

Changing botanical composition of species-rich meadows through variation of management

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“Nur was der Mensch kennt, lernt er lieben.

Nur was er liebt, verteidigt er.“

Konrad Lorenz

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List of Acronyms

a	year
Ca	calcium
CO ₂	carbondioxide
DMY	dry matter yield
e.g.	exempli gratia (for example)
et al.	et alii (and others)
EU	European Union
FFH	Fauna Flora Habitat
ha	hectare
K	potassium
kg	kilogramm
LAZBW	Landwirtschaftliches Zentrum Baden-Württemberg
LUBW	Landesanstalt für Umwelt Baden-Württemberg
Mg	magnesium
N	nitrogen
n	number of samples
NEL	net energy lactation
P	phosphorus
PAR	photosynthetically active radiation
t	ton

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Figure 1 Lowland hay meadow distribution and conservation status from green = favourable (good) to red = unfavourable (bad) of the EU member states (European Environmental Agency 2012).

Figure 2 Mean DMY (dt ha⁻¹) and proportions of forbs (%) in the first growth at both sites of the years 2016-2018 with standard deviation (n = 36).

Abstract

Species-rich hay meadows are threatened habitats for many plant and animal species. The biomass of these meadows has traditionally been used as forage for extensively kept ruminants. During the last decades, their habitat quality and area have been declining. On the one hand, the decline of species-rich hay meadows could be caused by increased fertiliser use as well as earlier and more frequent cutting. On the other hand, a reduction or abandonment of agricultural usage reduces their floristic diversity. This reduces important ecosystem services provided by these habitats, e.g. pollination. Therefore, benchmarking data on botanical composition and biomass is needed to estimate effects of different fertilisation and cutting regimes.

This thesis aims at developing recommendations on how to manage species-rich hay meadows in order to conserve habitat functions and to enable agronomic usage at the same time. More specifically, management effects on botanical composition, biomass quantity and quality were investigated in a field trial running from 2013-2018. In this field trial, twelve different treatments were tested in a randomised block design at two sites in southern Germany. The different treatments consisted of a combination of three fertilisation levels (none, PK and NPK) and four different dates of first cut. The cutting date variants were chosen according to the phenology of dominant grasses before, at the beginning and at the end of flowering as well as a late cut at the seed-ripening stage. Both sites were species-rich hay meadows and each plot was fertilised annually and cut twice a year during the six-year field trial.

The first study investigates changes in botanical composition. The number of vascular plant species did not significantly change after four years of fertilisation or cutting-date treatments, but botanical composition was affected significantly. The proportion of grasses was promoted by NPK fertilisation at both sites and by early cutting dates at one site only. Forbs were replaced by grasses because this site was dominated by the annual species *Rhinanthus alectorolophus*, a hemiparasite relying on generative reproduction. The second study addresses the question if living conditions of small plant species are affected by changed management. It was shown that shading reduced the proportion of small plant species due to the increased dry matter yield (DMY) caused by NPK fertilisation. It was concluded that annual NPK fertilisation containing 35 kg N ha⁻¹ impairs the habitat function of species-rich hay meadows and, in the long term, excessive fertilisation could lead to reduced species numbers. Results of the third study revealed that although the date of first cut plays an important role in determining chemical composition of biomass, there is flexibility of choice at later cutting dates. Between the flowering and the seed-ripening stage, there were no significant differences in forage quality. However, late-cut hay is suitable as exclusive feed for horses only. Therefore, an early cut is recommended for use as biogas substrate and to be included into rations for extensively kept ruminants.

In conclusion, general patterns of management effects were detected: Annual NPK fertilisation decreases the habitat quality of species-rich hay meadows. Based on the three studies presented in this thesis, the date of first cut in perennial plant communities should be handled more flexibly. This would benefit farmers, because higher forage qualities can be achieved. However, it was shown that there are site-specific effects. Plant communities containing annuals can be severely affected by advanced cutting dates, and the phenology at the date of first cut is crucial for the survival of these species. Interacting negative effects of fertilisation and cutting date on habitat quality suggest that the combination of late cutting date and NPK fertilisation should be avoided. Therefore, the best management at whole-farm scale appears to be a rotational cut and an extensive fertilisation of single meadows.

Zusammenfassung

Artenreiche FFH-Mähwiesen sind als Lebensraum für viele Tier- und Pflanzenarten geschützt. Traditionell wurden sie als extensive Heuwiesen genutzt, doch ihre Habitatqualität und ihre Fläche gehen seit Jahrzehnten zurück. Zum einen sind die FFH-Mähwiesen durch die Intensivierung der Bewirtschaftung bedroht, z. B. durch einen verstärkten Einsatz von Düngern, einen früheren ersten Schnitt und eine höhere Schnitthäufigkeit. Zum anderen geht die floristische Vielfalt auch durch zu seltene Mahd oder Verbrachung bei Nutzungsaufgabe zurück. Dadurch werden wichtige Ökosystemdienstleistungen wie z.B. die Bestäubung verringert.

Ziel dieser Arbeit war es, Bewirtschaftungsempfehlungen zu entwickeln, die eine landwirtschaftliche Nutzung ermöglichen und gleichzeitig die Habitatfunktion der Mähwiesen sichern. Dabei wurden Auswirkungen variiertter Bewirtschaftung auf die botanische Zusammensetzung, die Trockenmasseerträge und Biomassequalität in einem Feldversuch von 2013 bis 2018 untersucht. Dieser Feldversuch wurde als randomisierte Blockanlage auf zwei Standorten innerhalb des FFH-Gebiets „Alb zwischen Jusi und Teck“ in Süddeutschland angelegt. Die zwölf untersuchten Behandlungen setzten sich aus einer Kombination von drei Düngungsstufen (ohne, PK und NPK) und vier Schnittvarianten (Zeitpunkt des ersten Schnitts) zusammen. Der Zeitpunkt des ersten Schnitts orientierte sich dabei an der Phänologie der Hauptbestandsbildner und variierte vom Stadium vor Beginn der Gräserblüte bis zur Samenreife. Während der gesamten Versuchslaufzeit wurden die Wiesen zweimal im Jahr gemäht und jährlich zum ersten Aufwuchs im März gedüngt.

In der ersten Studie werden Änderungen der botanischen Zusammensetzung durch Düngung und Schnittzeitpunkt untersucht. Es zeigte sich kein signifikanter Einfluss auf die Anzahl der Gefäßpflanzenarten, dennoch veränderte sich die botanische Zusammensetzung nach vier Jahren Versuchsdauer signifikant. An beiden Standorten wurde der Gräseranteil durch NPK-Düngung und an einem Standort zusätzlich durch einen ersten Schnitt vor der Samenreife gefördert. Hier erhöhte sich der Gräseranteil zulasten des Kräuteranteils, da die dort dominierende Art *Rhinanthus alectorolophus* als einjähriger Hemiparasit auf generative Vermehrung angewiesen ist.

Die zweite Studie untersuchte den Einfluss der Bewirtschaftung auf die Lebensbedingungen niedrigwüchsiger Pflanzenarten, die wichtig für die Habitatfunktion artenreicher Mähwiesen sind. Hier wurde gezeigt, dass die durch Düngung erhöhte Biomasseproduktion zu einer Beschattung der unteren Bestandschichten und gleichzeitig zu einer Abnahme niedrigwüchsiger Pflanzenarten führt. Daraus lässt sich schließen, dass schon eine NPK-Düngung mit 35 kg N ha⁻¹ pro Jahr die Habitatqualität der Wiesen verschlechtert und dass eine zu hohe Düngung langfristig die Artenvielfalt verringert.

Die Ergebnisse der dritten Studie zeigten schließlich, dass obwohl die Phänologie zum Zeitpunkt des ersten Schnitts die chemische Zusammensetzung des Aufwuchses bestimmt eine gewisse Flexibilität bei der Wahl des Schnittzeitpunktes gegeben ist. Zwischen der Hauptblütezeit der Gräser und dem Stadium der Samenreife bestand kein signifikanter Unterschied der Futterqualität. Dennoch ist das Heu ab dem Zeitpunkt der Gräserblüte nur für Pferde als alleiniges Futter geeignet. Daher wird ein früherer Schnitt für die Nutzung als Biogassubstrat oder für die Verfütterung in der extensiven Tierhaltung empfohlen.

Zusammenfassend zeigen die Erkenntnisse dieser Arbeit Handlungsoptionen zum Erhalt der artenreichen Mähwiesen auf: Eine Düngung wie in der getesteten NPK-Variante sollte vermieden werden. Der erste Schnitt kann vor der Samenreife der hauptbestandsbildenden Arten stattfinden, sofern nicht einjährige Arten den Bestand dominieren. Davon würden auch die Landwirte profitieren, da durch einen früheren Schnitt höhere Futterqualitäten erzielt werden. Der erste Schnitt sollte nicht

regelmäßig erst zum Zeitpunkt der Samenreife durchgeführt werden, um eine zu starke Beschattung niedrigwüchsiger Pflanzen zu vermeiden. Insbesondere die Kombination von NPK-Düngung und einem späten ersten Schnitt könnte zum Verlust wertgebender Arten führen. Daher wird als beste Bewirtschaftungsvariante eine angepasste Düngung alle zwei bis drei Jahre abgeleitet. Der erste Schnitt sollte im Zeitraum der Blüte der hauptbestandsbildenden Gräserarten (meist *Arrhenatherum elatius*) erfolgen. In einzelnen Jahren kann der erste Schnitt schon früher im Stadium des Rispschiebens erfolgen ohne negative Auswirkungen auf den Pflanzenbestand.

Chapter 1 General Introduction



Biodiversity is important because it maintains the adaptability of ecosystems to disturbances, like weather extremes caused by climate change. For example, long term studies show primary production of grassland with a higher number of plant species is more stable, e.g. resistant to drought (Tilman and Downing 1994). Therefore, biodiversity can be seen as an insurance (Baumgärtner 2008). Biodiversity secures the multiple functions that grasslands have and the services they provide for human well-being. For example, grasslands provide biomass, protection against erosion (Cerdan et al. 2010) and they can serve as a carbon sink (Jones and Donnelly 2004, Soussana et al. 2004). These services are called ecosystem services. According to the Common International Classification of Ecosystem Services (Haines-Young and Potschin 2018), they can be classified as provisioning, regulating and cultural services. Provisioning services are directly used by humans, for example biomass for various uses. Regulating services like for example carbon sequestration ensure ecosystem functions and represent indirect benefits like pollination, whereas cultural services, e.g. landscape and recreation value are noticed immediately. Pollination can be secured by a high number of flowering plant species (Ebeling 2008). Many ecosystem services like pollination depend on grassland biodiversity (Allan et al. 2015). However, it is not possible to maximise all ecosystem services at the same time because of trade-offs. These trade-offs will be explained in this chapter and discussed in the following chapters.

1.1 Botanical composition of species-rich hay meadows within the Natura 2000 network

Grassland area in Europe has been declining in the last decades (e.g. Pärtel et al. 2005). From 1967 till 2007 about 30 % of permanent grassland were lost in the EU, especially in Belgium, France, Germany, Italy and the Netherlands (Huyghe et al. 2014). Meanwhile, biodiversity losses have been increasing, leading to a mass extinction event that is caused by human intervention (Ceballos et al. 2015). Plant and animal species richness is still declining (Vickery et al. 2001), the main drivers being habitat loss caused by urbanisation, construction and intensification of land use (Socher et al. 2012, Habel et al. 2013). European grasslands are important for biodiversity, because they harbour many plant and animal species. Wilson et al. (2012) found the world record of plant species richness at small scale habitats was in managed, semi-natural temperate grasslands. These grasslands were traditionally used as hay meadows and are maintained by cutting or grazing. They serve as habitats for birds and many invertebrate species. For example, species-rich grasslands are very important for pollinators, especially lowland hay meadows (Kudrnovsky et al. 2020), because of their high flower density and diversity. In more intensively used meadows fewer plants reach the flowering stage before the first cut and fewer species occur. Furthermore, extensively managed hay meadows harbour more vascular plant species compared to abandoned meadows or woodland (Baur et al. 2006). Therefore species-rich hay meadows are protected by the EU Habitats Directive (European Commission 1992). The Habitats Directive aims at establishing a network of connected habitats throughout the EU, named the “Natura 2000 network” to facilitate movements and dispersal across borders. The directive states that a good conservation status of the habitats is to be maintained or re-established. For example, characteristic plant species must be present in certain amounts in a hay meadow. The conservation status of hay meadows depends on a high number of characteristic plant species, the canopy structure and possible disturbances (LUBW 2018). A favourable conservation status is reached if the habitat with its specific structure and functions is “likely to exist for the foreseeable future” (European Commission 1992). The main criterion for the conservation status of species-rich hay meadows is the botanical composition. It must be clearly assigned to the Arrhenatherion alliance / Polygono-Trisetion or Poion alpinae and contain a certain number of characteristic plant species. Each member state is obliged to report this conservation status each six years. Figure 1 shows that lowland hay meadows predominantly occur in Central Europe with Germany, Austria and the Czech Republic containing a great part of the area. Most of the EU hay meadows are found in Central Europe and their conservation status was bad in most member states by 2012 (Figure 1). This means, the habitat function is severely impaired, for example by losing plant species. In many cases, the conservation status was downgraded due to a change of botanical composition of these meadows, which leads to a loss of biodiversity. Recently, the European Commission filed suit against Germany for violations of the EU Habitats Directive, especially with regard to species-rich hay meadows (European Commission 2021).

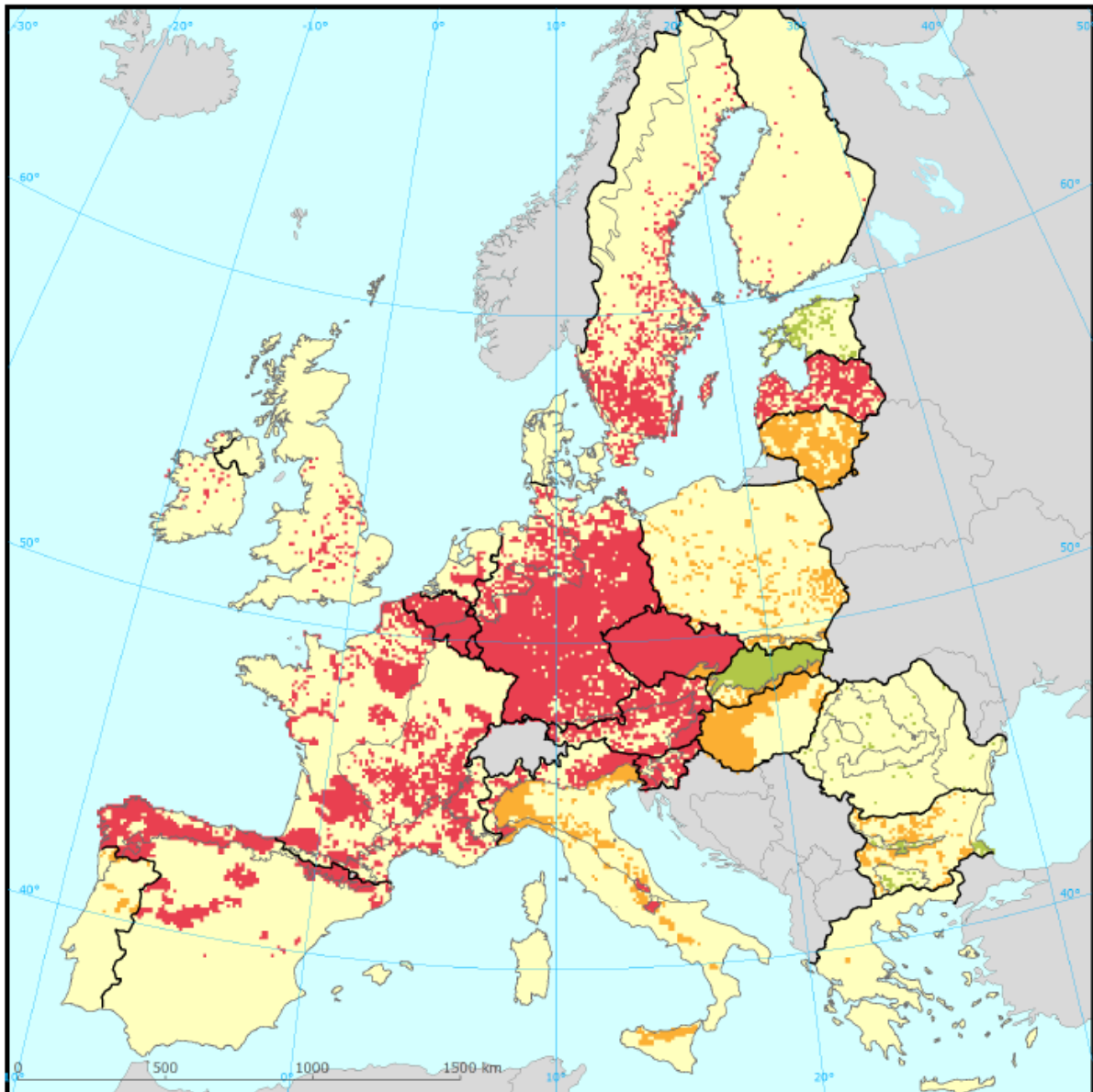


Figure 1 Lowland hay meadow distribution and conservation status from green = favourable (good) to red = unfavourable (bad) of the EU member states (European Environmental Agency 2012).

To improve the conservation status, it is important to identify the drivers of biodiversity loss. Hay meadows are multi-species communities and therefore they can react very differently to management changes. On the one hand, the decline in species richness can be due to intensification of management (e.g. Isselstein et al. 2005), for example intensive fertilisation (Crawley et al. 2005). On the other hand, too extensive management or abandonment threatens species-richness of meadows (Bakker and Berendse 1999, Lindborg et al. 2004). To maintain species-rich hay meadows, mowing is necessary (e.g. Valko et al. 2012). Concerning mowing, the time of the first cut can influence the botanical composition of meadows strongly (Kirkham and Tallowin 1995). Nowadays, an early cut is preferred for silage use instead of making hay and the haymaking period gets shorter (e.g. Jefferson 2005), due to the requirements of modern dairy cattle. Another aspect of management is fertilisation to compensate for nutrient removal by mowing and therefore secure dry matter yield (DMY). Tonn and Elsaesser (2014) recommend cutting these meadows twice and to fertilise them only extensively with farmyard manure or mineral fertiliser up to 35 kg P_2O_5 ha⁻¹ and 120 kg K_2O ha⁻¹ every two to three years. Annual

fertilisation is not necessary because organic fertilisers supply Nitrogen (N) beyond the year of application (Schröder et al. 2007). Hence, the fertilisation of previous years can affect DMY of species-rich hay meadows. Determining or calculating the limits of fertiliser amounts that are applicable to these meadows without reducing species richness is very difficult because the productivity depends on site-specific conditions as well. The challenge is to predict the influence of combined fertilisation and cutting regimes on botanical composition of species-rich hay meadows.

1.2 Competition in species-rich hay meadows

Species can coexist using different ecological niches. The term “ecological niche” is a functional term describing the sum of environmental variables influencing species and allowing them to exist (Silvertown 2004). The causes why changing the management of meadows influences their species richness and botanical composition are often plant-plant interactions like competition or facilitation (Harpole and Tilman 2007). If the supply of a resource is limited due to the interaction of two species, they compete with each other. Competition can take place above- or belowground. Competition can lead to niche separation, but also to competitive exclusion. Light is an important resource for plants, because they use it as an energy source. The availability of light to a plant within a meadow depends on the difference in height and the shape of its neighbouring plants. For example, a tall and a small species compete for light if they grow at the same spot. Most plant species can tolerate shade, but only to a certain degree. The species with the higher growth rate, here the tall one, will outcompete the other because it casts shade on the smaller species. Eventually, the small species gets locally extinct and the tall one dominates the spot. Moderate disturbances like grazing or mowing of meadows prevent this competitive exclusion, e.g. of grasses and shrubs in abandoned meadows. However, with increasing disturbance, e.g. mowing frequency, only a few adapted competitive species will remain. Therefore, many plant species coexist in a meadow because of a regular cut.

Plant availability of photosynthetically active radiation (PAR) can be distributed heterogeneously, especially in grasslands (del Pino et al. 2015). The heterogeneity of light distribution was examined vertically (Liira and Zobel 2000) and horizontally (Derner and Wu 2001). Especially vertical distribution of light can be crucial for slow-growing, small plants, because fast-growing, tall plants cast their shade aggravating the situation at high vegetation density (Tilman and Downing 1994). Hautier et al. (2009) showed in a glasshouse experiment that shade is limiting the productivity of small plants in fertilised grassland communities. In most cases, small plant species are low-nutrient indicating plant species, which are characteristic of lowland hay meadows and crucial for their habitat function. If small plant species get lost, biodiversity will get reduced and the conservation status of species-rich hay meadows will be downgraded. For this reason, competition for light could lead to hay meadow biodiversity loss, for instance due to increased vegetation density. For example, a seven-year study showed that the amount of 24 t manure reduced species richness, while half the amount (12 t per ha) applied annually maintained species richness of hay meadows at some sites in England (Kirkham et al. 2008). However, the composition of organic fertilisers like manure remains often unknown, whereas amounts and chemical composition of mineral fertilisers can be charged exactly. Mineral fertilisers affect the growth of grasses stronger than organic fertilisers because the provided nutrients are more readily available, especially during spring growth. Moreover, applying only mineral N may lead to a deficiency of other nutrients, e.g. phosphorus (P) or potassium (K). In the Rengen grassland experiment, species richness was reduced by NP-fertilisation and not by N application alone because the productivity at a two-cut regime proved to be P-limited (Hejcman et al. 2010). Here, an oligotrophic grassland dominated by *Nardus* species was converted into a mesotrophic hay meadow dominated by *Arrhenatherum elatius*

through fertilisation over decades. Another long-term fertilisation experiment showed only small fertilisation effects on botanical composition (Hejman et al. 2014). This experiment was performed on a relatively species-poor grassland under a three-cut regime, suggesting that botanical composition is less affected by fertilisation on more productive grassland. The Park Grass experiment in England showed that the most adverse effects on plant species richness can be achieved by combining N and P mineral fertiliser. Furthermore, this fertilisation treatment led to high DMY (Crawley et al. 2005). Therefore, it is important to assess fertilisation effects and interactions with cutting date on competition in grassland plant communities.

1.3 Biomass and potential uses of species-rich hay meadows

Although the habitat function is a focus of these species-rich hay meadows, other ecosystem services need to be considered, especially services like providing biomass for different uses. The meadows need management, this means a regular cut and to make this management economically viable biomass must be used. The biomass of species-rich meadows was traditionally used as forage. To integrate this biomass into modern livestock systems is a challenge because in many cases its energy and protein content is not sufficient to meet the requirements for example of dairy cattle (e.g. Hofmann et al. 2010, Donath et al. 2015). Biomass of species-rich hay meadows differs from that harvested at intensively used meadows in quantity and quality. The annual dry matter yield (DMY) of species-rich hay meadows varies between 4.7 and 5.7 t ha⁻¹ (Kirkham and Tallowin 1995, Tonn et al. 2010), but in general it is lower than the DMY of intensively used meadows. In addition, the quality of the biomass is often unknown, because it contains different plant species at different stages of maturity at the time of cut. Thus, the management, for example fertilisation, can influence chemical composition both directly and indirectly through the change of botanical composition. For extensively used hay meadows, alternatives like energy or material use are often discussed (Thumm 2018). The low protein content of biomass from extensively used meadows is beneficial for the use as biogas substrate. Furthermore, combustion of biomass could be of certain interest, because of the high fibre contents of late-cut hay, as suggested by Van Meerbeek et al. (2015) for heathland biomass.

1.4 Objectives and research questions

The aim of this thesis is to assess management effects on DMY, botanical and chemical composition of species-rich hay meadows. With this knowledge, specific management options and usage of the biomass can be recommended. The research questions were:

- How does botanical composition change through management of species-rich meadows?
- What influences have management practises on living conditions of small plants within these meadows?
- How does hay meadow management change quantity, quality and usability of biomass?
- Are there interactions between management, DMY and botanical composition?

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Chapter 2 Managing species-rich hay meadows for biodiversity: causes and effects



It is important to understand the underlying mechanisms of management effects to predict effects of fertilisation and cutting regime and to ensure future habitat quality. Therefore, I investigated on living conditions of small plant species within semi-natural plant communities. This chapter includes two studies on the causes and effects of changed botanical composition in species-rich hay meadows.

2.1 Management effects on botanical composition of species-rich meadows within the Natura 2000 network

Paper I

Title: Management effects on botanical composition of species-rich meadows within the Natura 2000 network

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Summary:

Species-rich hay meadows require regular cutting to maintain their botanical composition. Date of first cut and fertilisation both affect the botanical composition and agricultural productivity. The deviation from traditional cutting dates has reduced species richness in hay meadow field trials (Kirkham and Tallowin 1995, Smith et al. 1996). However, a combination with different fertilisation levels has rarely been tested.

At two species-rich hay meadows (Habitat Type 6510) in south-west Germany, a field trial was established to examine the effect of fertilisation and date of first cut on the botanical composition of the first growth. The first site (Swabian Jura) was located on the Swabian Jura and the second site (Foothills) at the foothills of this low mountain range. The plant community at Swabian Jura was dominated by *Rhinanthus alectorolophus* and the plant community at Foothills was comprised of typical lowland hay meadow species like *Arrhenatherum elatius* and *Salvia pratensis*. The conservation status was rated as “B” (good) at Swabian Jura and as “A” (excellent) at the Foothills site.

The randomised block design with three replications tested the three fertilisation variants none, PK and NPK, combined with four different dates of first cut based on growth stages. All plots were cut two times per year, with the second cut being performed at one day in September. Separate statistical analyses were performed for each site using R (R Core Team 2016), Excel 2013 (Microsoft Corporation) and SAS (version 9.3, SAS Institute). Multivariate statistics were performed using Canoco for Windows (ter Braak and Smilauer 2006).

At Foothills, species richness did not decline on unfertilised plots. After four years of field trial, species richness had declined by 11-20% due to NPK-fertilisation at both sites. Further possible causes for this decline can be the observer effect (Couvreur et al. 2015), the management prior to the field trial and the fact that biomass was removed from the plots immediately after the cut without drying on the field. Kirkham et al. (2008) found no differences in species richness with similar amounts of fertiliser after 7 years of application, but Socher et al. (2012) report a reduction of 19% with 35 kg N ha⁻¹ applied each year on the Swabian Jura. After four years of application, fertilisation has led to increased P and K contents in soil in our field trial.

At early dates of first cut, yield proportions of grasses increased at the expense of the annual *Rhinanthus alectorolophus* and the biennial *Tragopogon pratensis* at Swabian Jura. Therefore, the conservation status was downgraded. It was also graded down for NPK-fertilised plots at Foothills. Therefore, fertilisation of lowland hay meadows with mineral N should be avoided. The field trial demonstrates that meadows with low proportions of annuals are able to regenerate despite an early date of first cut.

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2.2 Different management practices influence growth of small plants in species-rich hay meadows through shading


Paper II

Title: Different management practices influence growth of small plants in species-rich hay meadows through shading

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RESEARCH ARTICLE

Different management practices influence growth of small plants in species-rich hay meadows through shading

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Abstract

Aims: The intensification of grassland management can cause a loss of biodiversity via alteration of the structure and micro-environmental conditions within the sward. The species richness of hay meadows protected within the Natura 2000 network is often endangered by high levels of fertilisation and relatedly, increased cutting frequency, which leads to decreased light availability for small plants and seedlings at ground level. The purpose of this study is to analyse how management practices and the interaction of fertilisation and cutting regime affect light distribution in grassland canopies as well as the growth of small plants.

Location: South Germany.

Methods: This was investigated by measuring the photosynthetically active radiation (PAR) and dry matter yield (DMY) of the first growth cycle over 2 years at two sites of a 5-year-old hay meadow trial. To calculate the relative light intensity, PAR was measured above the canopy and at ground level. Two factors, fertilisation (none, PK, NPK) and date of first cut (based on phenological stages), were manipulated.

Results: Our results show that the date of the first cut had no influence on light availability during the first growth cycle, whereas fertilisation decreased light availability for small plants due to a denser canopy. Fertilisation increased the DMY of the first growth cycle significantly. We show that NPK fertilisation (35 kg N, 35 kg P₂O₅ and 120 kg K₂O ha⁻¹ a⁻¹) decreased both the number and proportion of small plant species.

Conclusion: NPK fertilisation can lead to a loss of biodiversity. These results are of relevance for the improvement and management of species-rich hay meadows. Because of its effect on competition for light, the supply of plant-available nitrogen is a key driver in obtaining or restoring biodiversity in species-rich hay meadows. Therefore, careful site-adapted fertilisation is a basic requirement in maintaining the ecological value of grassland.

KEYWORDS

above-ground competition, fertilisation, grassland management, hay meadow, Natura 2000, relative light intensity, species richness

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1 | INTRODUCTION

In past decades, the species richness of hay meadows has been declining throughout Europe, as reported, for example, by Critchley et al. (2007) for England and Prince et al. (2012) for Spain. Hay meadows serve as habitats for many bird and invertebrate species (Vickery et al., 2001) and for this reason they are protected as habitat types by the European Union (EU) Habitats Directive (European Commission, 1992). At most sites in Central Europe, grassland management prevents succession (Ellenberg, 1982). However, with increased cutting frequency and/or fertilisation, only a few adapted competitive species remain owing to competitive exclusion. Stress tolerance and low-intensity disturbance allow small and large neighbouring plants species to co-exist (Grime, 1981). Competition occurs when shared resources are limited. Factors limiting plant growth include the availability of soil nutrients, water and light. Intensive fertilisation increases nutrient availability in soils and therefore above-ground biomass production. Increasing the productivity of grasslands leads to a shift from below-ground to above-ground competition (DeMalach & Kadmon, 2017; Grime, 1979). For example, fertilisation reduces competition for nutrients in the soil but intensifies competition for light. Hautier et al. (2009) demonstrated in a greenhouse experiment that shading limits the productivity of small plants in fertilised grassland communities. Hautier et al. stated that competition for light is the main cause of diversity loss through fertilisation. The availability of photosynthetically active radiation (PAR) is essential for the survival of plants, especially small plants and seedlings (e.g., *Salvia pratensis*, Hegland et al., 2001). Canopy shade can decrease the temperature at ground level, potentially leading to a change in microclimate at the soil surface and influencing, for example, seedling establishment (Leishman et al., 2000). The availability of PAR near ground level is important for seedlings and small plant species (Roscher et al., 2011) and its reduction can hinder seed germination completely (Voigtländer & Jacob, 1987). In addition, light can break the dormancy of buried seeds (Wesson & Wareing, 1967). PAR availability at the soil surface depends on canopy structure, for example, the density of individual plants, growth form and height of fully grown plants. In some cases, it can promote seed germination (Grime et al., 1981). If light is a limited resource, tall plants have a clear advantage compared with small plants. Shading is a type of stress that limits the dry matter yield (DMY) of smaller and slowly growing species (Grime, 1979). In mesic grassland, most species—in particular small species—require regular cutting to survive in the long term (Roscher et al., 2011), because each cut increases light availability at the ground level (Derner & Wu, 2001). On the one hand, an early cut promotes the growth of grasses, which can lead to a denser canopy in summer, casting more shade (Petersen & Isselstein, 2015). On the other hand, canopy stratification occurs only in tall, mature grassland (Liira & Zobel, 2000), so delaying the first cut can also lead to a denser canopy. This raises the question of how management practices affect light availability and distribution in species-rich grassland canopies at various points in time, and how these temporal changes in light distribution influence spatial and

temporal resource partitioning within the canopy as the standing biomass increases. To determine the effect of different management practices on light distribution in grassland canopies, we conducted a study that examined PAR availability and the biomass of small plant species in the fifth and sixth years of a hay meadow field trial. Taking the co-existence of large- and small-statured plant species within species-rich plant communities into consideration, we hypothesise that in fertilised plots, light availability is decreased. Furthermore, the observations suggest that the number of small plant species will be reduced by fertilisation due to a denser canopy. These hypotheses are explored below.

2 | METHODS

2.1 | Field trial

This study was conducted in 2017 and 2018 in a lowland hay meadow field trial near Stuttgart, south Germany. The field trial was established in 2013 at two sites and tested 12 treatments in a randomised block design with three replicates. The treatments were combinations of three fertilisation and four cutting-date variants. The fertilisation variants were: “none” (unfertilised), “PK” (35 kg P_2O_5 and 120 kg K_2O $ha^{-1} a^{-1}$) and “NPK” (35 P_2O_5 and 120 kg K_2O and 35 kg N $ha^{-1} a^{-1}$). These were combined with the four different dates for the first cut: C1 (before), C2 (at the beginning), C3 (at the end) and C4 (after the main grass flowering period). The second cut was performed on the same day in September on all plots. Each plot (25 m²) was cut twice a year at both sites. Biomass samples of a defined area were weighed fresh in the field and again after drying in an oven until constancy of weight was achieved (60°C, ≥48 h) to calculate DMY in t per ha, as described in Boob et al. (2019a).

2.2 | Experiment

PAR was measured at two experimental sites: “Foothills” (48°31′38.5″N, 9°31′53.9″E), a lowland hay meadow at 470 m a.s.l., and “Swabian Jura” (48°34′27.8″N, 9°26′29.7″E), located at 774 m a.s.l.

For all cutting variants (May–July), photosynthetically active radiation (PAR = electromagnetic radiation between wavelengths of 400 nm and 700 nm) was measured 1–9 days prior to each first cut date (Table 1). In 2017, all plots were measured on each measurement date (M), whereas in 2018 only the first growth was measured. This means that, in 2018, measurements were taken only on the uncut plots.

Radiation was measured when there were no clouds or slowly changing conditions in full sunlight, preferably within 3 h of solar noon, using a Sun Scan Canopy Analysis System (type SS1, Delta T Devices Ltd, Cambridge, UK) with a probe. The probe was 1 m long and pushed horizontally into the plots facing the sun (south) at three different spots per plot. For each spot, PAR was measured three

TABLE 1 Cutting variants (C1–C4) with dates of measurement at the two experimental sites in 2017

Site	Measurement date	Date of first cut
Foothills	10 May	17 May (C1)
Swabian Jura	16 May	
Foothills	23 May	31 May (C2)
Swabian Jura	26 May	
Foothills	19 June	21 June (C3)
Swabian Jura	13 June	
Foothills	4 July	5 July (C4)
Swabian Jura	26 June	

times at the soil surface (ground) and above the grassland canopy (above). The mean value of 26 photodiodes (photodiode 4–29) was calculated for each plot and height.

In addition, yield proportions of small plant species were estimated visually as a percentage of DMV according to the method of Klapp/Stählin described in Voigtländer and Voss (1979). Small plant species were defined as those with an average height ≤ 0.5 m, as defined in Jäger et al. (2013), and included *Ajuga reptans*, *Anthoxanthum odoratum*, *Bellis perennis*, *Briza media*, *Campanula rotundifolia*, *Cerastium holosteoides*, *Colchicum autumnale*, *Glechoma hederacea*, *Hypochaeris radicata*, *Lotus corniculatus*, *Plantago lanceolata*, *Plantago major*, *Plantago media*, *Primula veris*, *Prunella vulgaris*, *Ranunculus bulbosus*, *Taraxacum* sect. *Ruderalia*, *Veronica chamaedrys*, *Veronica filiformis* and *Vicia angustifolia*. The grassland communities of the trial were dominated by either *Arrhenatherum elatius* or *Trisetum flavescens*. The botanical composition of this trial is described in detail in Boob et al. (2019b) and nomenclature follows the Euro+Med (2021).

2.3 | Statistical analyses

The aim of the statistical analyses was to estimate light availability at ground level relative to that above the canopy, to ascertain how this ratio is influenced by differences in cutting date and fertilisation. Relative light intensity (RLI) can be calculated as

$$RLI_{hi} = \left(\frac{PAR_{g_{hi}}}{PAR_{max_{hi}}} \right) \times 100, \quad (1)$$

where $PAR_{g_{hi}}$ is the mean PAR at ground level of the i th fertiliser of the h th cutting date and $PAR_{max_{hi}}$ is the corresponding mean PAR above canopy. To estimate this ratio, the height of the measurement (two levels: ground and above) was treated as a third factor and the following additive three-way mixed model was fitted to each site-by-year combination:

$$y_{hijk} = \mu + b_k + \alpha_h + \beta_i + \gamma_j + (\alpha\beta)_{hi} + (\alpha\gamma)_{hj} + (\beta\gamma)_{ij} + (\alpha\beta\gamma)_{hij} + e_{hijk}, \quad (2)$$

where b_k is the random block effect of block k ; α_h , β_i and γ_j are the main effects of the h th cutting date, the i th fertiliser and the j th height level, respectively. $(\alpha\beta)_{hi}$, $(\alpha\gamma)_{hj}$, $(\beta\gamma)_{ij}$ and $(\alpha\beta\gamma)_{hij}$ denote the two- and three-way interactions of corresponding main effects. e_{hijk} is the error of observation y_{hijk} . A homogeneous error variance was assumed. All effects except for block and error were assumed as fixed. Note that data were assumed to be log-normally distributed and therefore were transformed logarithmically prior to the analysis. In this case, a difference in means on the log-normal scale (calculated using Equation 2) corresponded to the ratio after back-transformation (shown in Equation 1). Thus, to estimate ratios between ground and above measurements, the difference between both means was estimated on the transformed scale. To compare two ratios, the difference between two of these differences is required. The latter required the use of linear contrasts. Ratios and their differences were compared via Fisher's least significant difference (LSD) test in case of a significant global F -test of the corresponding interaction with height.

For DMV and the proportion of small plants, the model was simplified by dropping all effects containing height from the model. DMV values and proportions of small plants were logarithmically transformed prior to analysis. Estimates and their standard errors were back-transformed for presentation purposes only. The delta method was used to calculate the approximate standard errors.

For small plant species numbers, data from both years were analysed together. The mixed model can be described as follows:

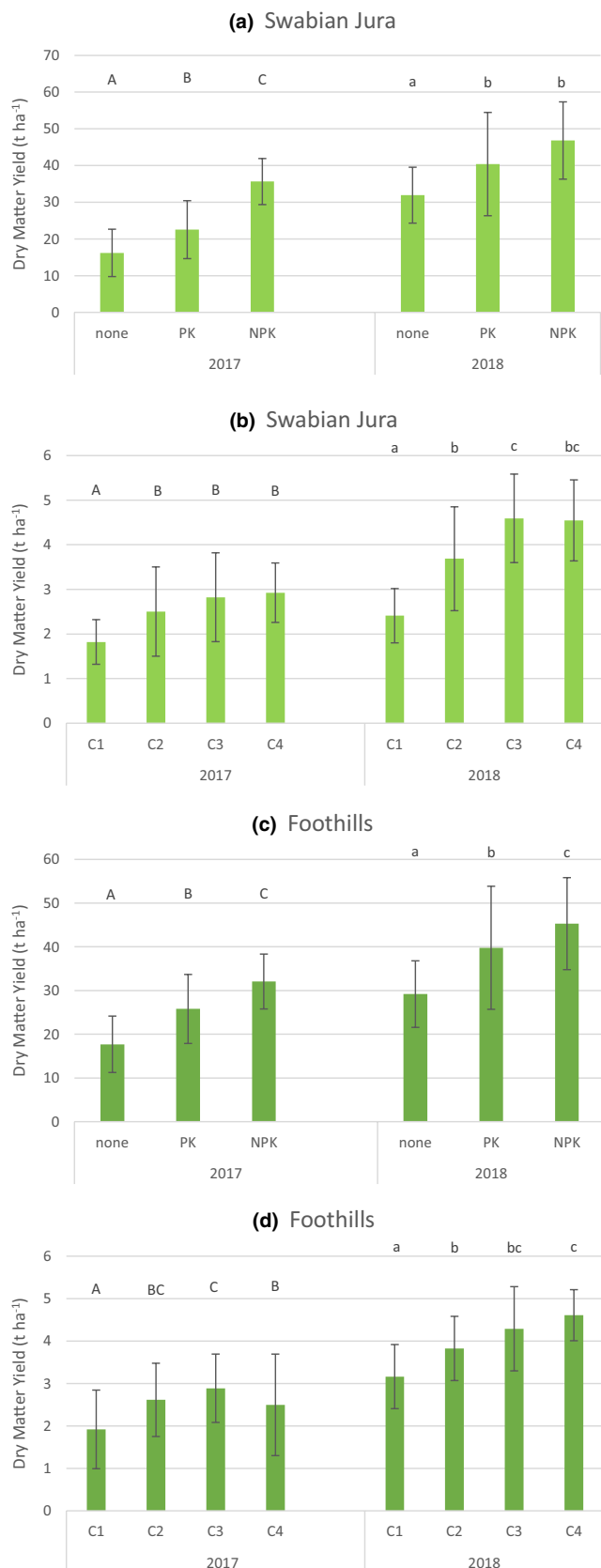
$$y_{hikl} = \mu + j_l + b_{kl} + \alpha_h + \beta_i + (\alpha\beta)_{hi} + (\alpha j)_{hl} + (\beta j)_{il} + (\alpha\beta j)_{hil} + e_{hikl}, \quad (3)$$

where j_l is the fixed effect of the l th year, $(\alpha j)_{hl}$, $(\beta j)_{il}$ and $(\alpha\beta j)_{hil}$ are the corresponding 2- and 3-year interaction effects with year and treatment factors fertilisation and cutting date, and all other effects are defined analogous to Equation 1. Because data were taken from the same plots in both years, a compound symmetry structure with year-specific variances was fitted for block and error effects. Again, Fisher's LSD test was performed in case of finding differences via global F -test.

3 | RESULTS

3.1 | Dry matter yield of the first growth

The “year \times fertilisation” and “year \times cutting date” interactions were significant (Appendix S1, Table A3). Therefore, DMV means were calculated separately for each year and fertilisation level/cutting date. The DMV of the first cut was slightly higher in 2018 (c. 4.5 t/ha on NPK-fertilised plots) than in 2017 (Figure 1). DMV was significantly increased by fertilisation on all dates at both sites. The DMV of the first cut was always increased by delaying the cutting date, with the exception of the Foothills site in 2017.



3.1.1 | Relative light intensity

RLI (%) at ground level was significantly influenced by measurement date. Interactions with the factor cutting date were not significant

FIGURE 1 Back-transformed means of dry matter yield (t/ha) of the first growth at Swabian Jura in (a) 2017 and (b) 2018, and at Foothills in (c) 2017 and (d) in 2018 with standard errors ($n = 3$). Values with at least one identical lower case letter indicate non-significant differences between cutting dates within a year and site. Values with at least one identical upper case letter indicate non-significant differences between fertilisation variants within a year and site ($\alpha = 0.05$)

in 2018, whereas in 2017 they were significant on the second, third and fourth measurement dates (Appendix S1, Table A1).

Because of differing light conditions on the measurement dates (M) in 2017, the means of the treatments are presented separately for each measurement date (Figure 2). RLI was significantly higher for unfertilised plots (none) in the first growth at both sites. At Foothills, RLI decreased between M3 and M4 in the variant that was uncut at the time of measurement (C4) for all fertilisation variants. This was not the case at Swabian Jura; here, RLI increased slightly on later measurement dates in 2017 at unfertilised plots (Figure 2a).

RLI (%) was c. 75%–100% when measured a few days after the first cut (Figure 3). In 2017, cutting variant C1 showed significant differences in RLI between fertilisation levels for the second growth. This was the case in variant C1 when measured 40, respectively 48 days after the first cut (M4) at both sites (Figure 3). Other cutting variants showed no significant differences. The DMY of the first cut is linked to the RLI (%) on the first measurement date in both years (Figure 4).

The “year \times fertilisation” interactions were significant for the proportion of small plant species (Appendix S1, Table A2). Therefore, means were calculated separately for each year and fertilisation level. NPK fertilisation had a significant influence on small plant species. It reduced the yield proportion of small species at both sites in 2018, but only at the Swabian Jura site in 2017 (Figure 5). There was also a significant influence of cutting regime on the proportion of small species (Appendix S1, Table A2).

The number of small plant species per plot was significantly influenced by fertilisation at both sites and by date of first cut at the Foothills site (Appendix S1, Table A4). Species numbers were decreased by NPK fertilisation (Figure 6). At the Foothills site, species numbers were significantly lower at late dates of first cut in 2018.

4 | DISCUSSION

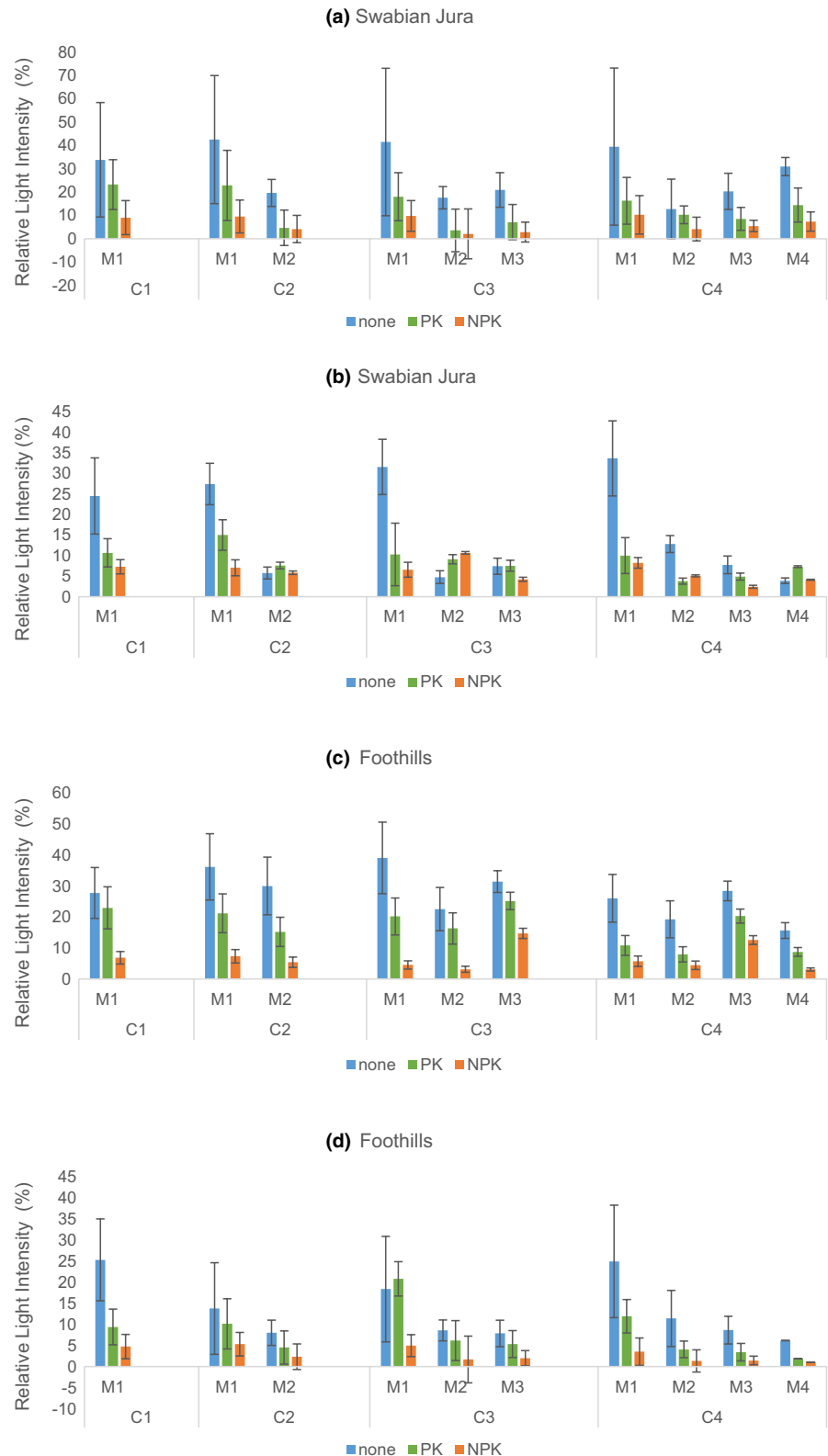
4.1 | Effects of cutting date

On the first measurement date before the first cut, cutting date had no significant influence on RLI. This means that the cutting date in previous years had no influence on the light availability at ground level in May of the measurement year. As expected, light availability was very high at subsequent measurement dates after the plots had been cut, because all plants were cut to the same height (c. 2–5 cm).

We were able to detect an effect of cutting date on the proportion of small plant species in both years of the study. Because



FIGURE 2 Back-transformed means of relative light intensity (%) per fertilisation level for each cutting-date variant (C1 = early cut, C4 = late cut) and development over measurement dates (M1–4), for the first growth at Swabian Jura in (a) 2017 and (b) 2018, and Foothills in (a) 2017 and (d) 2018 with standard errors ($n = 3$)



most of these species are perennials, they do not require annual seed ripening for their persistence. However, at one site, the species richness of small plants was decreased at later dates for first cut (Figure 6).

DMY of the first cut, that is standing biomass, increased at uncut plots with later measurements in 2018. In the same year, RLI of

variant C4 decreased at later measurement dates also (Figure 2b,d). In our experiment, depending on fertilisation, light availability decreased again in the regrowth after the first cut. The light availability in the second growth depended on the time between the cut and the measurement being taken (100% after 6 days; c. 80% after 19 days).

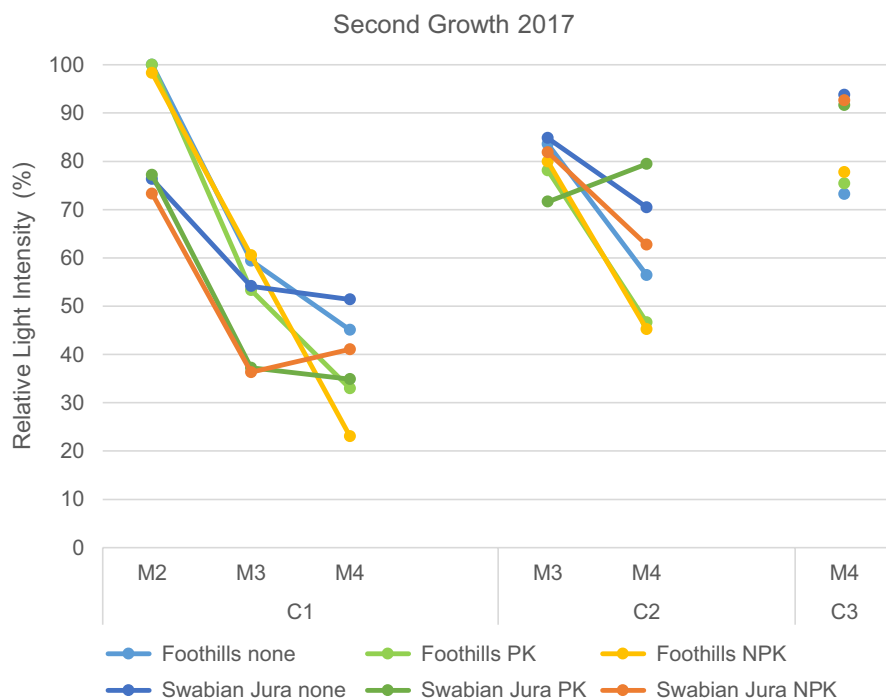


FIGURE 3 Back-transformed means of relative light intensity (%) per fertilisation level for each cutting date-variant (C1 = early cut, C3 = late cut) and development over measurement dates (M2–4), for the second growth at Swabian Jura and Foothills in 2017 ($n = 3$)

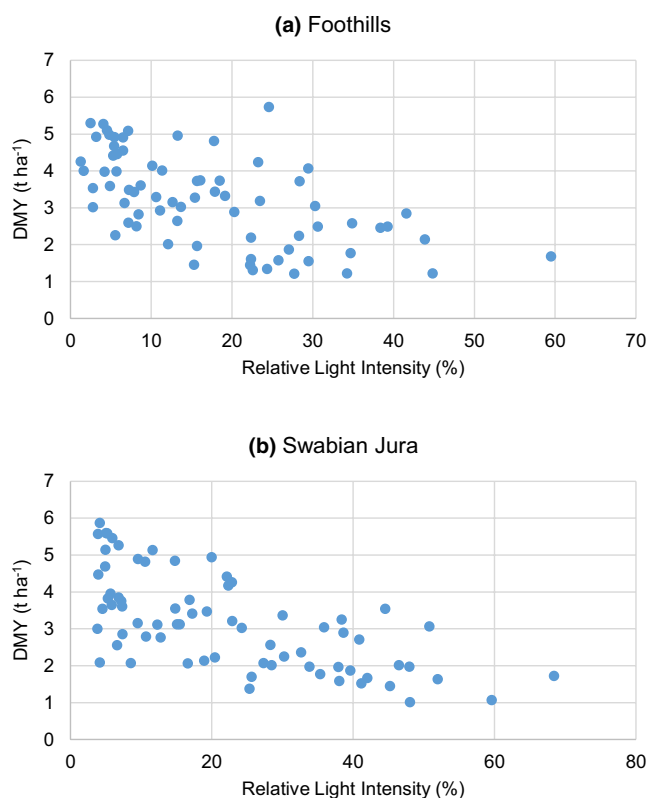


FIGURE 4 Relative light intensity at ground level (%) before first cut and dry matter yield (DMY) of first cut for 2017 and 2018 together at (a) Foothills and (b) Swabian Jura

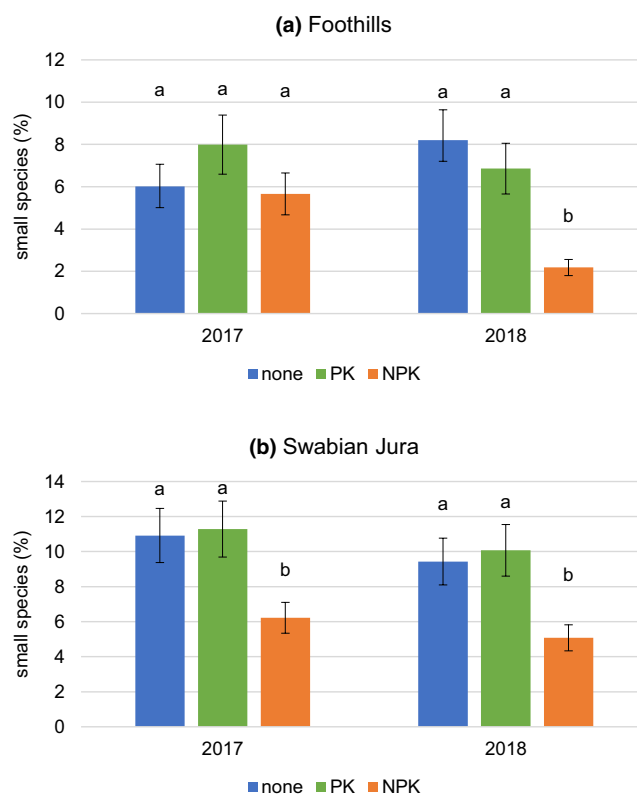
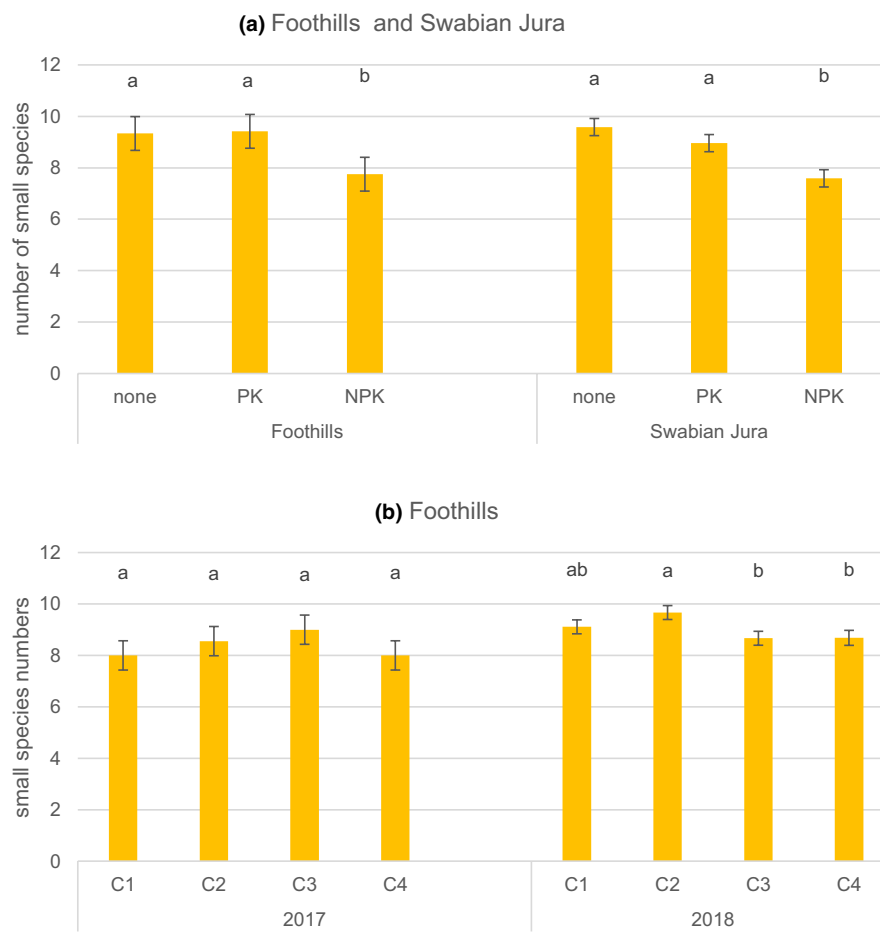


FIGURE 5 Back-transformed means of small plant species (%) per fertilisation level (none, PK and NPK) at (a) Foothills and (b) Swabian Jura in 2017 and 2018 with standard errors ($n = 3$). Values with at least one identical lower case letter were not significantly different from each other

FIGURE 6 Mean species numbers of small plants (per plot) at (a) Foothills and Swabian Jura in both years per fertilisation level and (b) Foothills in both years per cutting date variant with standard errors ($n = 12$). Values with at least one identical letter were not significantly different from each other



4.2 | Effects of fertilisation

There was a significant effect of fertilisation on RLI and DMY of the first growth. Fertilisation significantly increased DMY of the first growth, which is linked to the RLI of the first growth cycle (Figure 4). For example, at a DMY of c. 3.5 t/ha, the RLI at ground level decreased to below 10%. As reported in Boob et al. (2019a), NPK fertilisation increased the yield proportion of tall grasses. This effect should be due to the grass growing denser, higher and casting more shade when fertilised. The hypotheses could be confirmed. We showed that the biomass and diversity of small plant species is reduced by NPK fertilisation in a hay meadow field trial. This is probably an effect of reduced light availability to these plants due to increased biomass. This is in line with the results of Hautier et al. (2009), who found that additional light in the understory increases the DMY of small species in fertilised grassland, and thus concluded that small species are light-limited. However, a 4-year experiment showed that the negative effect of fertilisation on plant diversity is not caused by decreased light availability alone (Dickson & Foster, 2011). There could be competition for other resources, e.g. water or nutrients in the soil. High light availability in combination with fertilisation can stimulate the growth of grass shoots (tillering) after cutting (Simon & Lemaire, 1987).

4.3 | Further effects

Significant interactions of fertilisation and cut date were found for the third PAR measurement at Foothills only. This may be because this measurement was taken 33 days after the first cut compared with only 22 days at Swabian Jura. This means that if the measurement had been taken 11 days later, we would have expected this effect at Swabian Jura as well. Furthermore, the shading effect of fertilisation at the last measurement date (M4) was not detectable at Swabian Jura in 2018 (Figure 2b). This could be due to differences in botanical composition. There are two possible mechanisms of shading by plants. First, horizontally oriented leaves of tall-growing forb or legume species produce shade. Second, shading can be due to the dense growth of tall grasses. Whereas tall grasses like *Arrhenatherum elatius* and *Festuca pratensis* dominated the late-cut variants at Foothills, the annual forb *Rhinanthus alectorolophus* dominated the late-cut variants at Swabian Jura, in particular the unfertilised variants (C4 none). The weakening effect of *Rhinanthus* on grasses was also confirmed by Davies et al. (1997) who experimentally decreased *Rhinanthus* densities, resulting in increased grass proportions. Because of this effect, there should be less shading when *Rhinanthus* dominates the sward. The higher proportion of legumes due to PK fertilisation could explain the decreasing RLI in PK-fertilised plots of the late-cut variant, because legumes cast



more shade as their leaves are oriented horizontally. In a plant-removal experiment by Petersen and Isselstein (2015), grasses with steep-angled leaves and forbs with horizontally arranged leaves were found to shape the canopy structure. However, in semi-natural hay meadows, NPK fertilisation mostly promotes the dense growth of grasses and not that of forbs. Another possible explanation is the different altitudes of the two sites in our study, with delayed senescence at the more elevated site Swabian Jura. Denser vegetation and lying grasses presumably shadowed the ground on the late measurement date (end of June/July) at Foothills.

Because there were also significant differences between fertilisation variants in the second growth (except at Foothills after 48 days), an influence of fertilisation on the second growth can also be assumed. This is of relevance for small plants as well as seedlings, which could emerge after the first cut at the seed ripening stage. They could be hindered by low light availability in NPK-fertilised plots during the second growth cycle.

5 | CONCLUSIONS

This experiment conducted in a 5-year-old field trial showed that even low levels of fertilisation increase biomass growth and reduce light availability to small plants. This could lead to a loss of biodiversity through a reduction in small species and—in the specific case of lowland hay meadows—to unfavourable conservation status. It can be concluded that the habitat function of these meadows can be severely impaired by NPK fertilisation. To preserve the biodiversity of hay meadows and achieve an acceptable DMY, extensive fertilisation with no more than 35 kg P₂O₅ and 120 kg K₂O per year is recommended.

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CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

MB carried out the experiment, was involved in sample analysis and analysed the data. JH was involved in data analysis. UT, ME and IL were responsible for the original idea for the research and initial concept of the experiment. All authors contributed to the preparation of the manuscript.

DATA AVAILABILITY STATEMENT

All data generated during this study are available via the Zenodo data repository: <https://doi.org/10.5281/zenodo.5628431>.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Results of *F*-tests of linear mixed models

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Chapter 3 Management effects on hay meadow biomass



In this chapter, a paper on the DMV and the chemical composition of different biomasses is presented. The research question, how hay meadow management changes quantity, quality and usability of biomass will be addressed.

Paper III

Title: Harvest Time Determines Quality and Usability of Biomass from Lowland Hay Meadows

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Article

Harvest Time Determines Quality and Usability of Biomass from Lowland Hay Meadows

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Abstract: Species-rich hay meadows are usually managed extensively to maintain their biodiversity, with the harvested biomass traditionally being fed to ruminants for milk or meat production. The quality of the biomass is, however, variable, difficult to predict and often does not fulfil today's requirements. This study established a field trial at two species-rich hay meadows to investigate the combined effect of fertilisation (none, phosphorus and potassium (PK), nitrogen, phosphorus and potassium (NPK)) and date of first cut (at different phenological stages) on biomass quality and quantity. In addition, the most suitable uses of the biomass were explored, including the alternatives biogas and combustion. After four years of the field trial, the stage of maturity at the time of first cut had a greater influence than extensive fertilisation on biomass quality. Dry matter yield (DMY) of the first cut was about 40%–60% of annual DMY ($53.99 \pm 12.51 \text{ dt ha}^{-1} \text{ a}^{-1}$) depending on site, fertilisation and harvest time. Fertilisation had a stronger effect than harvest time on DMY and annual methane yield. In most cases, there was no significant difference in chemical composition between biomass harvested at the end of the grass-flowering stage and at the seed-ripening stage. Thus, a late cut for hay proved to be the most flexible option.

Keywords: grassland management; Natura 2000; forage quality; bioenergy; biorefinery

1. Introduction

Approximately 10% of the utilised agricultural area in the European Union (EU) belongs to the Natura 2000 network [1]. This network includes lowland hay meadows, which are extensively managed meadows protected as 'Habitat Types of Community Interest' by the EU Habitats Directive [2]. The directive stipulates that the typical botanical composition of these lowland hay meadows is to be maintained. This can only be achieved through extensive management, usually with two cuts per year and fertilisation without mineral nitrogen (N) [3]. The biomass harvested is different to that of intensively used grassland in that it contains, depending on site conditions, a diverse range of plant species at different stages of maturity. Therefore, the quality of biomass harvested from lowland hay meadows is variable, difficult to predict and often unknown.

One factor influencing the chemical content of herbage is its botanical composition. This is in turn significantly influenced by management practices and other site-specific factors such as soil. For example, a study examining the nutritive value of various grass and legume species found that legumes always had the highest N content [4]. Another found forbs to be rich in mineral nutrients [5].

It is known that the stage of maturity at the time of harvest has a great influence on forage quality, because cell wall components increase during plant development [6]. Thus, grassland management can influence the quality of cut herbage both directly and indirectly.

To integrate grassland biomass into modern agricultural systems and to determine which value chain is most suitable for its use, information on its quality, in particular chemical composition, is invaluable. Traditionally, grassland biomass has been used as forage. Freshly cut forage is conserved as hay or silage and fed to ruminants for the production of milk or meat. In modern livestock systems, however, this traditional usage is restricted because highly productive dairy cows have high nutritional demands, which cannot be met by extensively managed grassland [7,8]. The quality of ruminant forage is often estimated based on content of usable energy (measured as net energy for lactation, NEL) and protein content. Both are reduced with advancing maturity of grassland biomass as fibre content increases. Thus, the question arises on the extent to which biomass from species-rich hay meadows can be integrated into modern livestock systems.

There are alternative energetic uses of grassland biomass as solid fuel or as substrate for biogas production. Although the feedstock-specific methane yield (SMY) of biomass from extensively managed hay meadows has not yet been sufficiently established [9], it is known that the higher lignin content of grassland biomass harvested at a later maturity stage reduces its digestibility and biogas yield. The same is true for its use as forage. On the other hand, the management required for use in biogas production can be less intensive than for dairy use, especially with regard to nitrogen (N) content [10]. Biomass rich in fibre is more suitable for combustion than either fermentation or forage use [11]. Here, low amounts of ash-forming components and N content are preferable, as these can cause problems such as sintering in the combustion chamber and harmful emissions [12].

Finally, there is also increasing interest in the material use of grassland biomass. One example is the green biorefinery, which combines the production of materials and energy, and commonly uses fresh green herbage or silage as substrate. Other examples are the thermo-chemical splitting of grassland biomass through pyrolysis into gaseous, liquid and solid components [13], and hydrolysis, which is the enzymatic production of sugars for bioethanol [14]. For use in biorefineries, grassland biomass needs to be stored in the form of silage to ensure year-round availability. However, biomass from extensively used meadows often does not contain the necessary levels of components such as sugars and amino acids [10].

The economic feasibility of the use of grassland is mainly determined by the yield and quality of the biomass. The DMY of extensively used grassland depends on water availability [15]. Where water supply is sufficient, management practices such as fertilisation and cutting regime are the key factors regulating DMY. Long-term fertilisation experiments have shown that nitrogen, phosphorus and potassium (NPK) fertilisation increases DMY in both intensively (e.g., [16]) and extensively used permanent grassland (e.g., [17]). However, increased DMY has also been reported with PK fertilisation only [18] in an alluvial meadow fertilised for 25 years. Thus, the grassland productivity in this fertilisation experiment was not N-limited [19]. Extensively used meadows often exhibit low soil P (phosphorus) and K (potassium) contents. The fertilisation effect depends on the soil nutrient status and pH value. For example, a long-term fertilisation experiment by [20] found that N application to a hay meadow led to P limitation.

Kirkham and Tallowin [21] found a poorer forage quality of species-rich hay meadows at late dates of first cut. They compared unfertilised plots and plots previously fertilised with NPK and found no significant effects of previous fertilisation or fertilisation x date of first cut interactions. By contrast, [22] reported highest DMY for NPK-fertilised plots combined with a late date of first cut in September, even though in their experiment only low doses of NPK were used to restore former agriculturally improved meadows. Thus, when deciding on the optimal date of first cut of hay meadows, there is a trade-off between high forage quality and high DMY.

The aim of our study is to assess the influence of harvest date and fertilisation on biomass quality and quantity from species-rich meadows. This is discussed in the context of the usability of biomass provided by lowland hay meadows.

We hypothesized that the stage of maturity has a stronger effect than fertilisation on biomass quality, especially on NEL, protein and fibre content. Further, we expected a better suitability of an early date of first cut for forage use and a late date of first cut for combustion. We anticipated site-specific effects of the treatments and an influence of botanical composition on biomass quality and quantity. To test these hypotheses, a 5-year field trial with three replicates was established at two lowland hay meadows in south-west Germany. The influence of the two factors fertilisation (none, PK and NPK) and date of harvest (before, at beginning of, at end of and after flowering of main grasses) was investigated.

2. Materials and Methods

2.1. Field Trial Location

The field trial was established in 2013 on two species-rich hay meadows (Habitat Type 6510) in a special area of conservation, approximately 30 and 40 km respectively from Stuttgart, south Germany. The first site, “Swabian Jura”, is located on this low mountain range at 774 m above sea level. The second site “Foothills” (470 m a.s.l.) is located approximately ca. 10 km away at the base of the Swabian Jura (Table 1). Both meadows belong to the *Arrhenatherion* alliance, with typical species of the *Geranio-Trisetetum* association at Swabian Jura (mean \pm standard deviation, $n = 36$) and a typical *Arrhenatheretum elatius* community at Foothills [23]. In 2013, average soil N and C content (in % of dry matter (DM)) were 0.63 ± 0.07 N and 7.87 ± 1.09 C at Swabian Jura, and slightly higher at Foothills (0.79 ± 0.05 N, 8.42 ± 0.55 C). The soil is alkaline at both sites (mean pH 7.4). There were no significant differences in pH values between sites and treatments. Meteorological data are shown in Figure A1 (Appendix A).

Table 1. Site characteristics at the beginning of the field trial (in 2013).

	Swabian Jura	Foothills
Coordinates	48°34′27.8″ N, 9°26′29.7″ E	48°31′38.5″ N, 9°31′53.9″ E
Mean Annual Temperature	7.4 °C	9.6 °C
Mean Annual Precipitation	1040 mm	970 mm
Altitude	774 m a.s.l.	470 m a.s.l.
Soil N content	$0.63 \pm 0.07\%$ DM	$0.79 \pm 0.05\%$ DM
Soil C _{total} content	$7.87 \pm 1.09\%$ DM	$8.42 \pm 0.55\%$ DM
pH	7.2 ± 0.1	7.2 ± 0.1
Soil K ₂ O content	13.38 ± 1.43 mg 100g ⁻¹	8.97 ± 1.42 mg 100g ⁻¹
Soil P ₂ O ₅ content	4.10 ± 0.71 mg 100g ⁻¹	2.70 ± 0.68 mg 100g ⁻¹

2.2. Design and Management

A randomised block-design field trial with three replications was set up at both sites. Each block was divided into 12 plots of 25 m², one for each treatment. Treatments were a combination of three fertilisation variants (none, PK and NPK) and four cutting variants (date of first cut).

The three fertilisation variants were none (unfertilised), PK (35 kg P₂O₅ and 120 kg K₂O ha⁻¹ a⁻¹) and NPK (35 kg N, 35 kg P₂O₅ and 120 kg K₂O ha⁻¹ a⁻¹). Fertiliser amounts were chosen to simulate traditional manure application according to recommendations by the governmental institute LAZBW [3] and applied every year in March. The meadows at both sites were cut twice per year, with the first cut being performed on four different dates and the second cut on the same day in September. The dates of first cut were based on growth stages. The earliest date of first cut (D1) was timed to represent an early cut before the main flowering period, the second (D2) and third (D3) dates were in the main

flowering period and the latest date of first cut (D4) was chosen to represent an extensive cut for hay at seed-ripening stage (Table 2). Data were taken in four subsequent years (2013 until 2016).

Table 2. Cutting dates of first (variants D1 = early date of first cut to D4 = late date of first cut) and second cut 2013–2016.

Year	1st Cut				2nd Cut
	D1	D2	D3	D4	
	Before Flowering	Start of Flowering	Flowering Period	Seeds Ripening	
2013	May 28 and June 4	June 14	July 3	July 18	September 23
2014	May 8	May 26	June 12	July 1	September 16
2015	May 13	June 2	June 16	June 30	September 15
2016	May 19	June 2	June 17	June 29	September 14

2.3. Sampling and Laboratory Analysis

Sampling and laboratory analyses were performed each year from 2013 to 2016. All plots were mown with a sickle bar mower to a sward height of 5 cm. The fresh weight of the harvested biomass was determined directly on the field. A subsample (about 0.5 kg) was selected randomly for the determination of DM content (60 °C for at least 48 h), and then ground in a cutting mill.

The specific methane yield (SMY) in $\text{Nm}^3\text{kg}^{-1}$ organic DM (ODM) was determined using the Hohenheim biogas yield test [24]. Gas production potential (GP) was determined [25] by means of the Hohenheim feed value test. Net energy for lactation content (NEL in $\text{MJ kg}^{-1}\text{DM}$) was calculated using the following equation [26]:

$$\text{NEL} = 0.54 + 0.096 \times \text{GP} + 0.038 \times \text{CP} + 0.00173 \times \text{CL}^2$$

where CP is the crude protein content (g/kg) and CL the crude fat content (g/kg). Additionally, the dried samples were analysed for contents of ash (XA), fibre, and the mineral nutrients potassium (K), phosphorus (P), magnesium (Mg), and calcium (Ca) [25]. The dry matter fibre content (all cell wall components (NDF), consisting of lignin (ADL), cellulose and hemicellulose) was determined by the Van Soest method.

Soil samples were taken after the second cut in 2013 using a soil corer at a depth of 0–10 cm. For each plot, at least 10 soil samples were mixed together. Mixed samples were air-dried, sieved and the pH value, K_2O and P_2O_5 (extracted by calcium acetate lactate) contents determined [27]. C and N contents were determined in an elemental analyser (Vario Max CNS, Elementar, Langenselbold, Germany).

2.4. Statistical Analyses

A linear mixed-effect model was fitted to each response variable and each site and residuals were checked graphically for homogeneity of variance and normal distribution. Where the residual distribution was not normal, data were square root-transformed. In this case, means were back transformed for presentation purpose only. The single-site statistical model used for each response is as follows:

$$y_{ijkl} = \mu + a_l + b_{kl} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + (a\tau)_{il} + (a\varphi)_{jl} + (\tau\varphi a)_{ijl} + e_{ijkl},$$

where μ is the intercept, a_l is the fixed effect of the l th year, b_{kl} is the random effect of the k th replicate in year l , τ_i , φ_j and $(\tau\varphi)_{ij}$ are the fixed effects of the i th cutting regime, the j th fertiliser level and their interactions, respectively. $(a\tau)_{il}$, $(a\varphi)_{jl}$ and $(\tau\varphi a)_{ijl}$ are the corresponding fixed interaction effects with year l . e_{ijkl} is the error of observation y_{ijkl} . For b_{kl} and e_{ijkl} homogeneous or heterogeneous variances with independence or a first order autocorrelation were assumed. The best variance-covariance structure was selected via AIC (Akaike information criterion) [28]. In case of significant F tests, a multiple comparison of means was performed and presented via letter display [29]. Statistical analyses

were performed using the PROC MIXED procedure of the SAS system (version 9.4, SAS Institute, Cary, USA).

3. Results

Most results showed significant influences of treatments \times year interactions. While for most variables both two-way interactions (date of first cut \times year and fertilisation \times year) were significant for both sites, the three-way interaction was significant only for K. We found no common effects across the years 2013–2016 (except for fertilisation for CP at Foothills) or fertilisation \times date of first cut interactions across these years except for P at Foothills. Thus, year-specific means were calculated throughout the paper. As year four (2016) is the most recent year, means for 2016 are shown in this study.

3.1. Dry Matter Yield

There were significant year \times fertilisation and year \times date of first cut interactions ($p < 0.05$) at both sites. Regardless of date of first cut, annual DMY increased from 48.2 ± 3.6 or 48.3 ± 2.5 to 69.7 ± 3.6 or 77.3 ± 2.5 dt ha⁻¹a⁻¹ (1 dt = 100 kg, ha = hectare, a = year), respectively, with increasing DMY with increasing amount of fertiliser used (Figure 1).

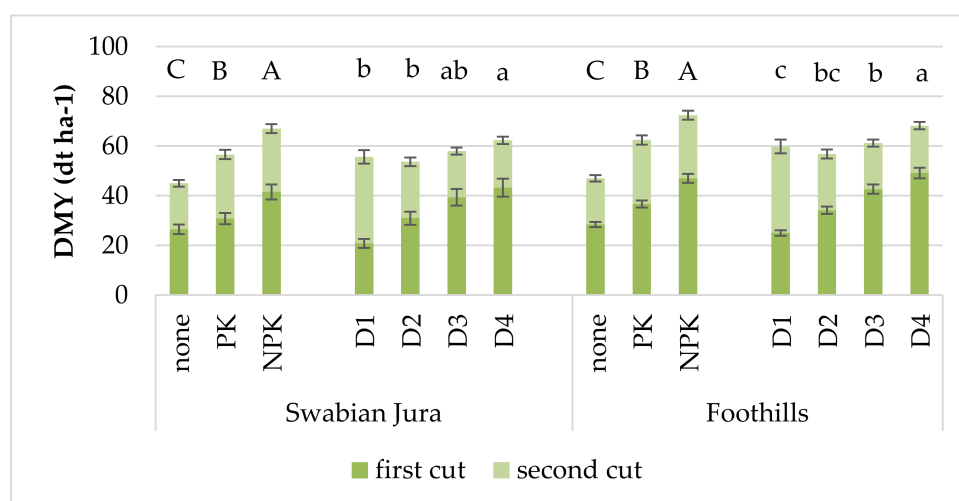


Figure 1. Means of dry matter yield (DMY) (dt ha⁻¹) at Swabian Jura and Foothills sites in 2016 with standard deviation. Data are shown for different fertilisation levels (none, PK, NPK) and cutting regimes (D1 = early date of first cut, D4 = late date of first cut). Identical lower-case letters between cutting variants ($p < 0.05$, $n = 9$) and identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) for each site.

3.2. Organic Components, Net Energy Content and Specific Methane Yield (SMY)

Fertilisation had no significant effect on ADL or SMY. NDF content was significantly influenced by fertilisation \times year interactions and date of first cut \times year interactions at both sites (Table 3).

ADL and SMY were influenced by date of first cut \times year interactions only in both sites. For CP and NEL, significant terms vary between sites. For CP at Foothills, fertilisation and year \times cutting interactions were significant, for Swabian Jura, the three-way interactions are significant. For NEL, year \times cutting interactions were significant at both sites while year \times fertilisation interactions were significant at Foothills only. In contrast, in 2016 date of first cut always had a significant effect on organic components, energy content and SMY of the first growth (harvested biomass of first cut), except for CP at Swabian Jura. There were significant year \times date of first cut interactions ($p < 0.05$) (Table 4).

Table 3. Least square means of organic components, net energy for lactation (NEL) and specific methane yield (SMY) of first cut in 2016 with standard error (\pm SE).

Site	Treatment	CP (Protein)		NDF (Fibre) g kg ⁻¹ DM		ADL (Lignin)		NEL MJ kg ⁻¹		SMY Nm ³ kg ⁻¹ ODM	
Swabian Jura	D1	135.2 ^a	± 2.2	498.5 ^c	± 0.8	58.63 ^a	± 0.19	5.81 ^a	± 0.10	0.320 ^c	± 0.003
	D2	104.6 ^b	± 2.2	527.3 ^b	± 0.8	66.00 ^b	± 0.19	5.39 ^b	± 0.10	0.300 ^b	± 0.0003
	D3	90.0 ^c	± 2.2	550.8 ^a	± 0.8	69.71 ^b	± 0.19	4.54 ^c	± 0.10	0.262 ^a	± 0.003
	D4	83.9 ^d	± 2.2	537.6 ^{ab}	± 0.8	69.81 ^b	± 0.19	4.64 ^c	± 0.10	0.269 ^a	± 0.003
	none	103.0	± 1.98	511.9 ^A	± 0.7	68.52	± 0.17	5.10	± 0.10	0.290	± 0.003
	PK	104.2	± 1.98	515.5 ^A	± 0.7	64.70	± 0.17	5.12	± 0.01	0.287	± 0.003
	NPK	103.2	± 1.98	558.2 ^B	± 0.7	64.89	± 0.17	5.07	± 0.01	0.283	± 0.003
Foothills	D1	112.5 ^a	± 3.26	500.0 ^c	± 0.8	53.66 ^a	± 0.15	5.56 ^a	± 0.08	0.312 ^a	± 0.004
	D2	86.2 ^b	± 3.26	528.6 ^b	± 0.8	60.46 ^b	± 0.15	5.22 ^b	± 0.08	0.288 ^b	± 0.004
	D3	78.8 ^c	± 3.26	557.7 ^a	± 0.8	63.03 ^{bc}	± 0.15	4.52 ^c	± 0.08	0.259 ^c	± 0.004
	D4	68.0 ^d	± 3.26	572.9 ^a	± 0.8	66.30 ^c	± 0.15	4.46 ^c	± 0.08	0.262 ^c	± 0.004
	none	81.3	± 3.04	497.0 ^C	± 0.8	59.65	± 0.13	4.94 ^A	± 0.07	0.283	± 0.004
	PK	88.1	± 3.04	550.2 ^B	± 0.8	61.83	± 0.13	4.88 ^B	± 0.07	0.280	± 0.004
	NPK	89.8	± 3.04	572.2 ^A	± 0.8	61.10	± 0.13	5.00 ^A	± 0.07	0.278	± 0.004

Identical lower-case letters between cutting variants ($p < 0.05$, $n = 9$) and identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) for each site.

Table 4. F statistics of three-way analyses of variance (ANOVAs) (factor year (Y), date of first cut (D) and fertilisation (F)) and significant interactions for organic components at Swabian Jura and Foothills site.

Site	Factor		CP	NDF	ADL	NEL	SMY
Swabian Jura	date of first cut	F value	373.81 ***	142.16 ***	75.29 ***	544.3 ***	288.1 ***
	Fertilisation	F value	0.67	27.37 ***	0.94	1.15	2.34
	Year	F value	1.75	11.70 **	182.6 ***	63.1 ***	204.27 ***
	Significant interactions		DxY	DxY, FxY	DxY	DxY	DxY
Foothills	date of first cut	F value	478.59 ***	117.59 ***	82.11 ***	652.97 ***	269.67 ***
	Fertilisation	F value	5.15 *	52.97 ***	1.60	4.91 **	1.59
	Year	F value	0.79	54.53 **	70.48 ***	11.23 **	32.11 ***
	Significant interactions		DxY	DxY, FxY	DxY	DxY, FxY	DxY

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. F and p values are results of data for years 2013–2016.

The first growth was rich in fibre, with cell wall components (NDF) constituting about 50% of DM. In 2016, the NDF and ADL content significantly increased with later date of first cut, while CP, NEL and SMY decreased at both sites. Furthermore, NDF increased with NPK fertilisation (Table 3).

Content of usable energy (measured as NEL) is often considered when estimating forage quality for ruminant feed. The NEL content was above 5 MJ kg⁻¹ DM at both sites each year at early date of first cut, but declined significantly at later date of first cut (Table 3).

The mean specific methane yield (SMY) was about 0.30 ± 0.003 Nm³ CH₄ kg⁻¹ DM at both sites. Fertilisation had no significant influence on SMY. As with NEL content, SMY decreased with later date of first cut at both sites (to 0.28 ± 0.02 Nm³ CH₄ kg⁻¹ DM).

Due to its better suitability for biogas production, the biomass of the first growth only was analysed for its biogas potential and taken into account in the assessment of methane yield per ha and year. Methane yield is calculated by multiplying DM yield by SMY. For this reason DM and methane yields showed similar results. However, calculated methane yields of the first growth (Nm³ CH₄ ha⁻¹) were comparable between sites, years and treatments. At Swabian Jura, the methane yields of the first growth cycle increased continuously during the four years of the field trial from 845 (2013) to 983 Nm³ CH₄ ha⁻¹ (2016). At Foothills, methane yields were generally higher than at Swabian Jura and in 2016 highest on NPK-fertilised plots. At this site, the highest methane yield was reached on the latest date of first cut in 2016 (1396 m³ CH₄ ha⁻¹). Additionally, annual methane yields (AMY) in Nm³ CH₄ ha⁻¹ a⁻¹ were calculated based on the SMY of the first growth cycle and the annual DMY to give a rough estimate for each site and treatment. In 2016, estimated AMY of D1 was 1355 (none), 1606 (PK) and 2177 Nm³ CH₄ ha⁻¹ a⁻¹ (NPK) at Foothills and 1565 (none), 1790 (PK) and 2087 (NPK) at Swabian Jura. At both sites, it was significantly increased by NPK fertilisation and there were significant differences between cutting variants in 2016 (Figure 2).

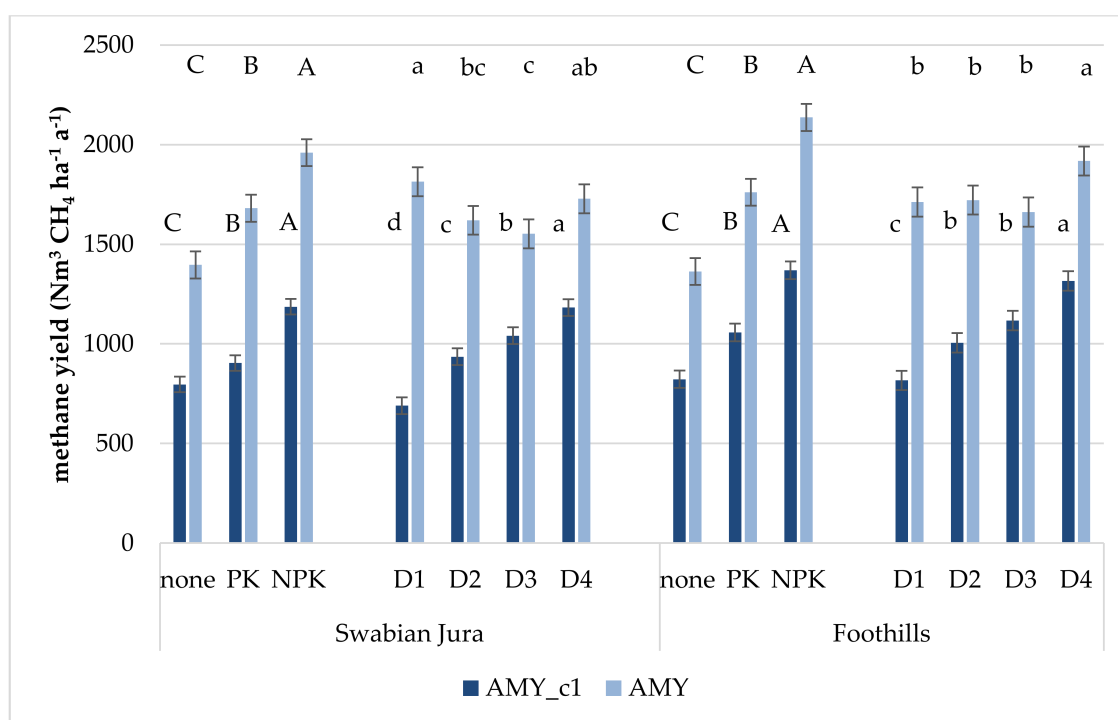


Figure 2. Mean methane yield from first growth (AMY_c1) and estimated annual methane yields (AMY) in Nm³ CH₄ ha⁻¹ a⁻¹ at Swabian Jura and Foothills sites in 2016. Data are shown for different fertiliser levels (none, PK, NPK) and cutting variants (D1 = early date of first cut, D4 = late date of first cut). Error bars indicate standard deviation. Identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) and lower-case letters between cutting variants ($p < 0.05$, $n = 9$) for each site.

3.3. Mineral Nutrients and Ash Content

There were significant differences between treatments in crude ash content and ash components of biomass harvested from the first growth and significant interactions with the factor ‘year’. Mean contents of mineral nutrients and ash are shown in Table 5.

Table 5. Least square mean content of mineral nutrients potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and ash (g kg⁻¹ DM) of first cut in 2016 with standard error (±SE).

Site	Treatment	g kg ⁻¹ DM									
		K		P		Ca		Mg		Ash Content	
Swabian Jura	D1	25.9	±1.4	3.41 ^a	±0.31	10.4	±1.0	1.50 ^b	±0.07	108.8 ^a	±4.5
	D2	23.3	±1.4	3.12 ^b	±0.31	10.2	±1.0	1.69 ^a	±0.07	103.7 ^{ab}	±4.4
	D3	19.1	±1.4	2.72 ^c	±0.31	11.1	±1.0	1.50 ^b	±0.07	105.3 ^{ab}	±4.4
	D4	19.5	±1.4	2.57 ^c	±0.31	10.7	±1.0	1.53 ^b	±0.07	93.9 ^b	±4.2
	none	22.2	±1.4	2.90	±0.31	12.0	±0.9	1.61	±0.06	115.2 ^A	±4.0
	PK	22.2	±1.4	2.99	±0.31	10.0	±0.8	1.53	±0.06	97.1 ^B	±3.7
	NPK	21.5	±1.4	2.98	±0.31	9.8	±0.8	1.53	±0.06	96.8 ^B	±3.7
Foothills	D1	24.9	±1.9	2.88 ^a	±0.35	10.0	±0.5	1.97 ^a	±0.15	112.5 ^a	±3.7
	D2	20.4	±1.9	2.33 ^b	±0.35	9.8	±0.5	1.87 ^{ab}	±0.15	95.0 ^b	±3.4
	D3	16.8	±1.9	2.16 ^c	±0.35	9.7	±0.4	1.64 ^c	±0.15	89.2 ^{bc}	±3.3
	D4	16.6	±1.9	1.92 ^d	±0.35	9.5	±0.4	1.72 ^{bc}	±0.15	84.7 ^c	±3.2
	none	19.0	±1.9	2.24 ^A	±0.35	9.0 ^B	±0.4	1.64 ^B	±0.14	92.0	±3.0
	PK	20.1	±1.9	2.43 ^B	±0.35	10.0 ^A	±0.4	1.83 ^A	±0.14	97.5	±3.1
	NPK	19.9	±1.9	2.29 ^{AB}	±0.35	10.4 ^A	±0.4	1.93 ^A	±0.14	95.8	±3.9

Identical upper-case letters indicate non-significant differences between fertilisation ($p < 0.05$, $n = 12$) and lower-case letters between cutting variants ($p < 0.05$, $n = 9$) for each site.

At both sites, date of first cut and fertilisation significantly influenced P, Mg, Ca content in 2016. For K, date of first cut and fertilisation interact, thus means for each combination should be compared in 2016. The P content of the harvested biomass was significantly decreased by later date of first cut (Table 5).

4. Discussion

4.1. Early Date of First Cut before Flowering Stage (D1)

As biomass cut early had the highest NEL and protein content, its use as forage would seem appropriate. However, although NDF and ADL values were lowest at early date of first cut, the fibre contents were comparable to those found for hay meadows in previous studies (e.g., [30]). For this reason, the biomass needs to be chopped if it is conserved as silage. Herrmann et al. [31] recommend silage additives for extensively used *Alopecurus pratensis* wetland meadows because the compactibility of fibrous material is poor.

In 2016, the biomass harvested at the earliest date of first cut had a CP content of 135.2 ± 2.2 g kg⁻¹ DM at Swabian Jura, but only 112.5 ± 3.3 g kg⁻¹ at Foothills. This was because the plants were at different stages of maturity. They were younger at Swabian Jura due to delayed development at this site (Figure A1). Roughage with a CP content of 160–220 g kg⁻¹ and below 22% NDF is suitable for dairy cows [32]. Forage of lower nutritional quality is not adequate. However, roughage with a CP content of at least 80 g kg⁻¹ DM can be fed to beef cattle and non-lactating sheep [32].

The net energy for lactation (NEL) of the biomass was calculated to estimate its forage quality. Lactating dairy cows require energy contents of at least 6.0 MJ NEL kg⁻¹ [7]. This level was not achieved at either site. In addition, protein and energy content losses can be expected during haymaking, especially with field-dried hay (e.g., [33]). Consequently, early-cut hays are not suitable as exclusive

forage for dairy cows. In terms of CP and NEL content, they would be preferable for more extensively kept animals such as suckler cows and sheep.

Due to the decreasing demand for grassland biomass as forage [34], there is also the option of using it for renewable energy production in biogas plants. Germany has the highest number of biogas plants in Europe. Of these, 50% use grass silage as a co-substrate [35]. At both sites of our field trial, the feedstock-specific methane yield (SMY) of the early first cut was 15%–18% higher than that of the later first cuts. The SMY of the early cut at both Swabian Jura ($0.32 \pm 0.003 \text{ Nm}^3 \text{ kg}^{-1}$) and Foothills ($0.31 \pm 0.004 \text{ Nm}^3 \text{ kg}^{-1}$) was similar to that of maize ($0.30\text{--}0.38 \text{ Nm}^3 \text{ kg}^{-1}$), the most commonly used feedstock, but maize can yield more biomass ($15\text{--}30 \text{ t ha}^{-1}$) [35]. DMY is the main determinant of high methane yields per hectare.

The AMY was significantly increased by NPK fertilisation at both sites. Similar to our field trial, [36] found an increased area-specific methane yield through an increase in biomass. In their experiment, different sown species mixtures yielded on average $1674 \pm 487 \text{ m}^3 \text{ CH}_4 \text{ ha}^{-1} \text{ a}^{-1}$ when cut twice a year. Compared to other long-term grassland experiments e.g., [37] the AMY in our study was relatively low. Only that of the NPK-fertilised plot at Foothills was comparable to levels, where $2157.3 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ were calculated for a two-cut system fertilised with 30 kg N per cut [37]. For intensive grassland, even higher methane yields of $2700\text{--}3500 \text{ m}^3 \text{ ha}^{-1}$ have been reported [38]. By contrast, extensive mountain grassland (890 m a.s.l.) yielded only about $910 \text{ m}^3 \text{ CH}_4 \text{ ha}^{-1} \text{ a}^{-1}$ when cut twice [38].

4.2. Cut at Beginning of Grass Flowering Stage (D2)

The biomass of cutting variant D2 showed a significant reduction in many of the quality parameters examined (NEL, SMY and Mg) compared to D1. The P, Ca and K contents were sufficient for forage use [6], but the Mg content was low ($1.72 \pm 0.72 \text{ g kg}^{-1}$, average of both sites). In agricultural practice, mineral nutrient contents are of minor importance for livestock, because they can be supplemented by mineral feed.

The feed energy content of the biomass from D2 was low, but could be included into feed rations if supplemented with concentrates or energy-rich silage [7]. Hay of about 5 MJ NEL kg^{-1} can be used for up to 80% of feed rations for pregnant suckler cows [39]. If forage quality is not sufficient, upgrading treatments can be applied. For example, adding ammonium salts to hay from species-rich meadows can improve its forage quality [6]. As the methane yield was similar to that of D1, the biomass from D2 can also be used for the production of biogas.

4.3. Late First Cut at Flowering (D3) or Seed-Ripening Stage (D4)

The first cut of species-rich hay meadows is often delayed for ecological reasons: the plants have time to develop, some species already produce seeds and there is a broader range of pollinators. This is why these meadows are protected as habitats. The main flowering period of grasses is in June, corresponding to our cutting variant D3. At the end of June, most grass species in our field trial had produced seed and many seeds were already ripening. At the same time, the NEL and SMY, but also N and K content, were continuing to decrease compared to D2. In 2016, there were no significant differences in chemical composition between D3 and D4, except for P at Foothills and CP. Thus, the first cut in the main flowering period can be delayed up to 14 days without substantially impairing forage quality. This is due to an increased proportion of forbs with later date of first cut as these do not mature as fast as most grass species [5].

At the latest date of first cut (D4), CP was reduced by 34%–41% and NEL by 20% compared to D1 (average of both sites). The fibre content increased, depending on fertilisation level. NDF content was significantly increased by NPK fertilisation due to the high percentage of grasses. Similar results were reported by [40]. Due to the NEL values below 5 MJ , the biomass from the D4 date of first cut cannot be recommended as forage for most types of livestock; biomass of this quality can only serve as exclusive feed for horses with low performance [7]. However, it should be kept in mind that hay

meadows cut late for several years in succession may contain plant species that are toxic to animals if eaten in large quantities. In European hay meadows, these include in particular *Colchicum autumnale* and *Senecio* species [41].

The mean AMY of D4 was significantly higher than D1 at Foothills. This is because the annual DMY was high at Foothills most likely due to better water availability, especially during the first growth cycle (April–June) in 2016 (Figure A1). In practice, chopped biomass conserved as silage is usually used as biogas substrate; however, problems with ensilaging of this fibrous material can occur. To achieve better usability of late-cut grassland biomass for biogas production, mechanical, chemical and biological pretreatments can be applied [42]. Another option is dry fermentation, which would be preferable due to the technical problems caused by high fibre content in wet fermentation.

Extensive grassland biomass from later date of first cut has often been suggested as a cheap resource for solid fuel [30,43] on account of its high fibre and low N content. These reduce its forage value, cause problems during the biogas process, for example with stirring devices, and also lower the SMY. Thus, biomass from a late cut could be more suitable for combustion. However, as N content leads to NO_x emissions, the threshold N value of 6 g kg^{−1} DM [12] for unproblematic combustion should not be exceeded. In our study, the N content of the biomass (calculated as CP × 0.16) from both sites was always above this limit, although it was comparable to values of dry hay meadows found in other studies e.g., [43].

Another important aspect for combustion is ash. In 2016, the ash content was very high due to soil contaminations related to the weather conditions at the time of harvest. This contamination was most likely caused by a larger area of uncovered soil this year through a higher occurrence of anthills (Foothills site) and voles (Swabian Jura site). In all other years, the ash contents were in the range of those reported in previous studies [30,43]. Mean K contents of later date of first cut were also similar to those reported in other studies [30,43]. K content was above the guideline value of 7% of ash content [12] at later date of first cut. These high K contents can lead to slagging and corrosion during the combustion process due to lower ash melting temperatures. Mean Ca content was comparable to that found in a dry hay meadow [43] and within the guideline range of 15%–35% of dry ash [44] at later date of first cut (D3, D4). However, in most cases, the Ca content was below this percentage, which can cause problems related to ash melting. Mg contents lower than 2.5% of dry ash can promote slagging [43]. At Foothills, Mg content was sufficient mainly at late dates of first cut in 2013 and 2014. At Swabian Jura, it was consistently low (1.6 ± 0.2 g kg^{−1} DM).

The quality of hay can be improved through upgrading treatments. For example, leaching can reduce unwanted contents of ash, Cl, K and Mg [45]. Contents of Cl, K and Mg can also be reduced by delaying the first cut until September, but this leads to substantial changes in species composition [46]. Therefore, this method is only suitable for maintaining the openness of the landscape and not for the preservation of species-rich hay meadows.

Another pretreatment is the IFBB (integrated generation of solid fuel and biogas from biomass) system, which was developed for the energetic use of late-mown grassland biomass. This technique separates silage into solid press cake and a liquid phase. The press cakes are rich in fibre and have better combustion properties as the detrimental mineral nutrients are concentrated in the press juice [47]. The drawback of this system is that it is only profitable if in spatial proximity to a biogas plant [48].

Our field trial showed that extensive fertilisation of species-rich hay meadows has only a minor influence on biomass quality, but harvesting time significantly influences chemical composition. Both the hypotheses that date of first cut has a strong effect on biomass quality and that there are site-specific effects were confirmed. The stage of maturity of grasses (the dominant plant functional type) needs to be considered for each site, because it depends for example on altitude.

Other newly developed conversion pathways, such as bioethanol fermentation and pyrolysis, have not yet been tested on biomass from extensively used meadows. One alternative use could be the production of paper. Late-cut biomass from the first growth cycle would be appropriate for this usage.

A first analysis identified water-soluble organic substances in wastewater as critical [49]. Further research is necessary on the usability of grassland biomass in this conversion pathway.

Where several biomass uses are possible, a combination of usage pathways could help alleviate trade-offs between agronomic and biodiversity goals, for example an early cut for forage and a late cut for energetic use. Depending on local conditions, a rotational use of several lowland hay meadows would allow an earlier cut in some years without changing their botanical composition. For the conservation of these meadows, new management and usage concepts are necessary. However, the usability of the biomass for farmers is limited and any additional income would not compensate for the extra expenditure. Therefore, the ecological benefit of extensive management concepts would need to be remunerated by public funds. With a continued increase in the price of fossil fuels, the energetic use of grassland biomass could become more economically viable.

5. Conclusions

An overview of the most recommendable uses of biomass from different cuts is provided in Table 6. The hypothesis that biomass from an early first cut is more suitable for forage use than that of a later first cut was confirmed. Early-cut biomass is suitable as forage for extensive animal husbandry systems, such as suckler cows and sheep, whereas the feeding of later harvests is limited to horses with low performance. The biomass needs to be supplemented due to its low energy content, especially at late dates of first cut. Forage quality can be improved through PK fertilisation; this leads to a higher proportion of legumes and thus higher protein content. However, it should be considered that an early cut before the main flowering period could lead to a loss of biodiversity in the long term.

Table 6. Recommendations for most suitable uses of biomass from harvesting dates of lowland hay meadows.

Harvest Time	Before Flowering (D1)	Main Flowering Period (D2)	End of Main Flowering Period (D3 and D4)
Components:			
Protein, energy content	High	low	low
Fibre content	Low	low	high
Usability for biomass			
Forage use	beef cattle, non-lactating sheep	suckler cows, sheep	as exclusive feed only for horses
Biogas use	suitable	suitable	only dry fermentation
Combustion	not suitable	not suitable	leached material suitable

A late cut is more suitable for biogas use than for feed application because any toxic species would not be problematic. The implementation is, however, restricted by economic considerations, because methane yields are comparatively low.

The most advisable use of biomass from a late cut is combustion. The hypothesis on the suitability of late-cut biomass for combustion was confirmed, the main problem here being the high N and ash contents. Other solid biofuels such as wood are more available and easier to combust than this late-cut grassland biomass. However, a late cut is more flexible than an early cut with respect to weather conditions. It was found in the field trial that the quality of the hay was not significantly reduced when the late cut was postponed by two weeks.

Author Contributions: Conceptualization, U.T. and M.E.; methodology, M.B. and J.H.; formal analysis, J.H. and M.B.; investigation, M.B.; data curation, M.B.; writing—original draft preparation, M.B.; writing—review and editing, M.B., U.T., M.E., I.L. and J.H.; visualization, M.B.; supervision, U.T. and I.L.; project administration, U.T.; funding acquisition, M.E. and U.T.

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Appendix A

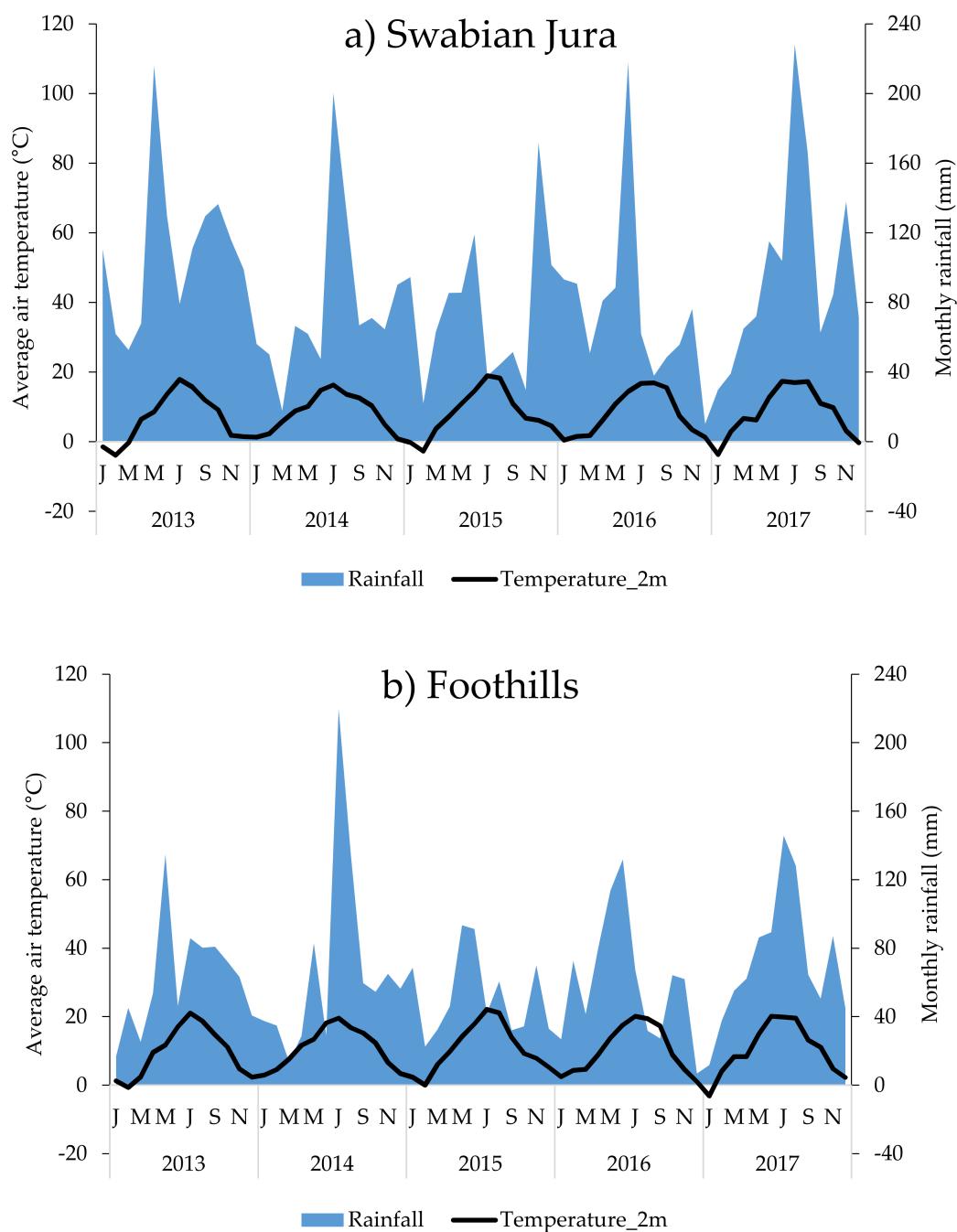


Figure A1. Mean air temperature (2 m) and total monthly precipitation [50] at (a) nearest weather station to Swabian Jura site, St. Johann (48°48'54.7" N, 9°33'86.2" E, 749 m above sea level) and (b) nearest weather station to Foothills site, Tachenhausen (48°64'96.1" N, 9°38'56.5" E, 330 m a.s.l.).

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Chapter 4 General Discussion



There is a lack of research combining both ecological and agronomical questions in grassland science (Bullock et al. 2020). Agronomical questions, e.g. how to enhance biomass quality, are mainly investigated on intensively used grasslands and research on extensively used grassland is focused on biodiversity. My research about species-rich hay meadows covers both topics and aims at a recommendation of optimal grassland management in the sense of conserving biodiversity and enabling agronomic usage at the same time. Species-rich hay meadows can deliver multiple ecosystem services. These services are measured by using different indicators like dry matter yield for provisioning ecosystem services and species richness for biodiversity (Garland et al. 2020). This thesis includes three studies about management effects on the delivery of different ecosystem services of species-rich hay meadows. The following sections will discuss and relate the three presented studies and put them in the broader context of recent literature. The first chapter will discuss causes for the change in botanical composition. Based on this, management options for the conservation of species-rich hay meadows will be derived. The second chapter will take a closer look at the farmer's perspective to find the optimal usage of biomass regarding biomass quality. The chapter will close with recommendations for dealing with trade-offs between agronomic and conservation goals.

4.1 Managing species-rich meadows with the goal to conserve botanical composition

In Europe, natural grasslands occur at marginal sites only. Semi-natural grasslands of high species richness evolved through agricultural practices and were first documented during the Middle Ages (Hejcman et al. 2013). Many hay meadows were created through deforestation to provide winter forage for cattle and horses. Mown pastures dominated until the 18th century, when more hay was needed as more livestock was kept in barns throughout the whole year. The manure of these animals was mainly used for crop production, therefore hay meadows often remained unfertilised. Pure meadows that were not used for grazing exist in Central Europe only for about one hundred years (Kapfer 2010). For example, the spread of *Arrhenatherum elatius*, a characteristic grass species of hay meadows, was enabled by the decline of grazing during the 19th century (Hejcman et al. 2013). Since 1970, hay meadow management was intensified using drainage, liquid manure and ensiling techniques. These changes were necessary because of progress in animal breeding and to increase productivity. But at the same time there was evidence for a loss of biodiversity. Not only management but also changed environmental conditions could have led to a loss of biodiversity of species-rich hay meadows. This will be discussed alongside management effects in this chapter. For example, climate change or nutrient inputs from the atmosphere can alter botanical composition (e.g. Stevens et al. 2010, Wilkins et al. 2016). Additionally, biodiversity of former hay meadows was promoted by diverse land use. Compared to nowadays, connectivity of the landscape was higher during these ages, e.g. through smaller management units and more connecting landscape elements like hedges (Tschardt et al. 2005). Moving machines between meadows and transhumance connect grassland communities through dispersing seeds (Fischer et al. 1996, Poschlod et al. 2005). Fragmentation of habitats, however, leads to reduced genetic diversity of populations and may lead to biodiversity losses in hay meadows (Young et al. 1996, Hooftmann and Bullock 2012). However, biodiversity secures the delivery of grassland ecosystem services (Tilman and Downing 1994, Baumgärtner 2008, Allan et al. 2015). From a conservationist point of view, a high number of characteristic plant species are desired to secure the ecosystem service as a habitat for flora and fauna. These plant species are often low-nutrient indicating forbs like for example *Leucanthemum vulgare*, *Centaurea jacea* and *Salvia pratense*. In Germany, these characteristic species are listed for each federal state separately (e.g. LUBW 2018), making prescriptions more flexible. As management is one main factor influencing botanical composition, the effects of cutting regime and fertilisation of species-rich hay meadows will be discussed in this chapter.

Cutting regime

Recommendations on number of cuts for species-rich hay meadows range from one to three cuts per year (Bochi-Brum et al. 2009, Bassignana et al. 2011), dependent on produced biomass and site conditions. In most cases, the traditional mowing regime was mowing twice a year (Szépligeti et al. 2018). At a two-cut regime, the highest plant species richness was found for the Swabian Jura region (Socher et al. 2013). Therefore, the best option for species-rich hay meadows in the study region was proven to be a two-cut regime. In the following, a two-cut regime, as it was tested in our field trial, will be discussed.

There have been observations of advanced dates of first cut in meadows all over Europe during the last decades (e.g. Eriksson et al. 2015). The question is whether global warming can compensate for this change or if meadows are cut at earlier phenological stages. Global warming advances the phenology of plants for about 1-2 days per decade (Renner et al. 2021). In most cases, advanced cutting dates are due to intensification of management. This means, the meadows are cut at an earlier

phenological stage to secure forage quality or to increase the number of cuts per year. Besides, climate change further threatens particularly mountain hay meadows (Buse et al. 2015, Janssen et al. 2016). Therefore, connectivity between these meadows needs to be improved to enable the migration of species that get extinct due to climate change. Landscape planning tools and the establishment of the Natura 2000 network within the EU help to improve the connectivity of species-rich hay meadow habitats (Arponen et al. 2013).

The date of first cut can influence total number of cuts per year and botanical composition. Therefore, many agri-environment schemes prescribe an earliest date of first cut to conserve species-rich hay meadows. The phenology of plants at the time of the first cut can influence botanical composition through reducing the production of seed. An early date of first cut could lead to overaging in perennial plant communities or completely hinder annual species in reproducing. To test this hypothesis, results of our field trial with respect to cutting date effects on reproduction are discussed in the following.

The results showed no influence of cutting date on species richness at one site, where only perennial species grow (**Paper I**). This is in line with some other studies, which showed no influence of cutting date on vascular plant species numbers (Camska et al. 2012, van Klink et al. 2017). On the one hand, these results are due to the time span of different cutting dates tested. Field trials where the first cut is delayed for several months instead of only several weeks show reduced species richness, especially if fertiliser is applied (e.g. Kirkham and Tallowin 1995). Though postponing the first cut by several weeks may benefit ground-breeding birds, it decreases plant species richness (Smith et al. 1996). And one must consider that if the first cut is performed after the seed ripening stage, plants do not flower again during the second growth (Nowak and Schulz 2002).

On the other hand, unchanged species richness at different dates of first cut are due to the adaptability of the examined plant communities. The adaptability of perennial, multi-species communities is high and therefore cutting dates advanced or postponed for some weeks do not affect their botanical composition. However, we found significant effects of advanced date of first cut on the reproduction of the annual plant species *Rhinanthus alectorolophus* (Paper I). Another experiment with hay meadows on peat soil showed a similar cutting date effect on species that regenerate via seeds (Kirkham and Tallowin 1995). Moreover, in our field trial the meadow's conservation status had to be graded down due to the loss of *Rhinanthus alectorolophus*. This means the habitat function is impaired by an early date of first cut, because it promoted the growth of grasses. Even if left unfertilised, the plots were dominated by grasses after this annual species disappeared because it was prevented from setting seed by an early cut. *Rhinanthus alectorolophus* is a hemiparasite that often infects grasses, hence it probably diminished the dominance of grasses at a late date of first cut after the flowering stage. For this reason, restoring species-poor meadows by introducing *Rhinanthus* seeds has been suggested (e.g. Hellström et al. 2011, Bullock et al. 2005). Before creating species-rich meadows with the help of *Rhinanthus*, however, one must bear in mind that even a recommended date of first cut in June can harm conservation status after just a few years due to the spread of grasses. Since *Rhinanthus alectorolophus* is not (yet) an endangered species, there is no reason to actively promote this species at sites where it does not occur naturally from a conservationist point of view.

During the last years, many agri-environment schemes in Germany have been changed from prescribed cutting dates to more flexible, result-oriented schemes (Schoof et al. 2019). It had been noticed that prescribed cutting dates lead to more homogeneous management of hay meadows or even delayed cutting dates for several weeks. Furthermore, the phenology differs between regions and depends on site conditions like altitude and climate. Weather conditions that differ between years influence phenology and therefore cutting dates have to be handled more flexibly.

Fertilisation

Eutrophication due to N fertilisation is reducing species richness worldwide (Soons et al. 2017). Additionally, studies showed that the species richness of mesotrophic grassland can be reduced through the deposition of nitrogen (N) from the atmosphere (Stevens 2010, Van den Berg et al. 2016). It is suspected that a loss of species in hay meadows can occur even below critical N levels (Wilkins et al. 2016). Therefore, conservationists often demand to completely pass on fertilisation in hay meadow management. Other authors exclusively accept fertilisation with PK to prevent the dominance of grasses (Jäger et al. 2002). Paper I showed no short-term fertilisation effects on plant species richness of the examined hay meadows. Similar to our six-year field trial, Kacorzyk and Głąb (2017) found no effect of NPK-fertilisation on plant species richness of mountain hay meadows at a ten-year field trial. However, this reflects only short-term effects of fertilisation. Fertilisation experiments running over decades show that NPK fertilisation leads to a significantly lower number of plant species of hay meadows due to N and P enrichment (Schellberg et al. 1999, Pierik et al. 2011, van Dobben et al. 2017). All these long-term grassland experiments in Europe use relatively high amounts of fertiliser, e.g. a minimum of 80 kg N ha⁻¹, 20-120 kg P ha⁻¹ and 60 kg K ha⁻¹ per year. In our field trial, NPK-fertilisation (35 kg N, 35 kg P and 120 kg K ha⁻¹ per year) enhanced the proportion of grasses in species-rich hay meadows (Paper I). Many grassland soils are P- (Ceulemans et al. 2013) or N-limited, especially if they are exclusively used as meadows. In our field trial, PK fertilisation had no detrimental effects on botanical composition (Paper I), but it increased DMY. PK fertilisation promoted legumes in our field trial, which could increase N content in soil through nitrogen fixation. This could promote the growth of grasses in the long term and could lead to a reduction in species richness. A long-term fertilisation experiment of a hay meadow indeed showed increased soil N contents in PK-fertilised plots (Hopkins et al. 2011). However, PK-fertilised plots of this experiment contained more plant species than plots fertilised with manure or mineral N (Kidd et al. 2017). So future losses of species due to PK-fertilisation seem rather unlikely. Many long-term experiments of grassland fertilisation show a stronger decline in species richness due to mineral NPK. This effect was attributed to soil acidification caused by mineral fertilisation over decades without the counterbalancing application of lime (Hejman et al. 2014). But what are the underlying mechanisms decreasing species numbers where the pH remained constant in soil?

Grasslands are communities of competitive plants. Fertilisation could lead to a loss of species because it increases below- and aboveground competition between plant species. In our field trial, the pH remained constant in soil over the course of six years. Nevertheless, results of Paper I show reduced proportions of forbs due to NPK fertilisation. This is in line with other studies showing a shift from forbs to grasses with increased fertilisation (Duffkova et al. 2015, Raus et al. 2014), even where lime was applied to prevent acidification (Berendse et al. 2021). Moreover, we showed that low-nutrient indicating plants and small plant species were reduced due to NPK fertilisation (**Paper I and II**). The amount of fertiliser applied in meadows cut twice per year depends mainly on nutrient removal via biomass as well as on-site conditions like soil fertility. For example, Schob (2017) recommends PK-fertilisation in amounts compensating for nutrient removal by cutting the meadows twice. Here, the application of fertiliser after the first cut proved to be beneficial for the conservation status of a lowland hay meadow on half-bog soil. The cause for different recommendations is that each site has a different potential to produce a certain amount of biomass. Therefore, choosing the amount of fertiliser in accordance with nutrient removal of biomass seems to be a reasonable solution.

4.2 Management of species-rich meadows for agricultural use

Regarding the frequency of mowing, most studies agree that hay meadows need a regular cut to maintain their species richness (e.g. Socher et al. 2013, Raus et al. 2012, Oomes 1981). Mulching of the meadows or composting the harvested biomass has been shown to be detrimental for biodiversity and to be uneconomical (Blumenstein et al. 2012). If the cut biomass is not removed, a litter layer casts shade and hinders germination and establishment of seedlings probably leading to a loss of light-demanding species. For this reason, the mown material must be used in some way. In 2018, the total area of species-rich lowland hay meadows in Germany covered about 140000 ha (BfN 2019). Such large areas cannot be mown without the help of local farmers. This means that farmers need financial support as well as information on how to manage the meadows. Studies all over Europe show that traditional farming knowledge gets lost (Babai and Molnar 2014, Burton and Riley 2008), especially in regions where livestock and farm numbers decline (Wehn et al. 2018). Therefore, knowledge on managing species-rich hay meadows for agricultural usage is important and will be discussed in the following section.

Energetic or forage use?

The highest dry matter yield (DMY) under a cutting regime of two cuts per year can be achieved via fertilisation. In our 6-year trial, maximum annual DMY was reached at NPK-fertilised plots, whereas the DMY of unfertilised plots declined over the course of five years. The biogas potential of biomass from species-rich meadows mainly depends on DMY and biomass quality (**Paper III**). A case study on biogas from landscape management grass in Germany showed that the profitability mainly depends on DMY, subsidies for land use and low distances of transport (Blokhina et al. 2011). The use of biomass from lowland hay meadows can be a sustainable alternative to replace energy maize in biogas plants and could help to reduce CO₂ emissions (Auburger et al. 2017). For example, Meyer et al. (2018) suggest a co-digestion of manure with straw and excess biomass from permanent grassland. If biomass of these meadows is used in biogas plants and closed nutrient cycles are intended, the spread of digestates on the meadows has to be considered as well. A field trial of digestate application did not show detrimental effects on biodiversity of hay meadows after five years, but reduced proportions of forbs at annual amounts of 25-54 kg N ha⁻¹ (Hensgen et al. 2016). Another field trial with slurry and digestate application on a species-rich hay meadow resulted in reduced proportions of low-nutrient indicating species compared to the unfertilised control (Seither et al. 2017). Therefore, similar effects to NPK-fertilisation in our field trial, namely reduced proportions of forb species, can be expected when digestates are applied to species-rich hay meadows.

Results of Paper III show that biomass of species-rich hay meadows is not suitable for combustion. This is due to the chemical composition of biomass. High K and too low Ca and Mg contents cause problems related to ash melting and slagging. A late date of first cut at the seed ripening stage indeed reduced K content of biomass, but nevertheless it remained above the guideline value of 7% of ash (Paper III). These findings are in line with those by Van Meerbeek et al. (2015) who showed hay cut in August is more suitable for combustion compared to earlier cut hay. The usage of hay from species-rich meadows is not recommended for small-scale combustion units (Tonn et al. 2010). Another challenge for the implementation of this usage option is the storage of hay before combustion.

In forage use of grassland biomass, there is often a trade-off between quality and quantity of biomass. The phenology of species-rich meadows at the time of first cut determines the forage quality (**Paper III**). This has been shown in several studies (e.g. Hermann et al. 2013, van Klink et al. 2017). At a two-

cut regime the farmers must decide which quality of biomass they need. Based on a compromise between these two goals, requirements of their livestock and depending on local weather conditions, the farmers decide on the date of first cut. Through physiological development of the plants the DMY increases, but at the same time fibre content of biomass increases at the expense of energy density. Forage containing a low proportion of cell wall constituents, however, is digested more efficiently than forage with a high fibre content and therefore low energy density.

In general, hay from species-rich meadows can meet the requirements of cattle, sheep and horses (French 2017). Hay from species-rich meadows can be fed to dry cows or heifers (Jilg 2011) but does not meet the requirements of lactating dairy cows. This means, dairy farmers will have problems integrating high amounts of forage from species-rich hay meadows into ruminant nutrition without compromising their performance. For dairy cows, a share of 40 % of species-rich silage should not be exceeded to maintain production (Bruinenberg et al. 2006). Excess hay can be sold to other farms or owners of hobby horses. Another marketing strategy is herbage hay for small animals like rabbits, although rather small amounts of this product are demanded by consumers.

Flexibility of management

Farmers often demand flexibility in their management of species-rich hay meadows, because they want entrepreneurial freedom (Riley 2006). This means for example the decision on the date of first cut, but also of conservation method, because weather conditions are often not optimal for haymaking. Biomass of species-rich meadows can be conserved as silage, but the use of silage additives is recommended (e.g. Herrmann et al. 2013). A better option proved to be hay making. Here, our results showed that a first cut at the stage of flowering is the most flexible option without compromising forage quality (**Paper III**).

The demanded flexibility of management includes grazing of species-rich hay meadows. Some authors argue that grazing of species-rich hay meadows leads to unwanted changes of botanical composition (e.g. Bonari 2017). These changes are caused by trampling, dunging and the selective grazing of animals (Farruggia et al. 2006, Critchley et al. 2007). Some plant species are preferably eaten by grazing animals or are not resistant to trampling (e.g. the species *Arrhenatherum elatius*) and can therefore get extinct.

But one has to consider that many of today's hay meadows formerly were at least partly grazed (Kapfer 2010), as was the meadow of our field trial at the Foothills site (**Paper I**). Studies all over Europe examining hay meadows cut once with aftermath grazing showed positive (Garcia 1992), negative (Smith 1994) or no effects on plant species richness (Briemle 2007). Continuous grazing has been shown to reduce biodiversity (Gilhaus 2017), but there have been positive results of rotational grazing by horses in Germany (Wagner and Luick 2005). In a review about possible grazing options of species-rich hay meadows, Drobnik and Poschlod (2012) stressed the importance of recovery periods and cutting for pasture care. They concluded that basically, grazing is an option to maintain conservation status, but must be fitted to the specific conditions. This means, for example, short grazing periods with a high stocking rate are suitable to imitate a mowing event. The crucial point is a rest period to ensure vegetation development and flowering. Positive effects of grazing are gaps for newly establishing species created by trampling and seeds transported by grazing animals. However, extensive grazing of species-rich hay meadows will only be applied where it is economically viable.

4.3 How to manage species-rich hay meadows best to balance biodiversity and agronomic demands

In the management of species-rich hay meadows, there is a trade-off between provisioning and regulating ecosystem services. For example, biomass production and the proportion of forbs showed oppositional reactions on fertilisation in our field trial (Figure 2).

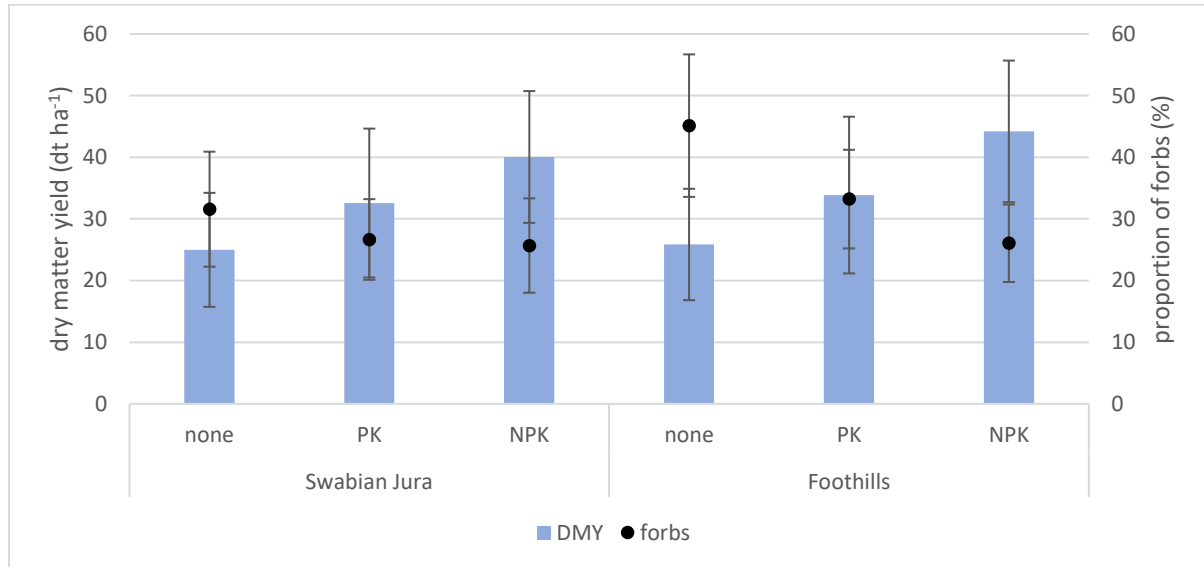


Figure 2 Mean DMY (dt ha⁻¹) and proportions of forbs (%) in the first growth at both sites of the years 2016-2018 with standard deviation (n = 36).

Most studies show a hump-shaped relationship between plant species richness and plant biomass (Mittelbach et al. 2001), confirming Grime's hump-backed curve theory (Grime 1973). The trade-off between biomass and biodiversity can be balanced with a compromise. Species-rich hay meadows should be fertilised with PK or manure to secure DMY, but farmers should be careful about using NPK fertilisers, digestates or liquid manure. Maximising all ecosystem services at the same time is not possible for one meadow. For an optimised delivery of all ecosystem services, it is useful to consider species-rich hay meadows at the landscape or whole farm level. This means, for example spatial variation of cutting dates.

Based on the results of my research, the first cut can be performed before the flowering period to secure forage quality for dairy farmers depending on the botanical composition of the site. To conserve species richness including annual and low-growing plants there is no exact date of first cut. Rather, a temporal variation of cutting date would be useful for maintaining the habitat function of species-rich hay meadows. It has been shown that interannual variation of management can promote biodiversity even in more intensively used grassland (Allan et al. 2015). For farmers, alternating dates of first cut can be recommended to achieve forage qualities of mixed rations sufficient for lactating animals. This can only be achieved if enough meadows are yielding higher forage qualities within the farm. Especially if annuals dominate the meadow, a late first cut at the seed ripening stage can be recommended every second year to secure the habitat function of species-rich hay meadows. To convert this recommendation into practice, the early cut should be best performed in the year following fertilisation. This means, a late cut at the flowering stage every other year is optimal regarding yield, forage quality and habitat function.

4.4 Conclusions

Hay of species-rich meadows can be integrated into rations of modern animal husbandry, even to a small degree into rations of lactating dairy cows and should be used as cut forage preferably. Extensive aftermath grazing is an option. The chemical composition of late-cut hay meets the requirements of hobby horses. If cut earlier, the hay can be fed to more extensively kept cattle, goats or sheep. A challenge is the increasing loss of livestock in many regions of Europe (e.g. Wehn et al. 2018), leading to a standardisation of cutting dates or even the abandonment of meadow usage. If it cannot be used for feed production, it is a source of biomass for a growing bioeconomy (Lewandowski 2018). For example, the co-digestion of grass and manure to produce biogas. In future, new technologies for an energetic usage are possible, which now are not economically viable. These technologies like for example the generation of solid fuel briquettes via mechanical separation are being tested with promising results (Joseph et al. 2018). Currently, there is a new usage option for hay cut at the seed ripening stage, although this is not a widespread practice. It is used for the restoration of species-rich hay meadows via hay transfer, because it contains valuable and regionally grown seed (e.g. Buchwald et al. 2007). In general, low-intensity farming can be economically viable, if farmers are paid for all ecosystem services they provide including biodiversity. Agri-environment schemes combined with management plans (Lakner et al. 2020) are a good possibility to achieve this goal.

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Supplementary Material (Paper I)

Table S1 List of vascular plant species. Mean yield proportions in first growth cycle of species in 2016 (n = 36) at the sites Foothills and Swabian Jura. Low-nutrient indicator species are marked with an asterisk (*) First column gives percentage means of different treatments in 2013 (n = 3). A „+“ stands for proportions below 1%.

Swabian Jura Vascular plant species	2013 mean	2016											
		none				PK				NPK			
		C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
<i>Achillea millefolium</i> agg.	+	.	+	+	+	+	+	+	+	+	+	+	.
<i>Ajuga reptans</i>	+	1	1	+	+	.	+	+	+	+	+	+	+
<i>Alopecurus pratensis</i>	+	+
<i>Anthoxanthum odoratum</i> *	3	6	2	2	2	4	+	1	2	8	1	+	1
<i>Anthriscus sylvestris</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Arrhenatherum elatius</i>	2	7	12	12	6	7	15	9	10	7	16	15	8
<i>Avenula pubescens</i> *	8	13	11	14	17	.	13	11	13	8	13	8	10
<i>Bellis perennnis</i>	+	+	+	.	.	+	+	+	.	+	+	.	.
<i>Briza media</i> *	.	.	+	+	+
<i>Bromus erectus</i> *	+	.	+	+	+	.	.	+	1	+	.	.	+
<i>Campanula rotundifolia</i> *	+	.	+	+	+	.	+	+	+	.	.	+	+
<i>Cardamine pratensis</i>	.	+	.	.	.	3	+	.	.	+	.	.	.
<i>Carum carvi</i> *	+
<i>Centaurea jacea</i> *	+	+
<i>Centaurea scabiosa</i> *	.	.	+	+	.	+	.	.
<i>Cerastium holosteoides</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Colchicum autumnale</i> *	+	+	.	+	+	+	+	+	+	.	.	+	+
<i>Crepis biennis</i>	6	+	.	+	+	+	.	+	.	+	+	.	+
<i>Cynosurus cristatus</i>	2	.	1	2	3	.	4	2	2	.	4	1	2
<i>Dactylis glomerata</i>	2	5	2	+	4	1	3	2	2	5	5	5	3
<i>Daucus carota</i> *	1	.	+	.	2
<i>Festuca pratensis</i>	3	6	13	10	6	23	13	14	5	15	12	13	8
<i>Festuca rubra</i> *	12	24	10	11	16	17	7	7	10	29	9	11	23
<i>Galium mollugo</i> agg.	2	8	11	9	9	13	7	11	8	11	13	9	8
<i>Geranium pratense</i>	1	5	+	1	2	3	+	5	1	2	1	3	3
<i>Glechoma hederacea</i>	+	.	.	.	+	+	.	.	.	+	+	.	.
<i>Heracleum sphondylium</i>	+	+	+	+	+	+	+	+	+	.	+	+	+
<i>Holcus lanatus</i>	+	.	1	+	1	1	2	2	3	3	+	7	8
<i>Knautia arvensis</i> *	.	.	.	+
<i>Leontodon hispidus</i> *	+	.	+	+	+	.	+	+	+	.	+	+	+
<i>Leucanthemum vulgare</i> *	+	+	1	4	4	+	3	2	+	+	+	+	+
<i>Lolium perenne</i>	1	.	+	1	+	.	+	+	+	.	.	+	1
<i>Lotus corniculatus</i> *	2	+	+	+	1	+	+	+	+	+	+	+	+
<i>Lychnis flos-cuculi</i> *	.	.	.	+
<i>Medicago lupulina</i>	2	+	+	+	+	+	+	+	3	+	+	+	+
<i>Myosotis arvensis</i>	+	+
<i>Onobrychis viciifolia</i> *	+
<i>Orobancha caryophyllacea</i>	.	.	.	+	.	.	.	+	+	.	.	+	.
<i>Phleum pratense</i>	.	3
<i>Pimpinella major</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Plantago lanceolata</i>	1	10	11	6	6	10	7	4	7	8	8	4	3
<i>Plantago major</i>	+
<i>Plantago media</i> *	+	1	+	+	+	+	+	+	+	+	+	+	+
<i>Poa pratensis</i>	1	.	+	1	+	.	2	+	1	.	1	1	1
<i>Poa trivialis</i>	2	6	1	2	+	8	3	3	1	.	3	+	+
<i>Ranunculus acris</i>	2	+	+	+	.	+	+	+	+	+	+	+	+
<i>Ranunculus bulbosus</i> *	+	.	.	.
<i>Rhinanthus alectorolophus</i> *	21	+	+	4	9	+	+	6	10	+	+	7	7
<i>Rumex acetosa</i>	+	+	+	+	+	+	+	+	+	.	+	+	+
<i>Salvia pratensis</i> *	+	.	.	.	+	+
<i>Scabiosa columbaria</i> *	+	+	+	+	.	.	+	+	2	+	.	.	+
<i>Silene dioica</i>	+	+	1	.	.	+	+	.	+	+	+	1	+
<i>Taraxacum</i> sect. <i>Ruderalia</i>	+	.	.	.	+	.	+	+	+	.	+	+	+
<i>Tragopogon pratensis</i> *	9	.	.	+	+	.	+	.	+	.	.	+	+
<i>Trifolium pratense</i>	4	.	+	+	+	+	+	+	+	.	+	+	+
<i>Trifolium repens</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Trisetum flavescens</i>	5	+	11	12	7	6	11	16	7	+	10	10	8
<i>Veronica chamaedrys</i>	+	+	1	+	+	+	+	+	+	+	+	+	+
<i>Vicia sepium</i>	1	+	+	+	+	+	+	+	+	+	+	+	+

Foothills	2013	2016											
Vascular plant species	mean	none				PK				NPK			
		C1	C2	C3	C4	C1	C2	C3	C4	C1	C2	C3	C4
<i>Achillea millefolium</i> agg.	+	+	1	1	3	+	1	2	2	1	1	2	1
<i>Ajuga reptans</i>	+	+	+	.	.	+	+	.	.	+	+	.	.
<i>Anthoxanthum odoratum</i> *	+	3	3	+	+	5	2	.	+	3	1	.	+
<i>Anthriscus sylvestris</i>	2	+	+	+	+	+	+	+	+	+	1	+	+
<i>Arrhenatherum elatius</i>	19	21	13	22	21	13	18	22	20	15	22	26	24
<i>Avenula pubescens</i> *	4	.	8	+	1	3	3	+	+	7	3	.	.
<i>Bellis perennnis</i>	+	.	+
<i>Briza media</i> *	.	.	.	+
<i>Bromus erectus</i> *	+	2	.	1	.	.	.	+	.	.	+	.	.
<i>Campanula glomerata</i> *	+	.	+	+	+
<i>Campanula patula</i> *	+
<i>Campanula rotundifolia</i> *	+	.	+	+	+	.	+	+	+	.	+	+	+
<i>Cardamine pratensis</i>	+	+	.	.	.
<i>Carum carvi</i> *	+
<i>Centaurea jacea</i> *	2	2	2	6	1	3	3	2	3	1	+	1	2
<i>Centaurea scabiosa</i> *	5	6	7	8	2	7	2	3	3	2	1	3	3
<i>Cerastium holosteoides</i>	+	+	+	+	+	+	+	.	+	+	+	+	.
<i>Colchicum autumnale</i> *	+	.	+	+	+	+	+	+	+
<i>Crepis biennis</i>	2	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cynosurus cristatus</i>	1	.	1	1	+	.	+	1	.	.	+	1	1
<i>Dactylis glomerata</i>	2	+	2	2	3	5	3	2	2	8	4	2	5
<i>Daucus carota</i> *	+	.	+	+	+	.	.	+	.	+	.	.	+
<i>Festuca pratensis</i>	2	6	7	8	7	15	5	8	5	6	8	9	7
<i>Festuca rubra</i> *	10	12	10	4	16	7	11	13	18	4	12	13	13
<i>Galium mollugo</i> agg.	6	4	6	6	7	4	6	8	6	3	5	6	5
<i>Geranium pratense</i>	+	+	1	+	2	+	1	+	2	1	+	+	1
<i>Heracleum sphondylium</i>	4	+	+	+	+	+	+	+	+	+	+	+	+
<i>Holcus lanatus</i>	3	+	3	2	4	1	7	4	5	3	11	6	8
<i>Knautia arvensis</i> *	6	3	6	7	6	+	5	3	5	1	5	5	4
<i>Leontodon hispidus</i> *	+	+	+	+	+	.	+	+	+	.	.	+	.
<i>Leucanthemum vulgare</i> *	+	+	2	6	+	+	+	+	2	+	+	1	+
<i>Lolium multiflorum</i>	.	2	.	.	1	+
<i>Lolium perenne</i>	2	+	2	+	1	2	3	+	1	13	2	+	1
<i>Lotus corniculatus</i> *	1	+	+	+	+	+	+	+	+	+	+	+	+
<i>Medicago lupulina</i>	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Medicago sativa</i>	+	+
<i>Pimpinella major</i>	+	+	+	+	+	+	1	+	+	+	+	+	+
<i>Plantago lanceolata</i>	+	6	6	4	6	6	5	4	3	4	5	2	2
<i>Plantago major</i>	+
<i>Plantago media</i> *	+	+	+	+	+	+	+	.	.	+	+	.	.
<i>Poa pratensis</i>	2	10	5	+	+	10	7	.	+	8	1	+	1
<i>Poa trivialis</i>	2	6	+	1	1	3	.	+	+	7	+	+	2
<i>Primula veris</i> *	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Prunella vulgaris</i>	+	.	+	+	+	.	+	+	+	.	+	+	+
<i>Ranunculus acris</i>	1	3	2	+	+	2	+	+	+	1	+	+	+
<i>Ranunculus bulbosus</i> *	+	.	.	.
<i>Rumex acetosa</i>	+	+	+	+	.	+	+	+	+	+	+	+	+
<i>Salvia pratense</i> *	5	5	6	2	2	2	3	3	3	3	6	3	1
<i>Scabiosa columbaria</i> *	.	+	2	.	.	.	2	.	.	+	.	.	.
<i>Taraxacum sect. Ruderalia</i>	+	+	+	+	+	+	+	.	+	+	+	+	.
<i>Tragopogon pratensis</i> *	2	.	+	+	+	+	+	.	+	.	+	.	+
<i>Trifolium pratense</i>	2	+	+	+	+	.	+	+	+	+	+	+	+
<i>Trifolium repens</i>	+	+	+	+	+	+	+	+	+	+	+	.	+
<i>Trisetum flavescens</i>	5	2	2	12	8	.	3	13	13	2	7	16	13
<i>Veronica chamaedrys</i>	+	+	+	.	+	+	.	+	+	+	+	+	+
<i>Vicia angustifolia</i>	+	.	.	.	2	.	.	.	+
<i>Vicia cracca</i>	+	.	.	+	.	.	.	1
<i>Vicia sepium</i>	+	+	+	+	+	2	1	2	1	+	+	+	+

Table S2 Cutting dates of first and second cut 2013-2016

Year	1 st cut				2 nd cut
	C1	C2	C3	C4	September
	Before flowering	Start of flowering	Flowering period	Seeds ripening	
2013	May, 28 and June, 4	June, 14	July, 3.	July, 18	23 th
2014	May, 8	May, 26	June, 12	July, 1	16 th
2015	May, 13	June, 2	June, 16	June, 30	15 th
2016	May, 19	June, 2	June, 17	June, 29	14 th

Table S3 Parameters for the assessment of conservation status in German lowland hay meadows.

Grade	A	B	C
<u>Botanical composition</u>			
Species typical for this habitat are:	present	limited	clearly depleted
Species richness	very species rich	species rich	mainly common species, few valuable species
Untypical species (e.g. nutrient indicator or ruderal species)	few present	not present in damaging amounts	present in damaging amounts
Sown species	none present	typical for this habitat type	not typical for this habitat type
<u>Habitat structure</u>			
Vegetation structure (proportion of grasses, dominance of one species)	excellent	medium	no typical structure
Site conditions (nutrients and water in soil)	natural	unfavourable	unnatural, unfavourable
Terrain or relief	natural	unnatural	very unnatural
Management	optimal (2-3 cuts, low amounts of fertiliser)	Favourable (mown irregularly, sometimes grazed)	Unfavourable (fallow, succession of wood, too much fertiliser, mulching or too many cuts)
Other disturbances	none	low	High (e.g. machine tracks)

Table S4 Parameter estimates and standard deviation (SD) of the difference between the two years 2013 and 2016 for fertilisation variants. Results are shown for variables with significant fertilisation x year interactions ($p < 0.05$).

		none	SD	PK	SD	NPK	SD
Swabian Jura	Grasses	23,83	±2,68	24,50	±2,68	31,92	±2,68
	Legumes	-2,11	±0,17	-1,67	±0,17	-2,46	±0,17
	Low-nutrient indicator	-19,78	±3,46	-26,45	±3,46	-20,07	±3,46
	-legumes	-1,85	±0,39	-1,68	±0,40	-2,21	±0,42
	<i>Poa trivialis</i>	-1,36	±0,46	-0,57	±0,46	-1,97	±0,46
Foothills	Grasses	0,50	±1,91	5,67	±1,91	14,83	±1,91
	Forbs	2,50	±1,77	-4,167	±1,76	-11,08	±1,77
	Legumes	-1,28	±0,16	-0,45	±0,16	-1,46	±0,16
	Low-nutrient indicators	-2,55	±3,45	-7,62	±3,38	-10,93	±3,38
	-grasses	-0,09	±0,17	0,09	±0,17	0,05	±0,17
	-forbs	-0,09	±0,14	-0,53	±0,14	-0,73	±0,14

Table S5 Parameter estimates and standard deviation (SD) of the difference between the two years 2013 and 2016 for cutting date variants. Results are shown for variables with significant cutting date x year interactions ($p < 0.05$).

		C1	SD	C2	SD	C3	SD	C4	SD
Swabian Jura	Grasses	29,11	±2,88	26,00	±2,88	24,89	±2,88	27,00	±2,88
	Forbs	-18,89	±3,61	-19,00	±3,61	-15,78	±3,61	-21,89	±3,61
	Legumes	-2,66	±0,19	-1,94	±0,19	-2,22	±0,19	-1,49	±0,19
	Species richness	-12,00	±1,07	-6,67	±1,07	-4,44	±1,07	-2,33	±1,07
	Low-nutrient indicators	-13,11	±5,67	-29,80	±3,59	-24,82	±3,56	-20,67	±2,23
	-grasses	0,67	±0,21	0,07	±0,21	-0,07	±0,21	0,30	±0,21
	-forbs	-3,94	±0,23	-3,09	±0,23	-1,62	±0,23	-1,50	±0,23
	-legumes	-2,10	±0,45	-1,98	±0,42	-1,94	±0,47	-1,64	±0,44
	<i>Rhinanthus alectorolophus</i>	-4,59	±0,25	-4,90	±0,25	-1,45	±0,25	-1,18	±0,25
Foothills	Legumes	-1,29	±0,18	-1,12	±0,18	-0,90	±0,18	-0,94	±0,18
	Species richness	-2,17	±1,39	-2,00	±1,35	-6,11	±1,35	-4,33	±1,35
	Low-nutrient indicators	-0,96	±3,88	-3,56	±3,77	-13,73	±3,77	-9,89	±3,77
	-grasses	1,10	±0,20	-0,15	±0,19	-0,75	±0,19	-0,13	±0,19

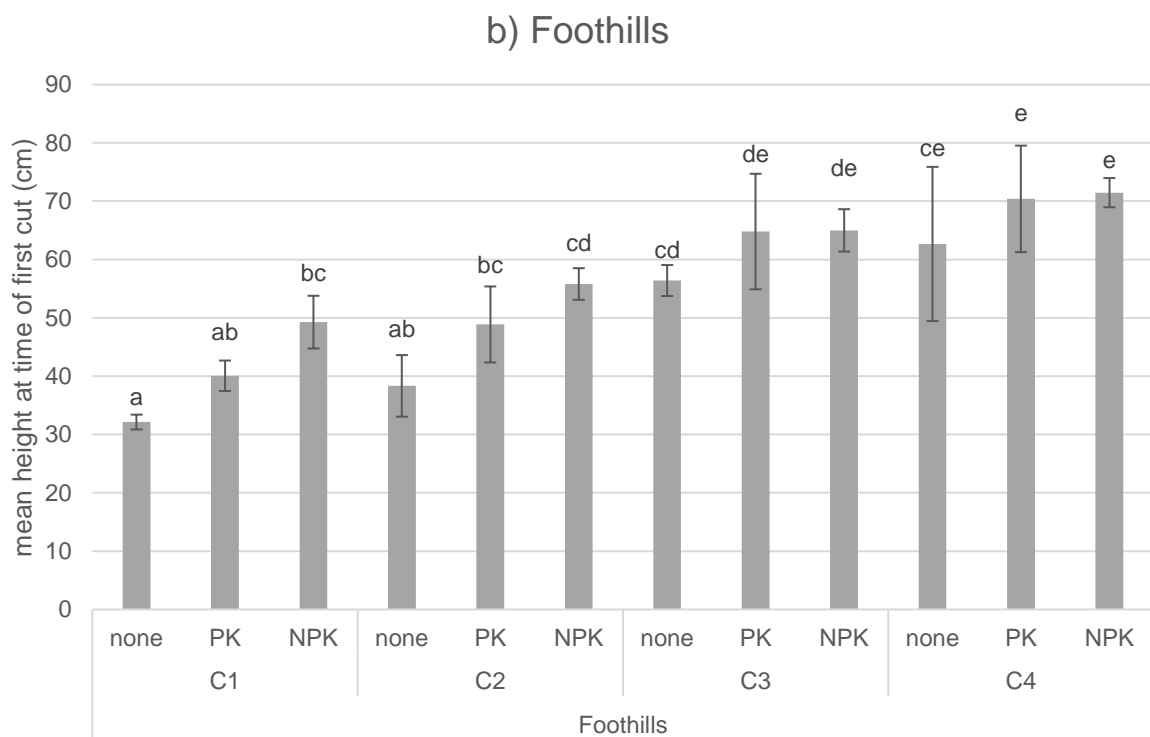
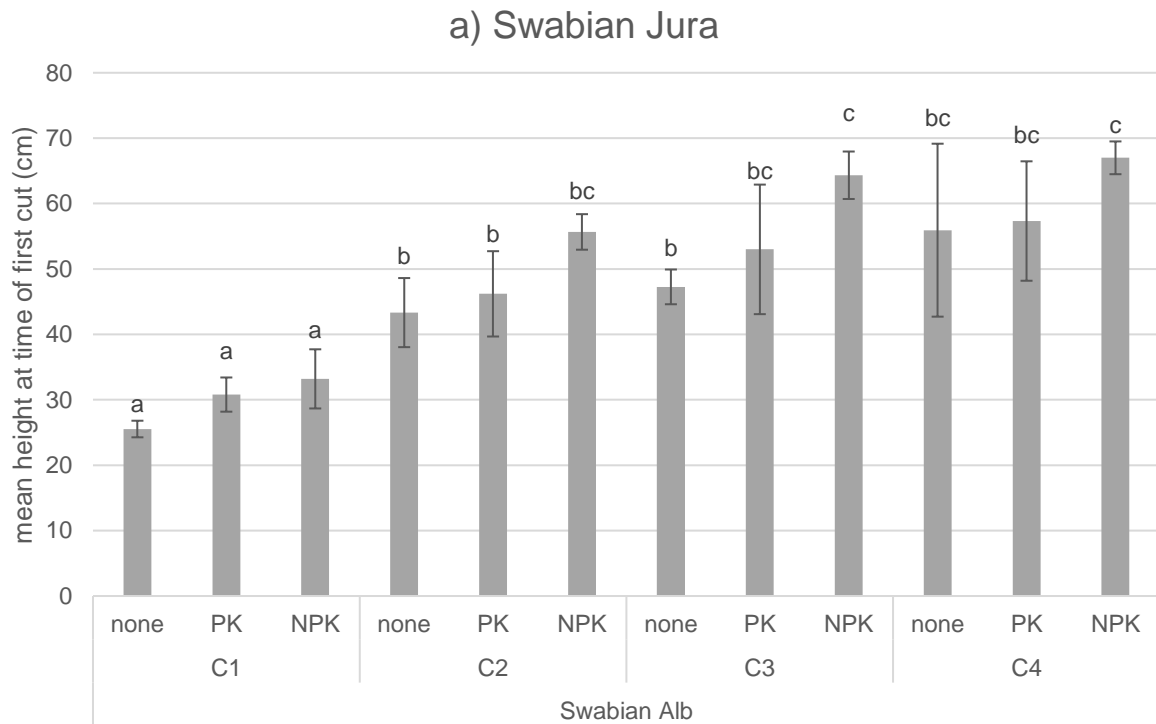


Fig. S1 Mean height at time of first cut for each treatment a) at Swabian Jura and b) at Foothills with standard deviation (n = 3). Different letters indicate significant differences ($p \leq 0.05$).

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Curriculum Vitae

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Professional experience

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Education

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2013-2015	Study of Agricultural Biology, University of Hohenheim
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Contributions to scientific conferences

Boob, M., Thumm, U., Lewandowski, I., Truckses, B., Seither, M. and Elsässer, M. (2017): Einfluss der Bewirtschaftung von FFH-Mähwiesen auf die botanische Zusammensetzung. Nachhaltige Futterproduktion auf Niedermoorgrünland 61. Jahrestagung der AGGF Berlin/Paulinenaue, Germany August 29th – 26th, 157-160. Available at https://www.lfl.bayern.de/mam/cms07/ipz/dateien/aggf_2017_boob_et_al.pdf (accessed on 06.06.2021)

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