S and Cl were all affected by at least two functional groups, while the MF was only affected by not more than one functional group.

In comparison to the functional-group effects reported for the PM (see Khalsa *et al.*, 2012a) most of the effects in the PM were also observed in the PC with Ca and N being identical while for K the legume and grass effect disappeared. For all the other constituents more effects were observed: (i) the concentration of Cl in the PC was affected by both the presence of legumes and small herbs, (ii) the concentration of S in the PC was also affected by legumes and (iii) the concentration of Mg in the PC was affected by small herbs. In conclusion, applying the IFBB procedure does not reduce the functional-group effects that occurred in the PM regarding Ca, Mg, N and S. It is only for K where two major effects vanished.

### 6.4.3 Solid fuel quality of the press-cake

The significant reductions in N, S and Cl concentration in PC, in comparison to PM, was either lower (56% instead of 83% for Cl) or higher (59% instead of 39% for S, and 28% instead of 19% for N) than in a study by Richter *et al.* (2010). Regarding the recommended concentrations by Obernberger *et al.* (2006, Figure 5.5), these reductions helped increase the solid fuel quality by bringing the Cl content in the all-functional-group mixtures near $1 \, g \, kg^{-1}$ DM, which reduces HCl emissions and corrosion to a tolerable level. The emission of polychlorinated dibenzodioxines and dibenzofuranes (PCDD/F) can be prevented as Cl concentrations were clearly below the $3 \, g \, kg^{-1}$ DM threshold. S concentrations in the PC of the all-functional-group mixtures were below the $1 \, g \, kg^{-1}$ DM and $2 \, g \, kg^{-1}$ DM thresholds, indicating a low risk of corrosion and a low risk of $SO_x$ emissions. N was significantly reduced by almost 30%, however, it was still higher than the $6 \, g \, kg^{-1}$ DM threshold, indicating that $NO_x$ emissions can be expected. The potential $NO_x$ emissions will have to be dealt with either through the use of filters or staged combustion techniques or both. Regarding the functional groups, grass monocultures had the best solid fuel quality regarding N, Cl and S.

Another important quality aspect of solid fuels is the AST that can lead, if too low, to slagging in the combustion plant. Of the major ash-forming elements of biofuels, concentrations of K, Ca and Mg are known to affect AST. K-rich salts in combination with phosphates and silica were found to be responsible for low AST (Steenari *et al.*, 2009) and the ratio between K, on the one hand, and Ca on the other hand, indicate whether low or high AST can be expected (Öhman *et al.*, 2000). The K/Ca ratio in the ash was significantly decreased from PM to PC and thus indicates an increased AST in the PC. Hartmann (2009) suggested that K, Ca and Mg concentrations in the dry matter of the biomass can be used to calculate AST. The observed reductions of K, Ca and Mg from the dry matter of the PM to the PC were all significant and were at least for K and Ca similar to Richter *et al.* (2010), while the reduction of Mg was lower (43% instead of 61%). The values for AST calculated according to Hartmann (2009) indicate a significant increase of AST by 80°C from PM to PC.

Obernberger *et al.* (2006) proposed thresholds for K and Ca concentrations in the ash which should be met if slagging is to be prevented (Figure 6.5). While PC values for K were, as desired, below the 70 g kg$^{-1}$ ash standard in the all-functional-group mixtures, Ca was a little lower than the required 150 g kg$^{-1}$ ash. Comparing the functional-group monocultures, grasses had the lowest Ca concentrations and therefore contribute to low ASTs, while mixture high in herbs and legumes would bare a low risk of slagging.

The experimental setup allows the investigation of potential correlations between functional-group abundances and expected concentrations of constituents either in the dry matter or the ash of the PC. The most accurate predictions could be made from grass abundance on Ca and Mg concentrations in the ash of the PC. However, this alone would not be sufficient to make any predictions on AST from grass abundance, as K was not correlated. Furthermore, the abundance of grass and legumes together could predict the N concentration in the PC fairly accurately.

### 6.4.4   Energy content and gross energy yield in relation to diversity

It is well known that HHV and ash content are negatively correlated (Jenkins *et al.*, 1996) and it was not surprising that the reduction of ash by 23% from the PM to the PC resulted in an increase of HHV by about 3.4%. Thus, through applying the IFBB procedure the energy content of the biomass available for combustion increased. However, as approximately 30% of the MF of dry matter is directed into the PF and thus not available for combustion, GE decreased by 27%. Those 30% DM will be used for anaerobic digestion as part of the IFBB

procedure. The resulting biogas is used in a combined heat and power plant to produce electricity and heat, which is then used for drying of the PC (Bühle *et al.*, 2012b). Thus, the reduction in GE is in fact a partial relocation of the energy from the combustion-directed path to the complementary biogas-directed path within the same system.

In terms of diversity effects ash, HHV and GE were all affected by SR in the same way as they were in the PM (see Khalsa *et al.*, 2012a). However, regarding the influence of the functional groups, several changes occurred: (i) while small and tall herbs used to increase ash and grasses decrease it, (ii) the ash in the PC is now only increased by the presence of grasses. This is probably due to the Si content of the grasses. Si significantly contributes to the ash content and is mainly retained in the PC, while Ca, Mg and K contents in the grasses are low and get transferred into the PF. For HHV the influence of the functional groups remained the same between PC and PM, while GE was no longer influenced by the presence of legumes.

### 6.4.5  Influence of harvest time

To conduct this study it was important to use the biomass from the first cut (which is harvested in the end of May) in order to have enough material for the IFBB-procedure. A management geared towards nature conservation, however, would have required a cut not earlier than the end of June, to ensure reproduction of most of the plant species. In the study by Richter *et al.* (2011), on the influence of sward maturity on solid fuel quality of PC from IFBB, N, Cl and S concentrations did not differ substantially between late May and the late June harvest. However, the ash content of the PC increased by 15% within that time period.

Thus, it can be concluded that the results presented here would probably not change much regarding N, Cl and S concentrations but the ash content could be higher and thereby reducing HHV (and consequently GE) slightly. The AST might also change, but no assumptions could be made to whether it would be increased or decreased.

## 6.5  Conclusions

Examining the PC of the experimental grassland communities made it possible to highlight (i) the diversity effect still relevant after hydro-thermal conditioning and mechanical dewatering of the biomass and (ii) the significant improvements in solid fuel quality through IFBB across a multitude of species mixtures. It can be concluded that high SR in extensive grassland will lead to high GEs, while SR has no effect on the solid fuel quality of the PC. Furthermore, the

functional groups provide the best solid fuel quality when they are in a well-balanced mixture of grass and non-grass species as e.g. grasses reduce the risk of emission and corrosion while the non-grass species keep the AST high. However, as GE is highly dependent on biomass yield and the presence of legumes was found to have an increasing effect on the biomass yield (Khalsa *et al.*, 2012a) legumes should, whenever possible, be present in the mixture.

# 7  General discussion

## 7.1  Energy content for combustion and anaerobic fermentation

While the energy content relevant for combustion, HHV, was not affected by changes in SR, the energy content relevant for anaerobic digestion, $CH_{4\ sub}$, decreased with increasing SR. This was due to the influence of different parameters on the respective energy content and their relation to the SR gradient.

The HHV is dependent on C, H and N content of the biomass, with C having the strongest impact (see 4.2.4). Neither C nor H change along the SR gradient while N had a slight negative slope with increasing SR. However, this was not enough to cause a SR effect on HHV. This is in line with the findings of Florine *et al.* (2006) who did not find a clear trend in HHV between sites with different SR.

Both CF and CP play a key role for the anaerobic digestibility and contributed to the equation for calculating $CH_{4\ sub}$ (see 5.2.4). Regarding $CH_{4\ sub}$, the decrease with increasing SR is a result of the CF and CP values changing along the SR gradient. CF increased with SR, which can be the result of increasing stress due to competition between plants of different functional groups but also within a functional group. Especially the small plant species are forced to grow taller in order to intercept more light at higher SR and thus might increase fibre contents. It is known that fibre and especially lignin plays an important role for the strength and the rigidity of plant cell walls (Buxton and Redfearn, 1997). CP decreased with increasing SR, which was interpreted as a result of the increasing competition of plants for the limited availability of nutrients such as nitrogen (N = CP/6.25) and the limited access of N fixing plants to light and thus a reduced N supply to the system (Vitousek and Field, 1999). Furthermore, in an earlier study on the Jena-biomass, it was found that the plant-available N in the soil solution decreased with increasing SR (Oelmann *et al.*, 2007).

The most relevant functional group for HHV were the legumes which significantly increased HHV when present in a mixture (Figure 4.2A). This is due to the legumes having a higher lignin content than grasses (also observed by Jung *et al.*, 1997) or the other functional groups. Lignin is known to have a carbon content of 640 g $kg^{-1}$ lignin, as compared to cellulose with a carbon content of 420 g $kg^{-1}$ cellulose (Lewandowski, 2009), and therefore, a higher lignin content results in a higher HHV.

For $CH_{4\ sub}$ both grasses and legumes played an important role as legumes significantly increased CP and grasses increased CF. This is not surprising as grass species are known to have higher CF contents than non-grass species (Buxton and Redfearn, 1997), and legumes increase CP through fixing atmospheric N via symbiotic root bacteria and by accumulating high concentrations of N in their tissue (Tomm *et al.*, 1995)

## 7.2 Solid fuel quality

Three parameters relevant for combustion were found to be affected by SR. Mg, N and S, all decreasing with increasing SR, however, the effect for Mg and N was not independent of the presence/absence of grasses (Figure 4.2B, Figure 4.6). While a lower Mg content at higher SR may have no further effect on any of the bioenergy parameters, and if so only on the AST (Steenari *et al.*, 2009), a lower content of N and S could be beneficial regarding $NO_x$ and $SO_x$ emissions. However, the observed levels of N and S concentrations, even at highest SR, were in relation to the recommended thresholds by Obernberger *et al.* (2006) still problematic.

Many functional-group effects could be identified and put in relation to the possible risk of emissions, corrosion and ash softening. Regarding several parameters legumes and grasses had opposite effects. While legumes increased N, Ca and Mg content, grasses decreased it. On the other hand, legumes and grasses also had similar effects which were in contrast to the herb functional groups. For instance, legumes and grasses both decreased K and ash content while small and tall herbs increased it.

Regarding functional-group effects on solid fuel quality, the two key factors seem to be: (i) the difference between dicotyledonous herbaceous plants, which are able to take up nutrients that are available as divalent ions (such as Ca and Mg) through their roots (Asher and Ozanne, 1961; Drake *et al.*, 1951), and monocotyledonous grass species; (ii) the difference in morphological traits of the plants, as higher nutrient concentrations are expected in biomass with a high leave-to-stem ratio (Pahkala and Pihala, 2000) such as herbaceous species.

Regarding the ash melting behaviour, the ratio between K and Ca indicates whether low or high AST can be expected (Öhman *et al.*, 2000). Also, Obernberger *et al.* (2006) gave guidelines for K and Ca concentration which should be met if problems with ash melting and consequently with slagging in the combustion chamber are to be prevented. Legumes, both increasing Ca and decreasing K in the mixtures, were found to be very important for keeping the AST high. Grasses on the contrary, especially as functional-group monocultures, had an unfavourable K/Ca ratio and exceeded the guidelines for K and Ca (Figure 4.5).

However, as much as the legumes seem to be superior regarding a high AST, grasses need to be abundant in the mixture to keep N and consequently $NO_x$ emissions low. In general, all the functional groups were found to have some enhancing effects on fuel quality as well as effects that were disadvantageous.

Since concentrations of N, K, Cl and S in the biomass, irrespective of the FGC, were above the recommended thresholds, a solid fuel improvement through IFBB was considered. The concentrations were successfully reduced for N, K, Cl and S (by 28%, 85%, 56% and 59%; for comparison see Richter *et al.*, 2010) and did now meet the thresholds proposed by Obernberger *et al.* (2006), except for N, which was still above 6 g kg$^{-1}$ DM. Ash was reduced by 23% and the K/Ca ratio in the ash of the PC (0.57) was lower than the K/Ca ratio in the ash of the PM (2.09). On almost all the constituents the functional groups still had the same effects as before applying the IFBB procedure. However, Mg, N and S were all three no longer affected by SR, which can be interpreted as the IFBB procedure resulting in equal levels of concentrations across all SR levels (Figure 6.4).

## 7.3 Energy conversion and output

Both GE and $CH_{4\,area}$ increased with increasing SR, which had been reported for GE before by Tilman *et al.* (2006) in a study on perennial grassland. The main driver behind this positive diversity – bioenergy relation is the biomass yield. HHV did not change with SR and $CH_{4\,sub}$ even decreased with increasing SR. However, GE and $CH_{4\,area}$ still had a positive slope along the SR gradient. A positive relation between SR and biomass yield has been reported in other studies (Hector *et al.*, 1999; Tilman *et al.*, 2001), whereby it is important to distinguish between the 'sampling effect' (the greater chance of including more productive species with increasing SR), or 'complementarity' (a combination of (i) 'facilitation', the presence of a species increases the growth or survival of another species, and (ii) 'niche-separation', the plant community can utilize more of the total nutrient pool and habitat space) being the driver for this effect.

Several factors indicated that in this study the increase in biomass with SR was due to complementarity mechanisms, such as: (i) the evenness increasing slightly with SR, which indicates that high-diversity plots had a more balanced dominance structure than low-species plots, (ii) transgressive overyielding (the productivity of a mixture is higher than the productivity of the most productive monoculture of the species contained in the mixture) could be observed by Marquard *et al.* (2009), who analysed five years (2003-2007) of

biomass data from the Jena-Experiment, (iii) no functional-group term in the statistical model interacted with SR regarding the biomass yield, which was tested by fitting the presence of each functional group before SR in the model.

As legumes had a significant positive effect on biomass yield independent of SR, and the evenness not being affected by the presence of legumes, it can be assumed that legume presence enhanced total biomass through complementarity, or more specifically, through facilitation. The facilitation mechanism of legumes is related to their ability to fix atmospheric N and accumulate high concentrations of N in their tissue (above and below ground), which is released into the soil during decomposition of their below-ground biomass (as the above-ground biomass in this experiment was harvested and is therefore unavailable for other plants). Non N-fixing plants (grasses and herbs) can directly benefit from these processes by taking up the plant available N released into the soil (Haynes, 1980; Spehn *et al.*, 2002). As N was found to be primarily limiting biomass production in the Jena-Experiment (Oelmann *et al.*, 2007), it can be assumed that legumes have provided plant available N for the system which promoted productivity and thereby GE and $CH_{4\,area}$. Therefore legumes play a key role regarding the energy yield of extensive grassland sites by increasing biomass yield and also by increasing HHV and $CH_{4\,sub}$, while grasses don't affect biomass yield but decrease $CH_{4\,sub}$.

Regarding the most relevant SR levels for extensive grasslands in agricultural practice (8 to 16 to 60 species) the annual GE in this study increased by 26% from 8 to 16 species ($SR_{8\rightarrow16}$) and increased by 65% from 8 to 60 species ($SR_{8\rightarrow60}$) (Figure 4.4). For the annual $CH_{4\,area}$ the increase was 22% from $SR_{8\rightarrow16}$ and 49% from $SR_{8\rightarrow60}$ (Figure 5.6).

If annual GE and $CH_{4\,area}$ are expressed by a common unit (MWh $ha^{-1}$ $year^{-1}$), combustion will have a higher gross energy yield than anaerobic digestion across all levels of SR, with the gross energy yield from combustion being almost double as high at SR = 16 and 60 (Khalsa *et al.*, 2012b). Therefore, combustion of extensive grassland biomass is the superior conversion technique regarding the energy output. However, as discussed before, the risk of emissions, corrosion and slagging has to be addressed and will therefore require a certain amount of additional input when looking at the overall feasibility of the conversion.
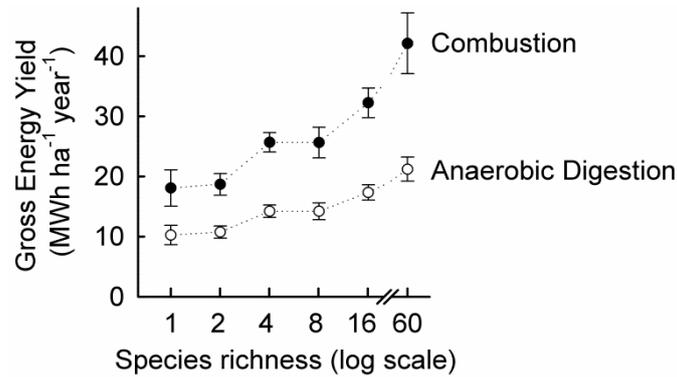
**Figure 7.1** Annual gross energy yields along the species richness gradient from biomass converted to energy through combustion or anaerobic digestion (from Khalsa *et al.*, 2012b). Error bars indicate standard error and values are means of both years.

Through applying the IFBB procedure the HHV increased by 3.4%, while GE was decreased by 27%. This is due to the MF of dry matter from the PM into the PF, which was at 30%. The reduction in GE is not a loss of energy but a partial relocation from the combustion-directed path to the complementary biogas-directed path within the IFBB system. The resulting biogas is used in a combined heat and power plant to produce electricity and heat, which is then used for drying of the PC (Bühle *et al.*, 2012b). In terms of conversion efficiency, in an IFBB stand-alone plant 44.7% of the biomass could be converted to heat and be exported while the combustion of hay would yield 53.8% of exportable heat (Bühle *et al.*, 2012b). However, the combustion of hay from a regular cutting date would require certain adaptations and maintenance of the plant, to deal with emission, corrosion and slagging, which is not reflected by these numbers. If biomass from delayed harvest (in February or March) is considered for hay combustion, which will have similar fuel quality as the IFBB material, the numbers can better be compared. However, delayed harvest biomass may have been subject to large biomass losses in the field of 30-70% owing to particle breakdown (Christian *et al.*, 2002; Tonn *et al.*, 2007). If the IFBB procedure is combined with an already existing biogas plant (IFBB add-on) the amount of exportable energy (as heat) from the biomass could be as high as 52.9% (Bühle *et al.*, 2012b) and such an IFBB add-on would also be more profitable than hay combustion (Blumenstein *et al.*, 2012).

Thus, applying the IFBB procedure to the extensive grassland biomass allows the farmer to utilize both material from early and late cutting dates for combustion, and in combination with an existing biogas plant, the conversion of biomass to energy is equally efficient as for hay combustion, while the risk of emission and corrosion problems is strongly reduced.

## 7.4 Experimental approach and future research

A distinct advantage of studies with artificial communities, as used here, is that the diversity gradients can be precisely maintained, and the variance in abiotic conditions and community structure between replicates is kept small. Many diversity experiments all over the world have used artificial grassland communities to investigate ecosystem mechanisms (Hector *et al.*, 1999; Tilman *et al.*, 2001; Kirwan *et al.*, 2007). However, there has been a debate whether the results of such studies can be applied to natural communities (Huston 1997; Loreau *et al.* 2001; Diaz *et al.* 2003; Pfisterer *et al.* 2004). When interpreted in the framework of agricultural management the relevancy of these studies has to be assessed, especially since the low species sites (SR ≤ 4) do not resemble naturally occurring conditions.

To better relate the diversity – productivity relation to agricultural management, Weigelt *et al.* (2009) added a management and a fertilization gradient to the diversity gradients (SR and FGR) of an artificial grassland experiment. Others have suggested that removal experiments should be used instead to investigate the role of functional groups under conditions relevant for practice (McLaren and Turkington 2010, Petersen *et al.*, 2012). However, Diaz *et al.* (2003) concluded from a comparison of removal experiments and experiments with artificially assembled communities that both experimental approaches should be used to complement each other, rather than one approach being favoured over the other.

The results presented in this study have relevance for extensive, non-fertilized grassland. However, in future experiments that investigate the relation between diversity and agriculturally relevant parameters from low-input management, the SR gradient could be more focused on the range of naturally occurring species numbers. Functional-group monocultures (as a measure to observe the functional-group effects in isolation) have proven to be useful to understand diversity effects on agricultural parameters, however, species monocultures and plant compositions with SR ≤ 4 are unlikely to have relevance for extensively managed grasslands.

Furthermore, to verify some of the results obtained here regarding bioenergy production, and especially, to further the knowledge on anaerobic digestion from grassland biomass it could be useful to investigate the digestibility and the $CH_{4\,sub}$ along the diversity gradients in a series of batch experiments. Regarding the solid fuel quality, obtaining the AST through standard methods along diversity gradients will bring new insight to the field of ash melting behaviour of grassland biomass.

# 8  Conclusions

Using artificial grassland communities to identify diversity effects on bioenergy parameters relevant for combustion or anaerobic digestion of extensive grassland biomass was successful and supports the following conclusions:

(i)    Increasing SR had several effects on bioenergy parameters, for example, decreasing $CH_{4\,sub}$, N (and CP), Mg and S content (even though they could not always be fully disentangled from functional-group effects), while other parameters were not affected (HHV) or increased (CF). However, the most relevant and positive effect of SR is the increase of energy yield for both combustion (annual GE increased by 26% from $SR_{8\rightarrow16}$ and by 65% from $SR_{8\rightarrow60}$) and anaerobic digestion (annual $CH_{4\,area}$ increased by 22% from $SR_{8\rightarrow16}$ and by 49% from $SR_{8\rightarrow60}$) through the strong interaction of SR with biomass yield. Thus, high-diversity, low-input grasslands are more valuable for bioenergy production than low-diversity, low input grasslands, as they generate more energy output.

(ii)   Identifying one functional group as being solely beneficial or disadvantageous for combustion or anaerobic digestion was difficult, as they exhibit contrary effects with regard to different parameters. However, legumes play a key role for the utilization of grassland biomass for energy production as they increase the energy content of the substrate (HHV and $CH_{4\,sub}$) as well as the energy yield (GE and $CH_{4\,area}$), the latter through facilitating plant growth of the other species and thus increasing biomass yield.

(iii)  As annual gross energy yields from combustion are expected to be double as high (at SR = 16, 60) than from anaerobic digestions and will thus be the favourable conversion technique, the solid fuel quality needs be improved to reduce the risk of corrosion and emission-related problems, as well as slagging, which is best done through IFBB.

(iv)   The solid fuel quality was significantly improved through IFBB regarding ash (reduced by 23%), N (28%), K (85%), Cl (56%) and S (59%) and resulted in equal levels of concentrations along the SR gradient. Furthermore HHV was increased by 3.4% and the risk of ash-melting at low temperatures was reduced.

# 9 Zusammenfassung

Die bioenergetische Nutzbarkeit von extensiver Grünlandbiomasse ist schon seit längerem Gegenstand der Forschung. Die Möglichkeit Artenschutz zu gewährleisten, ökonomische Perspektiven für Landwirte zu schaffen und keine weitere Konkurrenz zur Nahrungsmittelproduktion zu erzeugen bietet einen starken Anreiz. Die botanische Zusammensetzung der Biomasse spielt eine erhebliche Rolle und wirkt sich zum einen auf die Brennstoffqualität der Biomasse aus, die für Korrosions-, Emissions- und Verschlackungsprobleme verantwortlich ist, und zum anderen bestimmt sie die Vergärbarkeit der Biomasse und damit das Biogaspotential.

In dieser Studie wurden die oberirdischen Biomassen der Jena-Experiment-Flächen (aus den Jahren 2008 und 2009) geerntet und auf ihre Brennstoffqualität und ihre Vergärbarkeit entlang eines Artenzahlen-(AZ)-Gradienten und eines Funktionelle-Gruppen-Gradienten (Gräser, Leguminosen, kleine und große Kräuter) untersucht. Die Brennstoffqualität wurden anhand folgender Parameter analysiert: Brennwert ($H_o$), Bruttoenergieertrag (BE), Asche-, K-, Ca-, Mg-, N-, Cl- und S-Gehalt. In Bezug auf die Biogasproduktion wurden folgende Parameter untersucht: substratspezifische Methanausbeute ($CH_{4\ Substrat}$), flächenbezogener Methanertrag ($CH_{4\ Fläche}$), Rohfaser- (CF), Rohprotein- (CP), Rohfett- (CL) Gehalt und stickstofffreie Extraktstoffe (NfE). Außerdem wurde eine Möglichkeit der Brennstoffverbesserung mittels der Integrierten-Festbrennstoff-und-Biogasproduktion-aus-Biomasse (IFBB) untersucht und in Beziehung zu den Diversitätsgradienten gestellt

Es zeigte sich u.a., dass (i) der bedeutendste AZ-Effekt der Anstieg der Energieerträge, sowohl für die Verbrennung (Zunahme des jährlichen BE um 26% von $AZ_{8\to16}$ und um 65% von $AZ_{8\to60}$), als auch für die Vergärung (Zunahme des jährlichen $CH_{4\ Fläche}$ um 22% von $AZ_{8\to16}$ und um 49% von $AZ_{8\to60}$) war, bedingt durch die starke Beziehung der AZ und des Biomasseertrages; (ii) Leguminosen eine Schlüsselfunktion für die Nutzung von Grünlandbiomasse zur Energiegewinnung haben, da sie den Energiegehalt ($H_o$ und $CH_{4\ Substrat}$) der Biomasse und den Energieertrag (BE und $CH_{4\ Fläche}$) erhöhen; (iii) durch Verbrennung die höchsten Energieerträge erwartet werden können, aber die Biomasse aufbereitet werden muss, um das Emissions- und Korrosionsrisiko zu minimieren. Dies wurde mittels des IFBB-Verfahrens erreicht und die Gehalte an Asche (um 23%), N (28%), K (85%), Cl (56%) und S (59%) wurden reduziert, während die jeweiligen Konzentrationen keinen Zusammenhang mit der AZ zeigten.

# 10 Kurzfassung

## 10.1 Hintergrund

Das Ziel der Europäischen Union (EU; 27 Mitgliedsstaaten) den Anteil der erneuerbaren Energien am Gesamtenergiemix auf 20% bis zum Jahre 2020 zu erhöhen (Status quo in 2008 war 10%; European Commission, 2010) und die Notwendigkeit nachwachsende Energiequellen als Ersatz für fossile Brennstoffe zu identifizieren, hat die verschiedenen Formen der Biomasse (landwirtschaftliche, forstwirtschaftliche und Abfallbiomassen) in den Vordergrund gerückt.

Auf lange Sicht wird in der EU der landwirtschaftlichen Biomasse das größte Energiepotential zugesprochen, auch im Vergleich mit der forstwirtschaftlichen Biomasse (European Environment Agency, 2006). Durch anaerobe Fermentation wurden in 2010 in der EU allein 10.9 Millionen Tonnen Rohöläquivalent (Mtoe) als Biogas produziert (Deutschland, als Hauptproduzent, produzierte 6.7 Mtoe), was ein Anstieg um 31,3% zum Vorjahr war (EurObserv'Er, 2011a). Durch Verbrennung von Biomasse wurden im selben Jahr insgesamt 79.3 Mtoe in der EU als Primärenergie erzeugt (auch hier war Deutschland Hauptproduzent mit 12.2 Mtoe), wobei etwa die Hälfte durch die Verbrennung von Holz entstand und nur etwa 8% durch andere Pflanzenmaterialien bzw. Abfall- und Reststoffe (EurObserv'Er, 2011b).

Die zunehmende Produktion von Energie aus Biomasse geht einher mit einem Anstieg im Anbau von Energiepflanzen, wie z.B. Raps (für Bio-Diesel), Mais (Biogasproduktion) und Miscanthus oder Kurzumtriebshölzer (Verbrennung). Insbesondere die Biogasproduktion hat die landwirtschaftlichen Praktiken stark beeinflusst. So werden in Deutschland bereits 18% der landwirtschaftlich genutzten Flächen mit Energiepflanzen bestellt, wovon 45% für die Biogasproduktion, 43% für die Bio-Diesel-Produktion und nur 0.3% für den Anbau von Pflanzen zur Verbrennung genutzt werden (FNR, 2012). Diese Veränderung in der Nutzung von landwirtschaftlichen Flächen, die sich nicht nur auf Deutschland beschränkt, hat eine weltweite Diskussion über die Konkurenz zwischen Energie- und Lebensmittelproduktion und möglichen negativen Implikationen für die Umwelt ausgelöst (Fargione *et al.*, 2008; Petersen, 2008; Tilman *et al.*, 2009).

Als Teil eines nachhaltigen Szenarios für die Energieproduktion, bei dem der Verlust von Biodiversität, z.B. durch Rodung oder eine Intensivierung der Nutzung vermieden wird und

wertvolle Ökosystemfunktionen erhalten bleiben sollten vor allem Biomassen von wenig bewirtschafteten Flächen mit niedriger Produktivität genutzt werden (Tilman *et al.*, 2009), wie z.B. extensive Grünlandflächen.

Traditionell wurde die Biomasse von extensiv bewirtschafteten Flächen für die tierische Veredelung verwendet. Allerdings ist die Futterqualität dieser Biomassen, besonders in den entwickelten Ländern, immer weniger geeignet für Tiere mit hohen Milch- und Fleischerträgen. Daher lassen viele Landwirte diese Flächen, besonders in den Mittelgebirgslagen, zunehmend ungenutzt (Isselstein *et al.*, 2005). Für einige Regionen in Deutschland wird mit einem Rückgang in der Bewirtschaftung von Dauergrünlandflächen von 25% gerechnet (Rösch *et al.*, 2007). Als Folge des Rückgangs bis hin zu einer Aufgabe der Flächen, wird die Pflanzenvielfalt zurückgehen (Mitchley *et al.*, 2001). Extensive Grünlandflächen sind allgemein bekannt für ihre reiche Biodiversität und sind wertvolle Kulturflächen, auch auf Grund ihrer Ästhetik und ihres Erholungswertes. Außerdem erfüllen sie wichtige Ökosystemfunktionen, wie den Schutz des Grundwassers, den Schutz vor Erosion und den Erhalt der Böden (Wilkins und Vidrih, 2000). Oftmals sind diese Flächen Teile von Schutzgebieten, die von der EU gefördert werden (z.B. NATURA 2000; European Commission, 2012). Die Pflege dieser Flächen verlangt eine regelmäßige Umsetzung der Biomasse durch Beweidung oder Ernte, um die Artenzahl (AZ) zu erhalten und Sukzession zu vermeiden. Daher bietet eine energetische Nutzung dieser Biomasse gleichzeitig die Gelegenheit für (i) eine regelmäßige Pflege und den Erhalt der Flächen, und für (ii) eine nachhaltige Energieproduktion. Außerdem schafft eine solche Nutzung finanzielle Anreize die unabhängig von landwirtschaftlichen Fördermitteln sind und gleichzeitig der Pflege dieser wertvollen, extensiven Flächen dient.

Zurzeit ist nicht bekannt in welchem Rahmen extensive Grünlandbiomasse zur Energiegewinnung beiträgt. In Deutschland ist Grünlandsilage ein wichtiges Co-Substrat für die Biogasgewinnung (es wird in etwa 40% aller Biogasanalgen eingesetzt), neben dem Mais (welcher in 90% aller Anlagen genutzt wird) (Weiland, 2006; FNR, 2009). Wie viel extensive Grünlandbiomasse letztendlich zu der in Biogasanlagen verwendeten Grünlandsilage beiträgt, ist unklar, genauso wie der Anteil extensiver Grünlandbiomasse, die zur Verbrennung verwendet wird.

Die Nutzbarkeit von Dauergrünlandbiomasse für die Energieproduktion beruht auf einigen Schlüsselfaktoren, wie dem Energiegehalt, der chemischen Zusammensetzung der Biomasse und dem Energieertrag, welche bereits weitläufig untersucht wurden (Prochnow *et al.*, 2009a,

2009b). Für die Verbrennung ist die Brennstoffqualität von großer Bedeutung. Sie beeinflusst u.a. die Lebensdauer des Ofens und welcher Ofentyp besonders geeignet ist. Während der Kohlenstoff- (C), Wasserstoff- (H) und Sauerstoff- (O) Gehalt im Holz (Fichte) etwa 99% ausmachen, sind es bei Grünlandbiomasse, in Abhängigkeit von der Artenzusammensetzung, nur zwischen 90-93% (Hartmann, 2009). Die restlichen Bestandteile sind Elemente, die zur Aschebildung beitragen oder in die Gasphase übergehen und zu Emissionen führen. Der Aschegehalt ist negativ korreliert mit dem Energiegehalt (Jenkins *et al.*, 1996) und wird daher das Energiepotenzial des Substrates bestimmen.

Ein ideales Substrat zur Biogasgewinnung sollte reich an leicht abbaubaren Kohlenhydraten (z.B. Zuckern), Lipiden und Proteinen und gleichzeitig arm an Hemizellulosen und Lignin sein (El Bassam, 1998). Außerdem sollte das Verhältnis von Faser zu Protein (oftmals ausgedrückt durch das C/Stickstoff- (N) Verhältnis) ausgeglichen sein, da bei zu hohen Fasergehalten die Vergärbarkeit eingeschränkt ist (Buxton und Redfearn, 1997) und zu hohe Proteingehalte zu Prozessstörungen durch Ammoniakanreicherung führen können (Zubr, 1986).

Generell ist Biomasse von Extensiv-Grünland sehr heterogen in seiner Artenzusammensetzung und der AZ, was eine weite Variation in wichtigen Bioenergieparametern zur Folge hat. Dies ist ein klarer Nachteil im Vergleich zu Holzhackschnitzel oder Maissilage. Es ist daher wichtig für die Ermittlung des Energiepotentials von Grünlandbiomasse, die Artenzusammensetzung (bzw. die funktionellen Gruppen zu denen sie gehören) und die AZ zu kennen und in Beziehung zu den Bioenergieparametern setzen zu können.

Ein Gruppe von Wissenschaftlern hat beim Vergleich verschiedener Grünlandbiomassen von Flächen mit unterschiedlicher AZ Zusammenhänge entlang des AZ-Gradienten in Rohfaser- (CF) und Rohprotein- (CP) Gehalten beobachten können (White *et al.*, 2004). Solch eine Beziehung hätte direkten Einfluss auf die anaerobe Vergärbarkeit der Biomasse und damit auch auf den substratspezifischen Methanertrag ($CH_{4\ Substrat}$), da CF und CP diesen direkt beeinflussen. Eine Studie von Florine *et al.* (2006) hat bei unterschiedlichen Grünlandflächen mit unterschiedlicher AZ Unterschiede in Asche- und C-Gehalt gefunden, wobei der Brennwert ($H_o$) sich kaum unterschied. Außerdem wurde in mehreren Untersuchungen eine zunehmender Biomasseertrag mit zunehmender AZ beobachtet (Hector *et al.*, 1999; Tilman *et al.*, 2001), sogar wenn Dünger zugegeben und die Bearbeitung intensiviert wurde (Weigelt *et al.*, 2009). Dies ist von großer Bedeutung für den Bruttoenergieertrag durch

Verbrennung (BE) und den Bruttoenergieertrag durch anaerobe Fermentation (CH$_{4 \text{ Fläche}}$), die sich aus Biomasseertrag und Energiegehalt errechnen.

Andere Studien haben gezeigt, dass funktionelle Gruppen eine wichtige Rolle bezüglich der Bioenergieparameter spielen. Tonn *et al.* (2010) beobachtete einen höheren Aschegehalt in der Kräuterfraktion im Vergleich mit den Leguminosen und Gräsern, was die Kräuter weniger vorteilhaft für die Verbrennung erscheinen lässt. Außerdem kann erwartet werden, dass Leguminosen einen starken Einfluss auf CP-Gehalt, NO$_x$-Emissionen und Biomasseertrag haben werden, da sie, durch ihre Fähigkeit N aus der Atmosphäre über symbiotische Bakterien an ihren Wurzeln zu binden und N in ihrem Gewebe anzureichern, den Bestand mit zusätzlichem N versorgen können (Tomm *et al.*, 1995).

Um ein besseres Verständnis von der Heterogenität der extensiven Grünlandbiomasse und deren Einfluss auf die bioenergetischen Parameter bezüglich anaerober Fermentation und Verbrennung zu erlangen, wurden in dieser Arbeit artifizielle Grünlandbestände untersucht, die entlang eines AZ- und eines Funktionellen-Gruppen-Gradienten angelegt wurden. Außerdem wurde eine Möglichkeit der Brennstoffverbesserung untersucht und in Beziehung zu den Diversitätsgradienten gestellt. Die Brennstoffaufwertung erfolgte mittels der Integrierten-Festbrennstoff-und-Biogasproduktion-aus-Biomasse (IFBB) (Wachendorf *et al.*, 2009). Dabei wird das Ausgangsmaterial (AM) separiert in einen Presskuchen (PK), welcher verbesserte Brennstoffeigenschaften hat (Richter *et al.*, 2010) und einen Presssaft (PS), welcher reich an leichtverdaulichen, gut für die Biogasproduktion geeigneten, Bestandteilen ist (Richter *et al.*, 2009). Da das AM abhängig ist von der Zusammensetzung der funktionellen Gruppen und der AZ, wird auch die Brennstoffqualität des PK und die Verdaulichkeit des PS davon abhängig sein.

## 10.2 Zielsetzung

Die zentrale Annahme dieser Arbeit war, dass Veränderungen der bioenergetische Parameter, bezüglich der Verbrennung und der anaeroben Fermentation von Grünlandbiomasse mit sich ändernder AZ und Zusammensetzung der funktionellen Gruppen zu erwarten sind. Außerdem wurde davon ausgegangen, dass sich diese Effekte am besten mit artifiziellen Diversitätsgradienten untersuchen lassen, während die Varianz von abiotischen und biotischen standortspezifischen Faktoren klein gehalten wird.

Aus diesem Grund wurde das Jena-Experiment als Versuchsgrundlage gewählt, da es mit seinen 82 Parzellen möglichst vollständig die Parameter Funktionelle-Gruppen-Kombination

(FGK; *n* = 13), Funktionelle-Gruppen-Anzahl (FGZ; *n* = 4) und AZ (1, 2, 4, 8, 16 und 60 Arten) variiert, und sich im Rahmen eines extensiv genutzten Grünlandes bewegt. Das experimentelle Design (Roscher *et al.*, 2004; siehe Appendix) basiert auf den Erfahrungen vorangegangener Studien (Hector *et al.*, 1999; Tilman *et al.*, 2001), wobei die Pflanzenarten nicht, wie im einfachsten Fall, nur in Monokotyle und Dikotyle unterschieden wurden. Die funktionellen Gruppen wurden etwas spezifischer unterteilt, beispielsweise in eine eigene funktionelle Gruppe für die Leguminosen, da diese überproportional starke Effekte auf die Ökosystemprozesse haben können (Spehn *et al.*, 2002). Außerdem wurden die krautartigen Pflanzen, die oftmals als eine Gruppe gesehen werden, hier in zwei funktionelle Gruppen unterteilt: kleine und große Kräuter. Darüber hinaus war der Fokus des Experimentes die Effekte von AZ, FGZ und dem Vorkommen/Nicht-Vorkommen von funktionellen Gruppen so weit wie möglich zu trennen.

Das Ziel dieser Studie war es, zu untersuchen,

(i)      wie AZ und funktionelle Gruppen die Brennstoffqualität (bezüglich Verbrennung) und die chemische Zusammensetzung (bezüglich anaerober Fermentation) beeinflussen,

(ii)     wie AZ und funktionelle Gruppen den $H_o$ (errechnet aus C-, H- und N-Gehalt) und den $CH_{4\,Substrat}$ (errechnet aus den Weender-Fraktionen) beeinflussen,

(iii)    wie AZ und funktionelle Gruppen die entsprechenden Bruttoenergieerträge beeinflussen,

(iv)    wie die Brennstoffqualität sich vor und nach einer Verbesserung durch IFBB unterscheidet und ob die Diversitätsgradienten weiterhin einen Einfluss auf die Brennstoffqualität haben.

## 10.3 Schlussfolgerung

Das Verwenden von artifiziellen Grünlandbeständen zur Identifizierung von Diversitätseffekten bezügliche verbrennungs- und vergärungsrelevanter Bioenergie-Parameter war erfolgreich und erlaubt folgende Schlussfolgerungen:

(i)      Eine zunehmende AZ hat verschieden Effekte auf die Bioenergie-Parameter gezeigt, z.B. nahm $CH_{4\,Substrat}$, N- (und CP-), Mg- und S-Gehalt ab (wobei nicht alle Effekte vollständig entkoppelt waren von den Funktionelle-Gruppen-Effekten), $H_o$ war nicht beeinflusst und CF nahm zu. Der wichtigste Effekte war jedoch die Zunahme des BE (jährlicher BE stieg um 26% von $AZ_{8\rightarrow16}$ und um

65% von $AZ_{8\to60}$), als auch des $CH_4$ Fläche (jährlicher $CH_4$ Fläche stieg um 22% von $AZ_{8\to16}$ und um 49% von $AZ_{8\to60}$), bedingt durch eine positive Beziehung zwischen AZ und Biomasseertrag.

(ii) Es ist schwierig einzelne funktionelle Gruppen als ausschließlich vorteilhaft oder nachteilig für die Verbrennung oder anaerobe Fermentation zu identifizieren, da sie oftmals gegenteilige Effekte haben bezüglich der verschiedenen Parameter. Trotz alledem spielen die Leguminosen eine Schlüsselrolle für die bioenergetische Nutzung, da sie sowohl den Energiegehalt ($H_o$, $CH_4$ Substrat), als auch den Energieertrag (BE und $CH_4$ Fläche) erhöhen. Letzteren beeinflussen sie vor allem durch die indirekte Förderung des Wuchses anderer Pflanzen, was den Biomasseertrag erhöht.

(iii) Es kann davon ausgegangen werden, dass der jährliche BE doppelt so hoch sein wird wie der jährliche $CH_4$ Fläche (bei AZ = 16, 60), was die Verbrennung zu der bevorzugten Konversionsmethode macht. Allerdings muss die Brennstoffqualität noch verbessert werden, um emission-, korrosions- und verschlackungsbedingte Probleme in den Öfen zu vermeiden. Dies lässt sich am besten mittels der IFBB-Methode bewerkstelligen.

(iv) Die Brennstoffqualität konnte durch IFBB signifikant verbessert werden bezüglich Asch- (um 23% reduziert), N- (28%), K- (85%), Cl- (56%) und S-Gehalt (59%) und war unbeeinflusst von dem AZ-Gradienten. Außerdem konnte der $H_o$ um 3.4% erhöht werden und das Risiko von zu geringen Ascherweichungs-temperaturen wurde gemindert.

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# Appendix

**Table A.1** List of 60 plant species used in the Jena-Experiment and the respective functional group they were regarded as. Total number of species in each functional group is indicated.

| Grasses | Small herbs | Tall herbs | Legumes |
|---|---|---|---|
| Alopecurus pratensis | Ajuga reptans | Achillea millefolium | Lathyrus pratensis |
| Anthoxanthum odoratum | Bellis perennis | Anthriscus sylvestris | Lotus corniculatus |
| Arrhenatherum elatius | Glechoma hederacea | Campanula patula | Medicago lupulina |
| Avenula pubescens | Leontodon autumnalis | Cardamine pratensis | Medicago × varia |
| Bromus erectus | Leontodon hispidus | Carum carvi | Onobrychis viciifolia |
| Bromus hordeaceus | Plantago lanceolata | Centaurea jacea | Trifolium campestre |
| Cynosurus cristatus | Plantago media | Cirsium oleraceum | Trifolium dubium |
| Dactylis glomerata | Primula veris | Crepis biennis | Trifolium fragiferum |
| Festuca pratensis | Prunella vulgaris | Daucus carota | Trifolium hybridum |
| Festuca rubra | Ranunculus repens | Galium album | Trifolium pratense |
| Holcus lanatus | Taraxacum officinale | Geranium pratense | Trifolium repens |
| Luzula campestris | Veronica chamaedrys | Heracleum sphondylium | Vicia cracca |
| Phleum pratense | *n* = 12 | Knautia arvensis | *n* = 12 |
| Poa pratensis | | Leucanthemum vulgare | |
| Poa trivialis | | Pastinaca sativa | |
| Trisetum flavescens | | Pimpinella major | |
| *n* = 16 | | Ranunculus acris | |
| | | Rumex acetosa | |
| | | Sanguisorba officinalis | |
| | | Tragopogon pratensis | |
| | | *n* = 20 | |
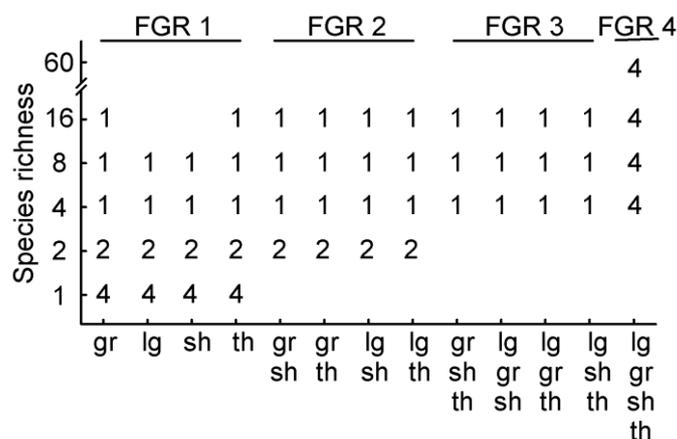


**Figure A.1** Combinations of functional groups and their replications (numbers) according to species-richness level. Each replication represents one of the plots used in this study; the total sum therefore is 82. The x-axis gives the different functional groups compositions (gr = grasses, lg = legumes, th = tall herbs and sh = small herbs). Functional-group richness (FGR) is shown on top.
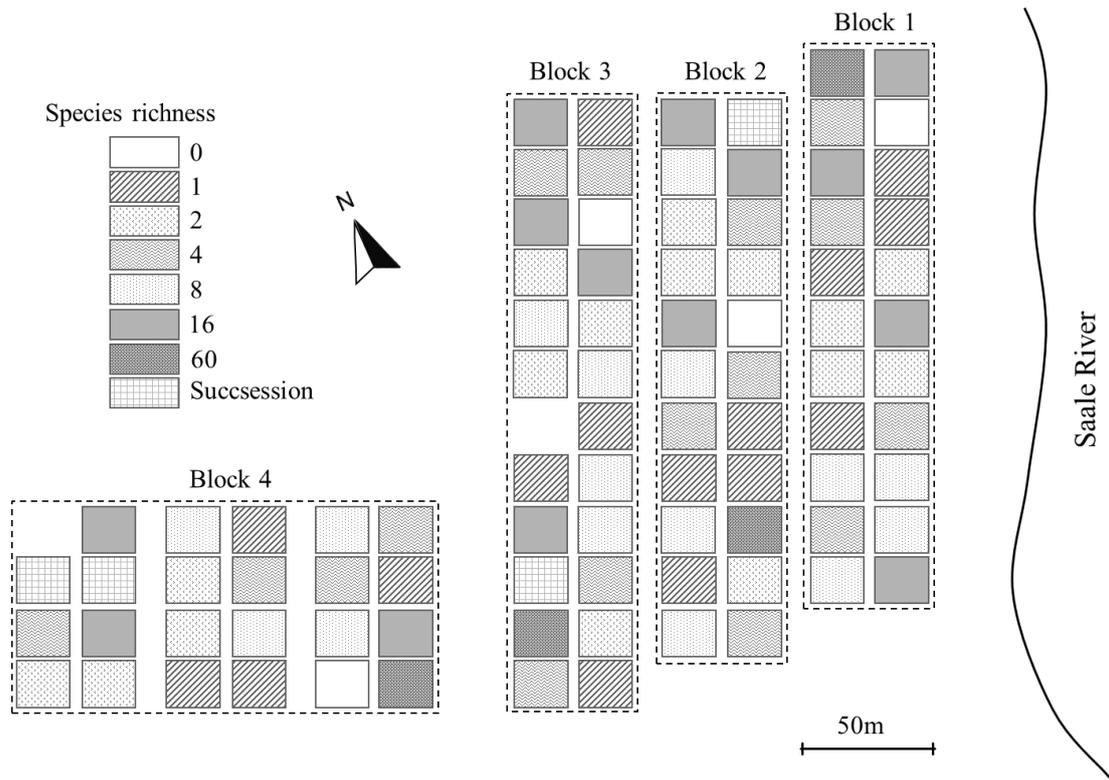
**Figure A.2** Map of the Jena-Experiment showing the different species richness treatments and their distribution across the four blocks.



**Figure A.3** Top-view on the Jena-Experiment site (© www.the-jena-experiment.de).