

Natura 2000 grassland conservation and restoration: fertilization and mowing regime in the interface between nature conservation goals and farmers' demands

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Restoration Ecology

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*“The world's longest-running experiments remind us that science is a marathon,
not a sprint.”*

Brian Owens, 2013, Nature

Summary

Semi-natural grasslands are the most species-rich habitats in the Central European cultural landscape. Tied to human activities, grasslands accumulated a huge amount of biodiversity during the thousands of years of extensive use. The provision of numerous ecosystem services, such as a sustainable feed base for livestock, characterize the multifunctionality of grasslands. However, the European semi-natural grassland area suffered a significant and ongoing decline since the early twentieth century. Agricultural intensification has resulted in many grasslands being converted to arable fields. On the other hand, the ongoing grassland improvement or abandonment leads to a shift in vegetation composition, coupled with a massive impairment of plant species diversity, and a loss in multifunctionality. Due to the threats, currently 32 natural and semi-natural grasslands formations have been protected by the EU Habitats Directive within the Natura 2000 network and deterioration must be avoided. However, almost half of the grassland habitat types are in unfavorable–bad condition. It is therefore essential to maintain appropriate grassland management, to restore grassland habitat types, and to reconcile agricultural and nature conservation interests.

Among the habitat types of the Habitats Directive that occur in Germany, the national conservation status of alluvial meadows (habitat type 6440) and lowland hay meadows (habitat type 6510) ranks as poor with a stable–deteriorating respective deteriorating trend. In the German floodplain regions, a tremendous change in the composition of plant communities has occurred in alluvial grasslands caused by agricultural intensification, and in particular nutrient input. For the remaining species-rich areas, guidelines for fertilization intensity are crucial for maintaining a good conservation status. Lowland hay meadows surface has declined dramatically in the last decades and massive efforts are now needed to restore these habitat types. Precisely because grassland is use-dependent, the question of how the conservation of species-rich grassland or its restoration can be reconciled with the fodder production is of fundamental importance for the future. In order to explore the trade-off between farmers' and conservationists' interests in grassland use intensity, three field experiments were conducted in Eastern Germany in the federal state of Saxony-Anhalt as part of this dissertation.

The Dessau grassland experiment evaluated the influence of different fertilizer variants (five treatments: 0 kg nitrogen (N) ha⁻¹ yr⁻¹ without and with phosphorus (P) and potassium (K), 60 kg N ha⁻¹ yr⁻¹ without and with PK, 120 kg N ha⁻¹ yr⁻¹ with PK) on the vegetation assembly and fodder quality of an ancient alluvial meadow in favorable conservation status over an 8-year study period. The target species persisted despite nitrogen application at this nutrient-poor site with a low soil phosphorus level. However, nitrogen fertilization resulted in a higher grass cover and, interestingly, reduced the cover of high-competitive target forbs. The negative effect on the target forbs could only be compensated by a moderate nitrogen fertilization of 60 kg ha⁻¹ yr⁻¹ which was combined with additional phosphorus and potassium application, resulting in a cover balance between grasses and forbs. The legume cycle could be maintained only for non-nitrogen-fertilized plots, which is an important finding for securing the feed supply for livestock with regard to future climatic conditions. The forage quality could hardly be improved during the study period with any of the applied fertilizer treatments. In order to

maintain the original good conservation status of the alluvial grassland, a maximum of 60 kg N should be applied in combination with a P and K application. However, this recommendation only applies to phosphorus-poor sites.

The Wulfen grassland experiment was established on a species-poor, formerly intensified mesic grassland where, despite extensification, the typical plant species failed to re-colonize. 18 site-adapted target forbs, typical of habitat type 6510, were sown after sward disturbance. The aim was to determine the effects of mowing frequency (three treatments: once, twice, three times) and fertilization intensity (three treatments: 0, 60, 120 kg N ha⁻¹ yr⁻¹) in the initial development phase of grassland restoration. In the crucial first year after sowing, frequent cutting was important for the establishment success at this nutrient-rich site. The target species number and individuals clearly benefited from the highest cutting frequency (three times). Moderate nitrogen fertilization of 60 kg ha⁻¹ yr⁻¹ did not lead to a significant lowering of the competitive ability of the sown species, which is important for restoration practice. It allows moderate fertilization if an adequate biomass removal by cutting will be applied. Therefore, the respective meadows can provide hay already during the restoration phase, which can increase farmers' acceptance of restoration actions.

The Hayn grassland experiment determined how different fertilizer levels (six treatments: 0, 60, 120 kg N ha⁻¹ yr⁻¹ without and with PK) and drought conditions affect target species performance and biomass production in the medium-term development in grassland restoration. 44 site-adapted target species, including grasses and forbs typical of habitat type 6510, were sown on a former arable land. The development was observed over a 5-year study period. In contrast to seeding after sward disturbance (Wulfen grassland experiment), target species number was not affected after seeding on former arable land despite higher nitrogen application of 120 kg ha⁻¹ yr⁻¹, which is in accordance to the Dessau grassland experiment. Fertilization resulted in higher cover of sown grasses as nitrogen intake increased, and forb cover benefited from the absence of nitrogen supply, resulting in a cover balance between grasses and forbs. The interaction of omitted nitrogen fertilization and improved phosphorus availability promoted the sown legumes, thus initiating the legume cycle. The first cut's biomass could only be significantly improved by the highest nitrogen fertilizer rate of 120 kg ha⁻¹ yr⁻¹. Interestingly, the forb biomass of the important first cut increased in the non-nitrogen fertilized treatments and in the 60 kg ha⁻¹ yr⁻¹ nitrogen-fertilized treatments despite drought, which is one of the key results in terms of climate adaptation. Six of the sown plant species appear to be particularly suitable for sustaining productivity over several drought years and should be part of high-diverse native seed mixtures.

Based on the results from the three grassland experiments, the following implications for practice and land use policy can be given: i) The compromise between grassland farmers' and conservationists' interests can be achieved by applying 60 kg ha⁻¹ yr⁻¹ of nitrogen in combination with phosphorus and potassium, ii) when designing management-based agri-environmental schemes, authorities should limit the amount of nitrogen as indicated above and provide extra salary for the additional application of phosphorus and potassium to under-supplied sites, iii) more frequent mowing in the first year after sowing ensures the successful establishment of the target species on nutrient-rich sites after sward disturbance, and 60 kg

nitrogen $\text{ha}^{-1} \text{yr}^{-1}$ can be applied if an adequate biomass removal is achieved by mowing in the initial and medium-term post restoration management phase, and iv) drought-tolerant matrix species should be included in high-diverse native seed mixtures when arable fields are converted to species-rich grasslands in order to maintain biomass production and strengthen climate resilience.

Zusammenfassung

Grünlandlebensräume sind die artenreichsten in der mitteleuropäischen Kulturlandschaft. Gebunden an eine Nutzung durch den Menschen, akkumulierte sich im Lauf der Jahrtausende währenden extensiven Nutzungsweise eine enorme Artenvielfalt. Das hohe Maß der Multifunktionalität ist durch die Bereitstellung zahlreicher Ökosystemleistungen gekennzeichnet, so dient Grünland beispielsweise als nachhaltige Futtergrundlage für Nutztiere. In Europa verzeichnete das permanente Grünland seit Beginn des 20. Jahrhunderts einen erheblichen Rückgang mit anhaltendem negativem Trend. Infolge der Intensivierung der Landwirtschaft wurden zum einen viele Grünlandflächen in Äcker umgewandelt. Zum anderen führten Maßnahmen zur Steigerung der Produktivität oder die Nutzungsaufgabe zu einer erheblichen Veränderung der Vegetation, gekoppelt mit einer massiven Beeinträchtigung der Pflanzenartenvielfalt, sowie zu einem Verlust an Multifunktionalität des Grünlandes. Aufgrund dieser Gefährdungen sind derzeit 32 natürliche und halbnatürliche Grünlandformationen durch die Europäische Fauna-Flora-Habitat-Richtlinie im Rahmen des Natura-2000 Netzwerkes geschützt und eine Verschlechterung des Erhaltungszustandes muss verhindert werden. Allerdings befindet sich fast die Hälfte der Grünlandlebensraumtypen in einem ungünstigen bis schlechten Zustand. Es ist daher unerlässlich, geeignete Formen des Grünlandmanagements beizubehalten, die Renaturierung voranzutreiben sowie landwirtschaftliche mit naturschutzfachlichen Anforderungen in Einklang zu bringen.

Der Erhaltungszustand der Brenndolden-Auenwiesen (Lebensraumtyp 6440) sowie der Mageren Flachland-Mähwiesen (Lebensraumtyp 6510) wird in Deutschland aktuell als unzureichend eingestuft, mit einem stabilen bis sich verschlechternden beziehungsweise ausschließlich verschlechternden Trend. In den großen Flusslandschaften veränderten sich durch die Intensivierung der Landwirtschaft die Pflanzengemeinschaften enorm, insbesondere verursacht durch den hohen Eintrag von Nährstoffen. Für die Bewahrung eines günstigen Erhaltungszustands des noch verbliebenen artenreichen Auengrünlands sind daher Richtlinien für eine maximal zulässige Düngeintensität von entscheidender Bedeutung. Die Ausdehnung der Flachland-Mähwiesen ist in den letzten Jahrzehnten drastisch zurückgegangen. Folglich sind nun massive Anstrengungen erforderlich, um diesen Lebensraumtyp wiederherzustellen. Gerade weil Grünland nutzungsabhängig ist, ist die Frage, wie die Erhaltung oder Renaturierung artenreicher Bestände mit der Futterproduktion in Einklang gebracht werden kann, für die Zukunft von existenzieller Bedeutung. Um darauf evidenzbasierte Antworten geben und Empfehlungen aussprechen zu können, wurden im Rahmen der vorliegenden Dissertation im Bundesland Sachsen-Anhalt drei Freilandexperimente durchgeführt.

Das Dessau Grünlandexperiment wurde auf einer Auenwiese in hervorragendem Erhaltungszustand eingerichtet und untersuchte über eine 8-jährige Versuchslaufzeit den Einfluss verschiedener Düngevarianten (fünf Varianten: 0 kg Stickstoff (N) $\text{ha}^{-1} \text{Jahr}^{-1}$ mit und ohne Phosphor (P) und Kalium (K), 60 kg N $\text{ha}^{-1} \text{Jahr}^{-1}$ mit und ohne PK, 120 kg N $\text{ha}^{-1} \text{Jahr}^{-1}$ mit PK) auf die Vegetationszusammensetzung und die Futterqualität des Aufwuchses. Die Zielarten persistierten auf dem nährstoffarmen Standort (niedriger Phosphorgehalt) in allen Varianten. Die Stickstoffgabe führte jedoch zu einer höheren Gräserdeckung und reduzierte

interessanterweise die Deckung der konkurrenzstarken Zielartenkräuter. Der vom Stickstoff ausgehende negative Effekt auf die Zielartenkräuter konnte nur für die moderate Gabe von $60 \text{ kg ha}^{-1} \text{ Jahr}^{-1}$ durch eine zusätzliche Gabe von P und K ausgeglichen werden und ein ausgeglichenes Gräser-Kräuter-Verhältnis erzeugen. Der Leguminosenzyklus konnte nur ohne Stickstoffgabe aufrechterhalten werden, was im Hinblick auf die Sicherung der Futtermittelversorgung für Nutztiere unter künftigen klimatischen Bedingungen eine wichtige Erkenntnis ist. Die Futterqualität verbesserte sich während des Untersuchungszeitraums bei keiner der getesteten Düngevarianten. Um den ursprünglich hervorragenden Erhaltungszustand des Auengrünlandes zu erhalten, sollten maximal 60 kg N ha^{-1} und nur in Kombination mit einer Grunddüngung aus P und K ausgebracht werden. Zu beachten ist, dass diese Empfehlung ausschließlich für phosphorarme Standorte gilt.

Das Wulfen Grünlandexperiment wurde auf einer artenarmen, ehemals intensiv genutzten mesophilen Wiese angelegt, auf der sich trotz Extensivierung typische Pflanzenarten nicht wieder ansiedeln konnten. Nach Störung der Grasnarbe wurden 18 standortangepasste und für den Lebensraumtyp 6510 typische Kräuter ausgesät. Anschließend wurden die Auswirkungen verschiedener Mahdhäufigkeiten (drei Varianten: einschürig, zweischürig, dreischürig) und Düngungsintensitäten (drei Varianten: 0, 60, $120 \text{ kg N ha}^{-1} \text{ Jahr}^{-1}$) im ersten Jahr nach der Aussaat, der initialen Phase der Grünlandrenaturierung, untersucht. Auf dem nährstoffreichen Standort war häufiges Mähen entscheidend für den Etablierungserfolg. Die Arten- und Individuenzahl der Zielarten profitierte deutlich von der höchsten Schnitffrequenz (dreischürig). Die moderate Stickstoffdüngung von $60 \text{ kg ha}^{-1} \text{ Jahr}^{-1}$ führte nicht zu einer signifikanten Verringerung der Konkurrenzfähigkeit der ausgesäten Arten, eine für die Renaturierungspraxis wichtige Erkenntnis. Wiesen können deshalb bereits während der Entwicklungspflege im ersten Jahr ausreichend Biomasse für die Heugewinnung liefern und zu einer Steigerung der Akzeptanz von Renaturierungsmaßnahmen bei Landnutzenden führen.

Das Hayn Grünlandexperiment untersuchte, wie sich unterschiedliche Düngeintensitäten (sechs Varianten: 0, 60, $120 \text{ kg N ha}^{-1} \text{ Jahr}^{-1}$ ohne und mit PK) und Trockenheit mittelfristig auf die Entwicklung von Zielarten und den Biomasseertrag auswirken. Auf einer ehemaligen Ackerfläche wurden dafür 44 standortangepasste, für den Lebensraumtyp 6510 typische Gräser und Kräuter ausgesät und die Entwicklung über einen Zeitraum von 5 Jahren beobachtet. Im Gegensatz zur Einsaat nach Grasnarbestörung (Wulfen Grünlandexperiment) wurde die Anzahl der Zielarten trotz hoher Stickstoffgabe von $120 \text{ kg N ha}^{-1} \text{ Jahr}^{-1}$ nicht beeinträchtigt. Diese Erkenntnis deckt sich mit dem Dessau Grünlandexperiment. Die Düngung führte mit zunehmender Stickstoffzufuhr aber zu einer steigenden Gräserdeckung. Ohne Stickstoffzufuhr profitierte die Deckung der Kräuter und resultierte in einem ausgeglichenen Gräser-Kräuter-Verhältnis. Die Interaktion von unterlassener Stickstoffdüngung und verbesserter Phosphorverfügbarkeit förderte im Experiment die Leguminosen und initiierte den Leguminosenzyklus. Der Biomasseertrag des ersten Schnitts konnte nur durch die höchste Stickstoffgabe von $120 \text{ kg ha}^{-1} \text{ Jahr}^{-1}$ deutlich verbessert werden. Interessanterweise stieg bei den Varianten ohne und mit moderater Stickstoffgabe von $60 \text{ kg ha}^{-1} \text{ Jahr}^{-1}$ die Biomasse der Kräuter zum ersten Schnitt trotz Trockenheit, eines der wichtigsten Ergebnisse hinsichtlich Klimaanpassung. Sechs Pflanzenarten sicherten die

Produktivität über mehrere trocknen Jahre hinweg und sollten Teil artenreicher Wildpflanzenmischungen sein.

Basierend auf den Ergebnissen der drei Grünlandexperimente lassen sich für Praxis und Politik folgende Erkenntnisse und Handlungsempfehlungen formulieren: 1) Ein Kompromiss zwischen landwirtschaftlichen und naturschutzfachlichen Interessen kann durch die Ausbringung von 60 kg Stickstoff $\text{ha}^{-1} \text{Jahr}^{-1}$ in Kombination mit der Gabe von Phosphor und Kalium erreicht werden. 2) Bei der Ausgestaltung von Agrarumweltmaßnahmen sollte die maximal zulässige Stickstoffgabe auf die zuvor genannte Menge begrenzt und die Grunddüngung mit Phosphor und Kalium auf unterversorgten Standorten zusätzlich vergütet werden. 3) Erfolgt bei der Grünlandrenaturierung auf nährstoffreichen Standorten die Einsaat nach Störung der Grasnarbe, sichert eine höhere Mahdfrequenz die erfolgreiche Etablierung der Zielarten im ersten Jahr. Wird durch Mahd ein adäquater Biomasseentzug gewährleistet, kann sowohl in der initialen als auch mittelfristigen Phase nach der Einsaat eine Stickstoffdüngung bis zu 60 kg $\text{ha}^{-1} \text{Jahr}^{-1}$ erfolgen. 4) Bei der Wiederherstellung von artenreichem Grünland auf ehemaligen Ackerflächen sollten hochdiverse Wildpflanzenmischungen zum Einsatz kommen, die trockenheitstolerante Matrixarten enthalten. Dadurch lassen sich der Biomassertrag sichern und die Klimaresilienz steigern.

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Unless otherwise indicated, the photos are by the author.

Chapter I

State of the art and research needs



Species-rich alluvial grassland in a favorable conservation status in the Elbe floodplain near Dessau.

1 European semi-natural grasslands – land use-dependent ecosystems and their services

Semi-natural grasslands are tied to human use and have emerged as a result of Neolithic husbandry around 7,500–6,800 years ago in Central Europe (Petermann and Buzhdygan, 2021). Up to the Iron Age, only pastures and forest pastures existed and even after the development of iron short scythes suitable for cutting grasslands, haymaking was of secondary importance for a long time (Hejcman et al., 2013). After the Middle Ages, mowing played an increasingly important role in the production of winter fodder and led to the development of systematic meadow management (Kapfer, 2010). The large-scale enlargement in many regions occurred from the 18th century (Hejcman et al., 2013), resulting in regionally distinct plant communities associated with hay meadow management (Dierschke and Briemle, 2002). Until agriculture was intensified in the mid-20th century, a fauna and flora had developed that is associated with grasslands under low-intensity management practice (Hopkins and Wilkins, 2006; Kapfer, 2010). More than 1,300 species of vascular plants in Central Europe are more or less closely associated with grassland ecosystems, and almost 30% of these species occur exclusively in grassland (Bruchmann and Hobohm, 2010). Maintaining appropriate grassland management is therefore crucial to support biodiversity conservation in European grasslands (Elliott et al., 2023).

In Europe, grasslands play an important role as part of agricultural production systems (Isselstein et al., 2005; Peyraud and Peeters, 2016). As feed base for livestock, today's use ranges from extensively to intensively used permanent grasslands to improved grasslands, based on sown and highly productive forage grasses and legumes (Hejcman et al., 2013). Permanent grasslands simultaneously provide multiple ecosystem functions and services (Garland et al., 2020; Manning et al., 2018; Zhao et al., 2020). In addition to biomass production for meat production and dairy as one of the most important provisioning services, regulating services, such as pollination and carbon sequestration and storage, as well as cultural services, characterize the multifunctionality of grasslands (Bengtsson et al. 2019). However, the intensity of use is crucial for multifunctionality, which decreases with increasing intensity (Schils et al., 2022), caused by the loss of non-productive multifunctionality (Klaus et al., 2024).

2 The situation of European grasslands

2.1 General situation

Grassland ecosystems are the third most dominant ecosystem within the European Union and account for about 16% of the area (European Environment Agency, 2016). The European semi-natural grassland area suffered a significant and ongoing decline since the early twentieth century (Auffret et al., 2018), amounting to over 90% in most European countries (European Environment Agency, 2016). The current distribution show only a fraction of original grasslands. For the recent past, Pazúr et al. (2024) found strong country-specific differences and identified western England, northern Germany and the border regions of Austria as

hotspots of declining trajectories from 1990 onwards. The key pressure among grasslands is land use change. Agricultural intensification has resulted in many grasslands being converted to arable fields (Emmerson et al., 2016), and grassland improvement or abandonment leads to a shift in vegetation composition (Diekmann et al., 2019; Kuhn et al., 2021), a loss in multifunctionality (Schils et al., 2022) and negatively influences plant biodiversity (Billeter et al., 2008, Elliott et al., 2023).

Due to the threats, currently 32 natural and semi-natural grassland formations have been protected by the EU Habitats Directive and deterioration must be avoided (Council of the European Commission, 1992; European Commission, 2013). However, almost half of the grassland habitat types are in unfavorable–bad condition (European Environment Agency, 2016). It is therefore essential to restore grassland ecosystems, as incorporated in the Nature Restoration Law (Council of the European Commission, 2024a), and to reconcile agricultural development and conservation through measures such as agri-environment schemes (European Environment Agency, 2016).

2.2 Alluvial meadows of river valleys of the *Cnidion dubii* (habitat type 6440)

Alluvial meadows (habitat type 6440) occur in Central to Southeastern Europe, with a distribution focus in the large German floodplains. Within Natura 2000 sites, alluvial meadows occur in Germany on an estimated 3,726 ha (Buchwald, 2008). In the northern German floodplain regions, a tremendous change in the composition of plant communities has occurred over the last 50 years, with a consistent trend towards species-poor communities caused by agricultural intensification, and in particular nutrient input (Wesche et al., 2012). This situation also applies to Germany's other floodplain regions (Hölzel et al., 2002; Bischoff et al., 2009; Bischoff et al., 2018). Therefore, the national conservation status of alluvial meadows is currently ranked as unfavorable–bad with a stable–deteriorating trend (Bundesamt für Naturschutz, 2019). Nevertheless, particularly in areas not subject to intensive dairy farming, species-rich alluvial meadows can still be found in a good conservation status.

2.3 Lowland hay meadows (habitat type 6510)

Lowland hay meadows (habitat type 6510) can be found in almost all of Europe, with a high occurrence in Germany (European Environment Agency, 2019). Within Natura 2000 sites, lowland hay meadows occur on an estimated 80,237 ha in Germany (Buchwald, 2008) and are the habitat type with the largest surface area. They have declined especially strongly since 1950 (Briemle et al., 1999). The national conservation status is currently ranked as unfavorable–bad with a deteriorating trend (Bundesamt für Naturschutz, 2019). Due to the systematic failure of the German authorities to monitor Natura 2000 sites hosting habitat types 6510 and 6520 adequately and regularly, and to take appropriate measures to avoid deterioration, the European Commission initiated an infringement proceeding against Germany in 2019 (Schumacher and Schumacher, 2019). In November 2024, the Court of Justice of the European Union recently ruled against Germany for failing to fulfil its obligations

under Article 6(2) and the second subparagraph of Article 4(1) of the Habitats Directive (Court of Justice of the European Union, 2024b). In addition to the mandatory implementation of management plans, the attractive remuneration of nature conservation measures and the improvement of abiotic conditions, massive efforts are now needed to restore these habitat types (Jedicke, 2024).

3 Fertilization of alluvial meadows and its impact on vegetation assembly and forage quality

During the historical land use practice of low-intensity management, grasslands accumulated a huge amount of biodiversity (Habel et al., 2013). Floodplains have a long tradition of high-quality hay production. Meadows were usually mowed twice a year, depending on weather conditions and flooding (European Commission, 2023). The historical management can often still be recognized in the current vegetation of never-intensified grassland, where more typical grassland species occur (Kuhn et al., 2021; Straubinger et al., 2021), which characterize the favorable conservation status. For several decades, fertilizer application has been a common method for agricultural improvement to increase yields and improve forage quality (Pavlů et al., 2022). It has been commonly found that nitrogen fertilization is a key driver of changes in grassland vegetation (Pavlů et al., 2022) that led to decreased plant species richness for the whole range of European grassland types (Soons et al., 2017). Starting from a species-rich condition, studies from mountain and alpine regions showed drastic changes in species assembly and reduced nature conservation value due to nitrogen fertilization (Scotton et al., 2014; Gillet et al., 2016). But there are different findings on the response of grassland forbs to fertilization (Hejcman et al., 2007; John et al., 2016; Busch et al., 2019). For lowland and mountain meadows, the promotion of grasses and the decrease of legumes by nitrogen application have been described prominently (Kacorzyk and Głąb, 2017; Dindová et al., 2019; Ranta et al., 2021). Legumes have a positive effect on forage quality (Wilman and Williams, 1993; Buxton, 1996; Lüscher et al., 2014) and occur cyclically in grassland (Schwinning and Parsons, 1996; Herben et al., 2017). However, the legume cycle and the effects of fertilization have hardly been studied so far (Herben et al., 2017).

Currently, there are few long-term studies on changes in the species assembly of species-rich alluvial meadows that have been subjected to different fertilization treatments. Existing studies often compare the effect of different fertilizer applications only after decades of use, while annual fluctuations and periodic oscillations were not evaluated (see Honsova et al., 2007; Hrevušová et al., 2009; Vargová et al., 2012; Hejcman et al., 2014). There are also few long-term studies on the development of forage quality in permanent grasslands (Mrkvička and Veselá, 2002; Pavlů et al., 2006; Hrabě and Knot; 2011, Štýbnarová et al., 2014). In many cases, only results from one- to three-year experimental studies have been published (see Ludewig et al., 2015; Pornaro et al., 2019; Schaub et al., 2020; Ranta et al., 2021), or the quantity and quality of forage production of different meadow types have been assessed (see Scotton et al., 2014, Loucougaray et al., 2015). However, consideration of these aspects is

important to improve our understanding of the relationships between fertilization, forage quality and species assembly in alluvial meadows.

4 Restoration of lowland hay meadows and post-restoration management

The unfavorable conservation status of grassland habitat types in large parts of Europe has led to the development of various techniques for the restoration of grassland communities (see Kiehl et al., 2010; Kirmer et al., 2012; Blakesley and Buckley, 2016). A natural and low-cost way is spontaneous recolonization (Prach and Hobbs, 2008), which can occur in the short term from persistent seeds in the soil seed bank (von Blanckenhagen and Poschlod, 2005) and in the long run from large target species populations in the immediate vicinity (Prach et al., 2021). However, if seeds are not available due to soil seed bank and dispersal limitations, this presents the main barrier to spontaneous grassland restoration (Öster et al., 2009a; Török et al., 2018). Pywell et al. (2002) also identified the presence of seeds of target species as a key factor for the development of species-rich meadow communities. To overcome these limitations, active introduction of target species during restoration is required (Bakker and Berendse, 1999; Walker et al., 2004). This can be done by transferring directly harvested seed-rich biomass or seed mixtures from seed production (Kirmer et al., 2012). The sowing of native seeds from regional propagation is one of the most commonly used method in Europe to restore species-rich semi-natural grassland on ex-arable land (Kiehl et al., 2010; Kirmer et al., 2012), where the soil seed bank tends to be dominated by ruderals, whereas typical grassland species are usually absent (Walker et al., 2004). However, it is also a successfully tested method for the restoration of species-poor grassland after seedbed preparation by topsoil disturbance (e.g., strip approach: Rayburn and Laca, 2013; Shaw et al., 2023, gap approach: Kiss et al., 2021).

Grassland restoration methods and their effects on ecological community reassembly have been extensively studied, while the importance of post-restoration management has so far received less attention (Tölgyesi et al., 2022). The first year after species introduction seems to be the most crucial period. Young seedlings are extremely sensitive to different biotic as well as abiotic factors resulting in a high loss rate (Fenner, 1987; Grubb, 1977). However, as shown by Öster et al. (2009b), a high first-year recruitment of sown species is very important for long-term establishment and therefore for the restoration outcome. It is thus a question how to manage this crucial first year to achieve high establishment rates of introduced species. Particularly if a major part of farmed grassland is embedded into restoration schemes, restoration projects are often confronted with farmers' demands. Because lowland hay meadows represent major forage sources for livestock feeding, farmers stipulate that the restoration sites are sufficiently productive and provide hay with a good forage quality already in the restoration phase. This would imply to fertilize restoration sites (mainly with nitrogen), cut them twice a year as well as roll them using large machinery, like under traditional grassland management practices. The question is, how would this affect the establishment of introduced target species? On the one hand, many grassland restoration projects cut their restoration sites only once a year (van der Putten et al., 2000; Hölzel and Otte, 2003;

Jongepierová et al., 2007; Török et al., 2010) or even refrain from cutting in the early restoration period (Nordbakken et al., 2010; Rydgren et al., 2010). A relatively low biomass production is given as one argument for this management (Hölzel and Otte, 2003). Other arguments may be the fear of increasing soil compactness by cutting with large machinery (Schäffer et al., 2007), which can reduce seedling establishment (Török et al., 2011) or the disturbances caused by cutting. On the other hand, some restoration studies have demonstrated that frequent cutting already in the first year can have positive effects on seedling establishment (Hofmann and Isselstein, 2004; Lawson et al., 2004). In particular, on sites with high soil nutrient contents seedlings of less competitive species can be suppressed by an increase in aboveground biomass and therefore increased light limitation for understory species (Hautier et al., 2009; Borer et al., 2014). Thus, frequent cutting may be a useful measure to reduce negative competition effects particularly in the first crucial year of establishment and on more productive sites (Lawson et al., 2004; Borer et al., 2014). Furthermore, effects of fertilization on seedling establishment might also be different depending on the intensity of biomass removal, i.e., cutting time and frequency. Therefore, testing the effects of different mowing regimes in combination with different nitrogen fertilization treatments is highly relevant for restoration practice, but has rarely been tested (Jones and Hayes, 1999; Smith et al., 2003; Foster et al., 2009).

The crucial initial phase is followed by management in the medium-term phase. During this period, the species composition continues to change. In particular, climatic and edaphic factors influence grassland composition and structure (Feurdean et al., 2018). However, sown species can continue to emerge well beyond the first year. Germination speed as well as seedling recruitment varies widely across species (Eriksson and Ehrlén, 2008; Wagner et al., 2011) and target species can be expected to establish for several years after sowing native seed mixtures. Moreover, undesirable species can emerge from the seed bank or arrive from the surrounding, with possible consequences for further assembly as well as ecosystem functions and services (Funk et al., 2008; Weidlich et al., 2021).

Regardless of the ongoing development processes of restored grasslands, there is often interest in using the biomass for animal feed as fast as possible. This is particularly the case in regions with suckler cow husbandry, where the feeding of hay is of great importance as a feed basis, especially in years of drought. The use of native seed mixtures on former arable land can lead to the rapid establishment of meadow species producing vegetation suitable for haymaking (Mitchley et al., 2012). To maintain biomass production, grassland has traditionally been fertilized to balance nutrient removal (Kapfer, 2010). In contrast, the higher use of nutrient concentrations, especially nitrogen, is a common practice in modern grassland systems to increase productivity (Oenema et al., 2012; Einarsson et al., 2021), and conflicts with biodiversity conservation goals (Boch et al., 2021; Ranta et al., 2021). In the context of different land use priorities, it is important to address the challenge of reconciling farmers' demands on biomass for hay production with the nature conservation goal to restore species-rich grasslands. The role of fertilization (e.g., Pecháčková et al., 2010) or the impact of drought (e.g., Cole et al., 2019) has been studied only in existing grasslands. Studies on fertilization in relation to species richness and the resilience of biomass production facing climate change in

the course of grassland restoration by seeding are lacking so far. Climate change is expected to increase drought frequency and severity in Western Europe (Spinoni et al., 2017), posing new challenges both for maintaining grassland productivity (Wu et al., 2021) and for the successful restoration of grasslands (Lyons et al., 2023).

5 References

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Chapter II

Research questions



Lowland hay meadow in the Wulfener Bruch near Köthen. Photo: Henriette John.

1 Motivation and aims of the thesis

The Global systems are in a state of profound crisis. Ongoing climate change is threatening food security and human well-being (Mirzabaev et al., 2023), and biodiversity is currently experiencing a dramatic mass extinction worldwide (WBGU, 2021). The trilemma of simultaneously ensuring food security, biodiversity conservation, and climate-change mitigation requires solutions for sustainable land stewardship (WBGU, 2021). Permanent grassland covers 30,5% of the European Union's agricultural area (Eurostat, 2020), are a source of fodder for livestock, contribute to carbon storage (Bengtsson et al., 2019) and host a huge amount of biodiversity (Wilson et al., 2012; Dengler et al., 2020). Grassland thus plays a particularly important role in tackling these major challenges.

Different demands on grassland have implications for management decisions, which in turn influence ecosystem services and biodiversity (Habel et al., 2013; Schollenberger et al., 2019). While production-oriented management requires intensive fertilizer input and high frequency of use, low-intensity use promotes non-production services and high biodiversity (Klaus et al., 2024). Precisely because grassland is use-dependent, the question of how the conservation of species-rich grassland or its restoration can be reconciled with the biomass production is of fundamental importance for the future and represents the central motivation of this thesis (Figure 1).

The study sites of this thesis are located in the eastern part of Germany in the federal state of Saxony-Anhalt. In Germany, grassland covers 28% of the agricultural area, 43% of which is used as meadow (Statistisches Bundesamt, 2024). Due to the historically predominant farm structures, the grassland proportion in the agricultural area in Eastern Germany is particularly low, and Saxony-Anhalt shows the lowest proportion at 15% (Umweltbundesamt, 2023). As part of the reporting process under Article 17 of the Habitats Directive, Saxony-Anhalt reported a total area of 3,053 ha for Alluvial meadows (habitat type code 6440) and 10,796 ha for Lowland hay meadows (habitat type code 6510) to the Federal Agency for Nature Conservation, including 19% and 32% respectively in an unfavorable conservation status (Landesamt für Umweltschutz Sachsen-Anhalt, 2025). Consequently, there is a need for both conservation and restoration efforts. And beyond that, the restoration of an unquantified but presumably very large grassland area that has been lost as a result of conversion to arable land.

In order to maintain grassland in a favorable conservation status in a targeted and area-effective manner, farmers must be supported with attractive and evidence-based agri-environmental schemes. Furthermore, prohibitions and requirements in Natura 2000 areas must be based on scientifically sound knowledge, but also give farmers leeway for production-oriented grassland management. In the context of restoration, it must be taken into account the farmers' need to re-use the restored grassland in a timely manner in order to produce sufficient biomass with a reasonable fodder quality for feeding livestock, even in times of severe drought.

In the context of the challenges and needs described, the thesis pursues the following objectives:

- Exploring the trade-off between farmers' and conservationists' interests in grassland use intensity with a particular focus on fertilization.
- Providing evidence-based thresholds for authorities for the further development of management-based agri-environmental schemes for grassland habitat types.
- Providing recommendations to practitioners on the intensity of grassland use at different post-restoration management phases without jeopardizing the expected restoration outcome.
- Identification of drought-tolerant species for seed mixtures used in grassland restoration on former arable land to strengthen the climate resilience of restored grasslands.

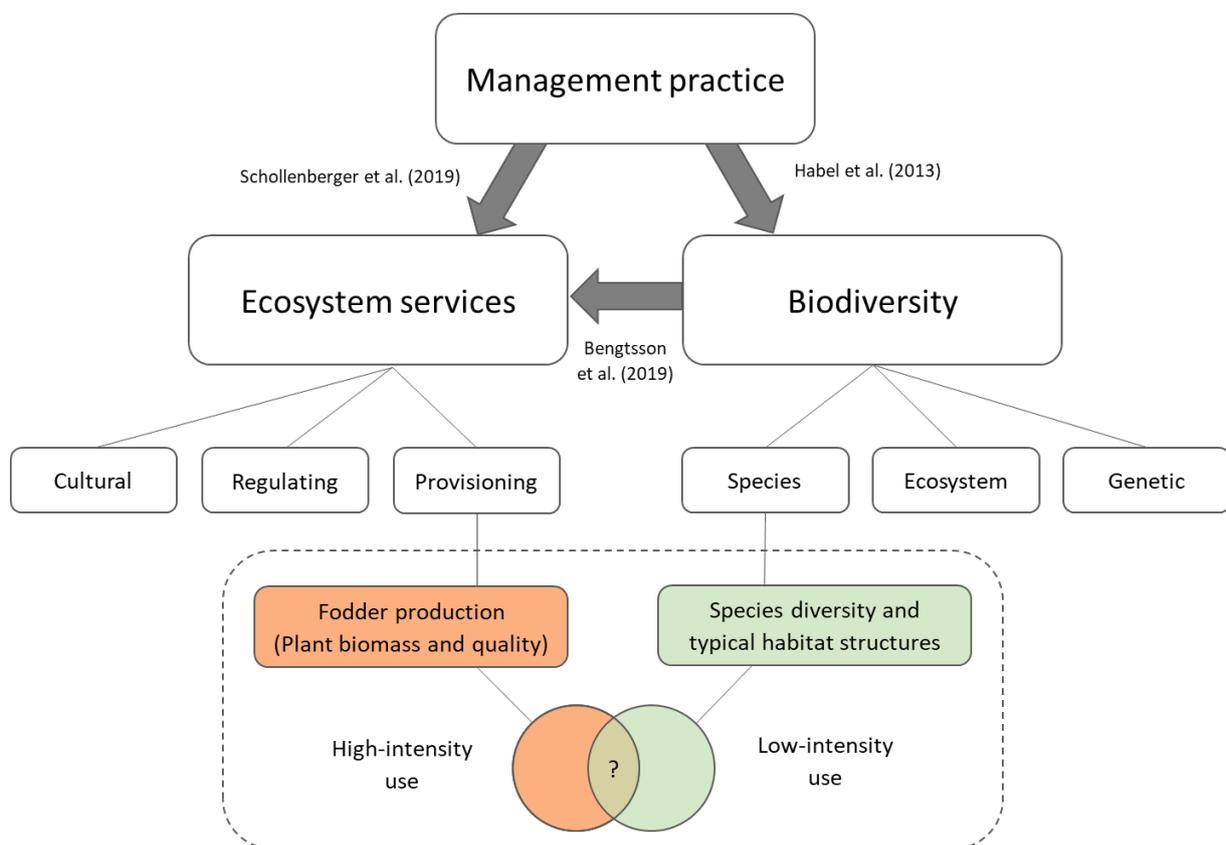


Figure 1 Illustration how the topic of the thesis fits into the complex system of grassland management, ecosystem services and biodiversity. Since the key pressure on grassland habitats comes from agriculture (European Environment Agency, 2020), but use is a mandatory requirement for semi-natural grassland maintenance (Elliott et al., 2023), compromises must be found by considering both agricultural and nature conservation needs. Orange = intensive management to meet farmers' demands, green = extensive management to meet nature conservation goals, mixed color = space for compromises.

2 Outline of the thesis and research questions

This thesis is based on three original research articles, all dealing with different aspects of grassland management (Figure 2). To assess the effects of different management treatments, all investigations were carried out in field experiments in the form of plot trials in Eastern Germany (see Chapter II 1.3).

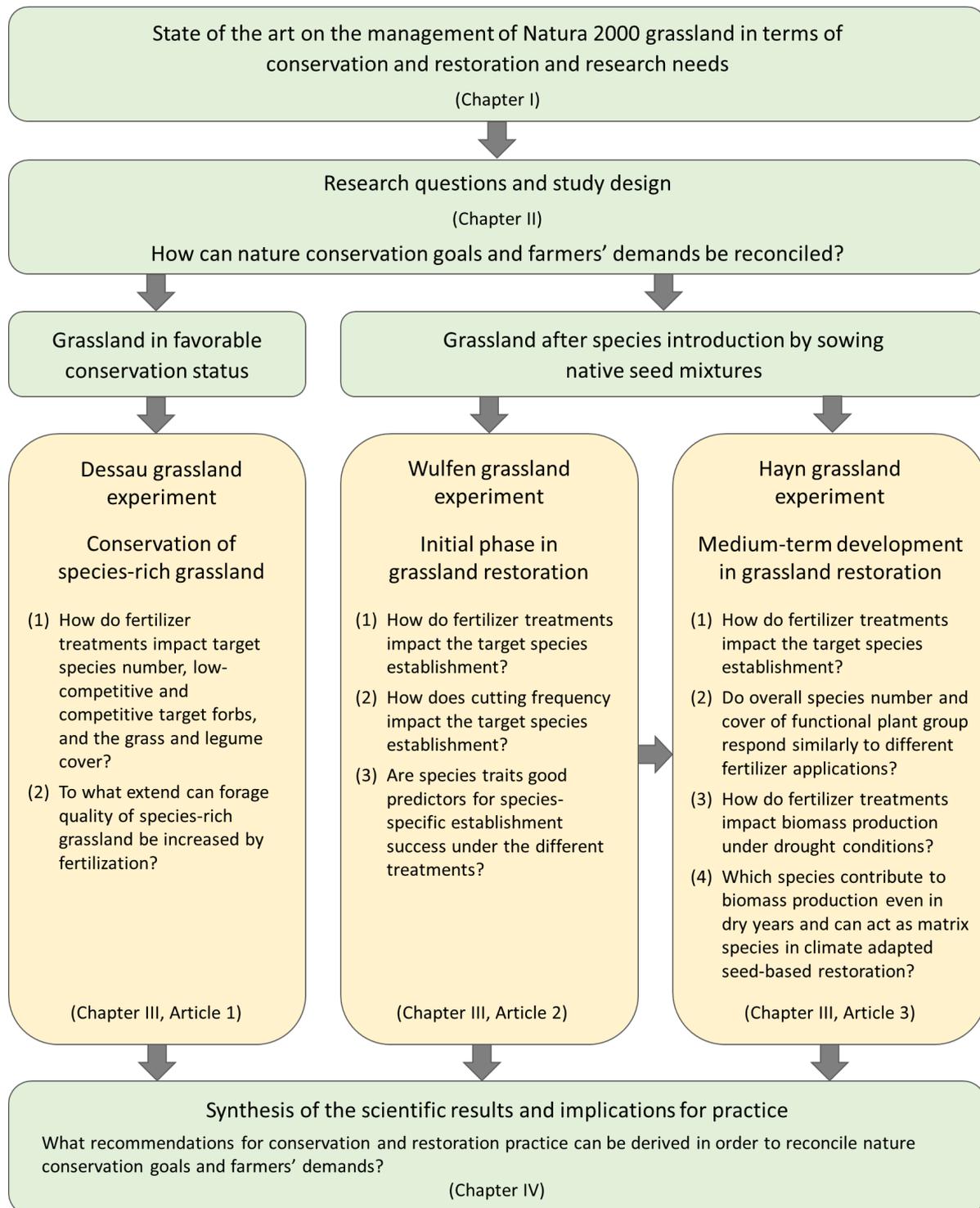


Figure 2 Outline of the thesis and research questions.

The first paper (Chapter III Article 1) presents the research results on the effects of different fertilizer applications on an ancient floodplain grassland (habitat type 6440). The focus is on the question of what amount of fertilizer can be applied without endangering the favorable conservation status. In addition, the extent to which fertilization improves the fodder quality for livestock is investigated.

The other two papers deal with questions regarding management options in grassland restoration. The focus is on the various phases following the introduction of target species by sowing native seed mixtures. The second paper (Chapter III, Article 2) shows the effects of different mowing frequencies and fertilization intensities on target species seedlings in the initial phase after species introduction in a species-poor lowland hay meadow (habitat type 6510). The third paper (Chapter III, Article 3) compares the medium-term development of target species under different fertilizer treatments, and also looks at the development of biomass production for feed production under increasing drought periods.

Chapter IV is the concluding chapter; it summarizes the results and brings the thesis to a close with recommendations on management practice for grassland conservation and restoration, while considering farmers' demands.

3 Grassland experiments

3.1 The Dessau grassland experiment

The Dessau grassland experiment site was situated on the Elbe floodplain near the city Dessau in the German federal state Saxony-Anhalt (Figure 3 and 4), 58 m above sea level. The climate is continental with a mean annual precipitation of 552 mm and a mean annual temperature of 10.0 °C (climatologic station: Jessnitz, period: 1981–2010, DWD, 2025). The Elbe River is 2.5 km away and used to flood the area in spring, but during the study period, the study site was not flooded every year. Dry soil conditions prevail in summer. The soil is an acidic gley, characterized by low phosphorus and a medium potassium level. The grassland belongs to the Natura 2000 habitat type 6440 (Alluvial meadows of river valleys of the *Cnidion dubii*), protected by the Habitats Directive 92/43/EEC, and to the plant association *Sanguisorbo officinalis-Silaetum silai* Klapp 1951 (Schubert, 2001). Before the experiment started, the permanent and never oversown grassland was in a favorable conservation status, contained many characteristic river corridor plants (according to Burkart, 2001) and a high plant species richness. The meadow was traditionally mown twice in the growing season for hay making, and, depending on the growth, additionally grazed by sheep in autumn and not fertilized for at least 30 years. Before the experiment started, the soil had a pH (0.01 M CaCl₂) of 4.9 ± 0.1, contained 0.5% ± 0.1 total nitrogen and 5.6% ± 1.0 total carbon. Phosphorus (double-lactate method) was measured at a very low level (1.2 ± 0.5 mg per 100 g soil, level A) and potassium at an optimal level (11.8 ± 2.0 mg per 100 g soil, level C) (classification follows LLG, 2019).

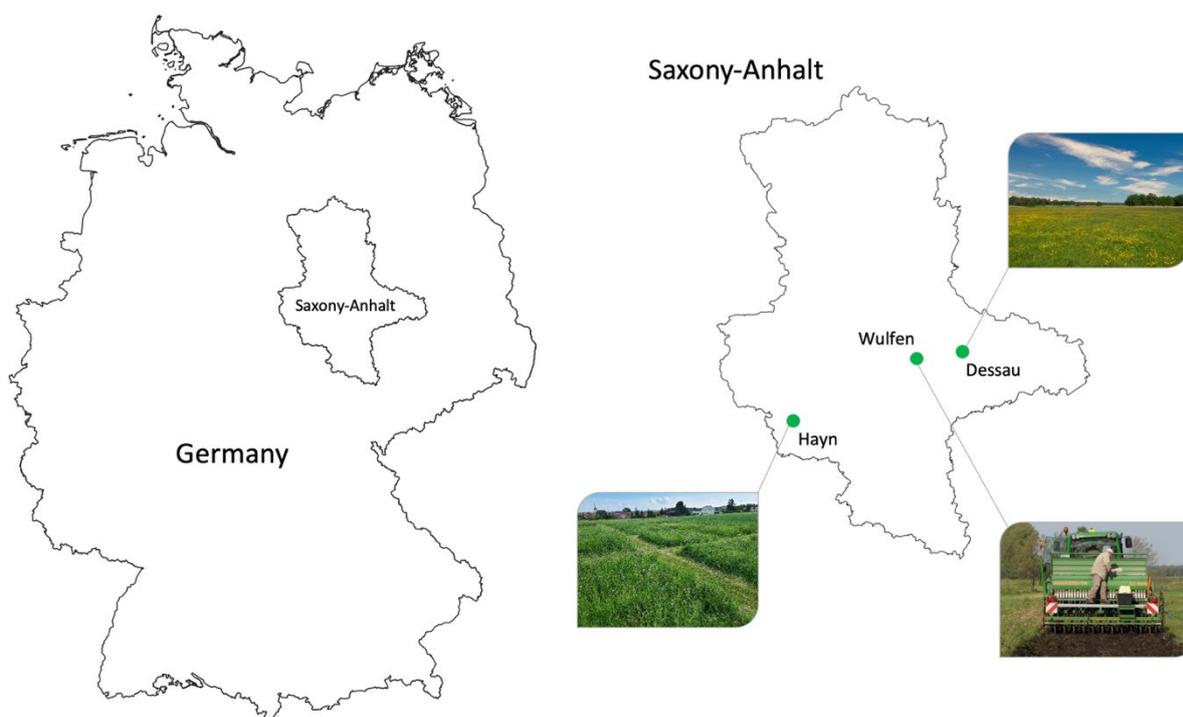


Figure 3 Location of the study sites (green dots) in Germany and at regional scale.

In 2010, the experiment was established in four completely randomized blocks with each block containing five square sample plots (5.0×5.0 m) each for one of the five fertilizer treatments (altogether 20). The meadow was mown twice, with a first cut in early June and a second cut in mid-August. Five fertilizer treatments were tested (see Table 1). Phosphorus and potassium were applied in the amounts that were removed with the harvest in the previous year, calculated for each treatment. We used mineral fertilizer applied in commercially available granules, nitrogen as urea in two doses before and shortly after the first cut, phosphorus in the form of triple superphosphate and potassium in the form of potassium chloride 60% in one dose before the first cut. The methods of data collection can be found in Chapter III Article 1, page 34.

Table 1 Experimental treatments of the Dessau grassland experiment.

Code	Fertilization treatment
N0	Unfertilized control
PK	Phosphorus and potassium as withdrawn
N60	Nitrogen $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$
N60PK	Nitrogen $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ plus phosphorus and potassium as withdrawn
N120PK	Nitrogen $120 \text{ kg ha}^{-1} \text{ yr}^{-1}$ plus phosphorus and potassium as withdrawn



Figure 4 The Dessau grassland experiment site on the Elbe floodplain.

3.2 The Wulfen grassland experiment

The study site was located in the Wulfener Bruch, which is part of a large lowland plain area of the Elbe River in the center of the German federal state of Saxony-Anhalt (Figure 3 and 5), 52 m above sea level. The area is characterized by a continental climate with mean annual precipitation of 552 mm and mean annual air temperature of 10.0°C (climatologic station: Jessnitz, period: 1981–2010, DWD, 2025). The study site was a former half-bog, but, as in many other regions, the site was long-since drained and used as a hay meadow. The soil is gley with high organic matter content (14.3%). The total nitrogen content was about $0.7 \pm 0.3\%$, phosphorus (double-lactate method) 11.6 ± 7.5 mg per 100 g soil (level E, classification follows LLG, 2019), potassium 21.4 ± 6.5 mg per 100 g soil (level D, classification follows LLG, 2019), and pH (0.01 M CaCl₂) of 6.5 ± 0.7 . The high nutrient level is due to intensive use during the times of the former GDR (high fertilization with slurry, several cuts per year). After German reunification, the meadow has been extensively managed (no fertilization, only one cut per year). This former management resulted in species poor stands (18.3 species per 16 m²) with a clear dominance of grasses and a dense vegetation structure, as well as high litter accumulation. The meadow can be characterized as a species-poor lowland hay meadow (Arrhenatherion, Habitats Directive code 6510) which shows some features of an alluvial meadow (Cnidion, code 6440).

We used a split-split-plot design with four blocks (replications) to study the effects of different fertilizer treatments on the number and individuals as well as composition of target species in the first year after sowing (Table 2). The cutting treatment was the main treatment of each block, split by the fertilization treatment (split-plot treatment) which was additionally split by the rolling treatment (split-split-plot treatment). All possible combinations of these single treatments resulted in 18 different management treatments. For each of the 18 treatments and their replications a sample plot with a size of 4.0 m × 4.0 m was installed (altogether 72).

Interspaces between sample plots were about 12.0 m to avoid reciprocal interference of the different fertilization treatments and to facilitate the use of large machinery for mowing and rolling. The cutting height was about 10 cm and cuttings were subsequently removed within one week. The nitrogen fertilizer (urea) was spread by hand only within the 4.0 m × 4.0 m sample plots. Rolling was carried out using machinery typically used by the local farmers for this purpose (manufacturer Güttler GmbH Germany, prismatic roll, width: 6 m, mass: about 3 t). All treatments represent typical management practices for this region. Cutting once represents the management practiced over the last 20 years at the study site, but came under criticism because of the unsatisfactory results in terms of the loss of many typical meadow species, particularly low-competitive forbs. Cutting twice is nowadays applied at nature conservation grasslands and cutting three times is mostly practiced at sites where farmers need high amounts of forage for cattle.

With regard to the fertilization treatments, no fertilizers are used at nature conservation grassland sites where the soil should be impoverished or where atmospheric nitrogen input is high, while 60 kg N ha⁻¹ yr⁻¹ are often used in already developed species-rich lowland hay meadows and alluvial meadows where atmospheric nitrogen input is low. Fertilization with 120 kg N ha⁻¹ yr⁻¹ is a typical practice if a high hay quality is required. Rolling is typically used at drained half-bog sites for soil consolidation and frost crack closing as explained above. All sample plots were ploughed and grubbed before sowing at the beginning of September 2011 and were afterwards sown with 18 target species (mixture composition see Chapter III Article 2: Table 2). To facilitate an even sowing pattern seeds were mixed with soymeal. Seeds were of regional origin and propagation, as recommended for example by Kiehl et al. (2014) and Tischew et al. (2011). The methods of data collection can be found in Chapter III Article 2, page 61.



Figure 5 Sowing the target species strips in the Wulfen grassland experiment.



Figure 6 Initial phase after sowing the target species. Juvenile individuals of *Geranium pratense* and *Selinum carvifolia* (left), and *Rumex acetosa*, *Daucus carota* and *Lathyrus pratensis* (right, both photos July 31, 2012).



Figure 7 The Wulfen grassland experiment had to be abandoned due to an exceptional flooding event. The site directly after the floodwaters receded (July 10, 2013, left). Some areas were still underwater at the end of August 2013 (right).

Table 2 Experimental treatments of the Wulfen grassland experiment.

Code	Treatment and dates 2012
Cutting treatments	
Ct 1	One cut June 18th
Ct 2	First cut May 19th and second cut August 13th
Ct 3	First cut May 19th, second cut July 10th, and third cut September 3rd
Fertilization treatments	
N0	Unfertilized control
N60	Nitrogen 60 kg ha ⁻¹ yr ⁻¹
N120	Nitrogen 120 kg ha ⁻¹ yr ⁻¹
Rolling treatments	
-r	No rolling
+r	Rolled once in April

Originally, the Wulfen grassland experiment was laid out as a long-term experiment. After the initial phase (Figure 6), it was planned to continue applying the treatment variants and to observe their influence on the vegetation assembly and fodder production. However, the experiment had to be abandoned in 2015 due to an exceptional and severe flooding event. From the beginning of June 2013, the entire Wulfener Bruch was flooded for six weeks and all vegetation was destroyed (Figure 7). In contrast to the Dessau grassland experiment, which was established on a floodplain grassland, the vegetation recovered only very slowly and the non-flood-tolerant target species of habitat type 6510 that were sown in the fall of 2011 had died. Therefore, the research questions regarding the medium-term management in lowland hay meadow restoration, which should actually have been investigated further, had to be examined in a follow-up experiment. The Hayn grassland experiment was therefore established in 2017 outside of a flood-prone area, and mesophilic species of habitat type 6510 were sown there.

3.3 The Hayn grassland experiment

The Hayn grassland experiment is located in the southern Harz region near the village Hayn in Saxony-Anhalt (Figure 3 and 8), Germany, 441 m above sea level. The experiment is part of the experimental station of the State Agency for Agriculture and Horticulture Saxony-Anhalt and was used for field trials in crop production until 2016. The climate is subcontinental with a mean annual precipitation of 742 mm and a mean annual temperature of 7.3 °C (long-term mean 1992–2021, LLG, 2022). Before the experiment started, the moderately acidic loam soil (pH value (CaCl₂) 5.28 ± 0.19), classification follows Ad-hoc-AG Boden, 2005) was characterized by a total nitrogen content of 0.2% ± 0.03 and a total carbon content of 2.1% ± 0.3, medium phosphorus (5.0 ± 1.4 mg per 100 g soil, double-lactate method) and medium potassium levels (16.5 ± 3.9 mg per 100 g soil, both class C, classification follows LLG, 2019).

In 2017, we sowed a regionally certified species-rich native seed mixture on the 0.5 ha restoration site. Following Kirmer and Tischew (2014), Arrhenatherion was selected as the target vegetation type. We aimed to establish a submontane mesic grassland of the plant association *Alchemillo vulgaris*-*Arrhenatheretum elatioris* (Oberd. 1957) Sougn. Et Limb. 1963 (Schubert, 2001), which belongs to the Natura 2000 habitat type 6510 (lowland hay meadows, European Commission, 2013) and is of interest for hay production for suckler cow husbandry. The mixture contained 44 native species, composed of 12 grasses and 32 forbs, including five legumes (mixture composition see Chapter III Article 3: Table A.1). We added three annual cultivar species as a nurse crop to ensure the establishment of native species by creating safe site conditions. In September 2017, we mulched the cultivars at a height of 20 cm to hinder their development and not to impede the sown target species.

In 2018, the experiment was established in a fully randomized design with 17 management treatments in six replications on 102 square plots (5.0 m × 5.0 m). This study focuses on the twice mown treatment with a first cut in mid-June and a second cut at the beginning of September. Six fertilizing treatments were tested (see Table 3). We used mineral fertilizer in commercially available granules, N as urea in two applications before and shortly after the first cut, P in the form of triple superphosphate 46 and K in the form of K₂O 60 in one application before the first cut. Both P and K were applied in the amounts removed by the previous year's harvest, which was calculated separately for each treatment, as it is common practice in agricultural meadow management. The methods of data collection can be found in Chapter III Article 3, page 79.

Table 3 Experimental treatments of the Hayn grassland experiment.

Code	Fertilization treatment
N0	Unfertilized control
PK	Phosphorus and potassium as withdrawn
N60	Nitrogen 60 kg N ha ⁻¹ yr ⁻¹
N60PK	Nitrogen 60 kg N ha ⁻¹ yr ⁻¹ plus phosphorus and potassium as withdrawn
N120	Nitrogen 120 kg N ha ⁻¹ yr ⁻¹
N120PK	Nitrogen 120 kg N ha ⁻¹ yr ⁻¹ plus phosphorus and potassium as withdrawn

In 2017, at the start of the experiment, precipitation was in line with the long-term mean. The following five years were repeatedly characterized by periods of drought. In the growing season from April to September 2018–2022, precipitation amounted to 161–349 mm, which was 10.0–58.4% lower when compared to the long-term mean (1992–2016: 387 mm). In contrast to precipitation, temperature showed increased values in 2018–2022. The long-term mean 1992–2016 was exceeded by 0.8–2.5 degrees. The monthly temperature was exceeded by 75% of the values.



Figure 8 The Hayn grassland experiment site in the southern Harz region.

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Chapter III

Publications



Twice mown and unfertilized plot of the Hayn grassland experiment in spring 2022.

The Dessau Grassland Experiment – Impact of fertilization on forage quality and species assembly in a species-rich alluvial meadow

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Abstract

Since alluvial meadows of river valleys of the *Cnidion dubii* are protected by the EU Habitats Directive, reconciling farmers' demands for forage quality with the objective of maintaining them in good conservation status is an important issue in grassland research. In a long-term experiment from 2010 to 2018, we investigated the impact of fertilizing on forage quality and species assembly on a species-rich and twice-mown alluvial grassland in the Dessau Elbe floodplain (Germany). The experiment was composed of an unfertilized control, PK, N60, N60PK and N120PK applications. A significant improvement in forage quality was achieved by nitrogen fertilization only for crude protein, with higher feeding requirements for sheep met only in individual years. The legume cycle was inhibited by the application of nitrogen and high grass cover was maintained, but not increased, at the highest nitrogen application after an exceptional summer flood. The target forbs persisted in numbers over the study period in all treatments. For cover, the low-competitive target forbs responded neutrally to nitrogen fertilization, whereas detrimental effects were demonstrated for the competitive ones. Thus, we recommend not applying more than 60 kg year⁻¹ of nitrogen and only in combination with phosphorus and potassium.

Keywords: alluvial grassland; fertilizer levels; feeding value; legume cycle; target forbs

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Introduction

In Europe, grasslands play an important role as part of agricultural production systems (Isselstein et al., 2005; Peyraud and Peeters, 2016). Grasslands have been acknowledged to simultaneously provide multiple ecosystem functions and services (Bengtsson et al., 2019; Garland et al., 2020; Manning et al., 2018; Zhao et al., 2020), such as feed base for livestock (Boval and Dixon, 2012; Berauer et al., 2020), pollination services (Orford et al., 2016), and as an important contribution to biodiversity (Dengler et al., 2014). Due to the drastic decline of grassland area during the last century (Van Dijk, 1991; Wesche et al., 2012; Diekmann et al., 2019), many grassland types have been protected by the EU Habitats Directive and deterioration must be avoided (Council of the European Commission, 1992; European Commission, 2007). The grassland habitat type 6440, alluvial meadows of river valleys of the *Cnidion dubii*, occurs in Central to Southeastern Europe, with a distribution focus in the large German floodplains. Within Natura 2000 sites, alluvial meadows occur in Germany on an estimated 3732 ha (Šeffer et al., 2008). In the northern German floodplain regions, a tremendous change in the composition of plant communities has occurred over the last 50 years, with a consistent trend towards species-poor communities caused by agricultural intensification, and in particular nutrient input (Wesche et al., 2012). This situation also applies to Germany's other floodplain regions (Hölzel et al., 2002; Bischoff et al., 2009; Bischoff et al., 2018). Therefore, the national conservation status of alluvial meadows is currently ranked as unfavorable—bad (BfN, 2019 a; BfN, 2019 b). Nevertheless, particularly in areas not subject to intensive dairy farming, species-rich alluvial meadows can still be found in a good conservation status. In the Dessau Elbe floodplain, a large part of the grassland belongs to habitat type 6440. Many farms participate in agri-environmental schemes and mow twice a year, which is in accordance with the habitat type management recommendations for mowing frequency (Šeffer et al., 2008). Due to species composition and mowing time, the grassland biomass is less suitable for dairy cattle (Franke, 2003) and is mainly used as hay for feeding sheep, robust cattle and horses. Therefore, the interest in nature conservation is often in conflict with the demand of regional farmers to fertilize species-rich grasslands in order to improve forage quality.

Currently, there are few long-term studies on the development of forage quality in permanent grasslands (Mrkvička and Veselá, 2002; Pavlů et al., 2006; Hrabě and Knot, 2011; Štýbnarová et al., 2014). In many cases, only results from one- to three-year experimental studies have been published (Ludewig et al., 2015; Pornaro et al., 2019; Schaub et al., 2020; Ranta et al., 2021), or the quantity and quality of forage production of different meadow types have been evaluated (Scotton et al., 2014; Loucougaray et al., 2015).

There are also few long-term studies on changes in the species assembly of species-rich alluvial meadows that have been subjected to different fertilization treatments. Three studies on alluvial *Alopecurus pratensis* L. meadows are unique: the Černíkovice experiment in the center of the Czech Republic (Honsova et al., 2007; Hrevušová et al., 2009), the Vel'ká Lúka experiment in the center of Slovakia (Vargová et al., 2012) and the Steinach experiment in SE Germany (Bavaria) (Hejcman et al., 2014). These studies compared the effect of different fertilizer applications only after decades of use, while annual fluctuations and periodic

oscillations were not evaluated. However, consideration of these aspects is important to improve our understanding of the relationships between fertilization, forage quality and species assembly.

Starting from a species-rich condition, studies from mountain and alpine regions showed drastic changes in species assembly and reduced nature conservation value due to nitrogen fertilization (Scotton et al., 2014; Gillet et al., 2016). For lowland and mountain meadows, the promotion of grasses and the decrease of legumes by nitrogen application have been described prominently (Ranta et al., 2021; Dindová et al., 2019; Kacorzuk and Głąb, 2017). Legumes have a positive effect on forage quality (Buxton, 1996; Wilman and Williams, 1993; Lüscher et al., 2014) and are in a periodic system in grassland (Schwinning and Parsons, 1996a; Herben et al., 2017). The legume cycle and the effects of fertilization have hardly been studied so far (Herben et al., 2017), and to our knowledge, have not been investigated for grasslands with flooding events. Finally, the change in alluvial meadow target species is also an important issue. It has been commonly found that nitrogen fertilization led to decreased plant species richness for the whole range of European grassland types (Soons et al., 2017). In addition, target species numbers are expected to decrease (Galka et al., 2005; Socher et al., 2012; Allan et al., 2015; Humbert et al., 2015; Korevaar and Geerts, 2015; Müller et al., 2016; Molina et al., 2021). There are different findings on the response of grassland forbs to fertilization (Hejcman et al., 2007; John et al., 2016; Busch et al., 2019). Of interest here is the effect of plant strategy types on target forbs performance, assuming that low-competitive forbs will benefit if no nitrogen fertilizer is applied due to reduced light competition (Grime, 1979; Hautier et al., 2009).

Based on the results of previous grassland studies on the effect of fertilization, there is a certain discrepancy between the demand for improving forage quality through fertilization and the conservation of species assembly in species-rich floodplain grassland. In a largescale field experiment, we examined the effects of different fertilization treatments on the development of forage quality and species assembly of a species-rich alluvial meadow over eight years. We focused on the following questions: (Q1) Can forage quality be increased by fertilization to the extent that it is suitable for higher performance phases of sheep? (Q2) Which fertilization treatments have a negative influence on target species number development? (Q3) How does the grass and legume cover change with different fertilizer applications? (Q4) Do low-competitive and competitive target forbs respond similarly to different fertilizer applications? We discuss our findings in a wider perspective, the challenge to reconcile farmers' demands on the forage quality of alluvial grasslands with the nature conservation goal of maintaining them in good conservation status.

Materials and methods

Study area

Our study area is situated on the Elbe floodplain near Dessau in Saxony-Anhalt (N 51°5'09.35", E 12°17'57.12", 58 m above sea level). The climate is continental with a mean annual precipitation of 571 mm and a mean annual temperature of 9.4 °C (long-term mean 1981–

2010, Deutscher Wetterdienst). The Elbe River is 2.5 km away and used to flood the area in spring, but during the study period, the study site was not flooded every year. Dry soil conditions prevail in summer. The soil is an acidic gley, characterized by low phosphorus and a medium potassium level. The grassland belongs to the Natura 2000 habitat type 6440 (alluvial meadows of river valleys of the *Cnidion dubii*), protected by the Habitats Directive 92/43/EEC, and to the plant association *Sanguisorbo officinalis*-*Silaetum silai* Klapp 1951, a vegetation type with low fodder value (Schubert, 2001). Before the experiment started, the permanent and never oversown grassland was in a favorable conservation status. Species richness was high with a mean number of vascular plants of 30.0 (\pm 3.4) and forbs of 23.8 (\pm 2.9) on 25 m². The study site contains the characteristic river corridor plants (according to Burkart, 2001) *Allium angulosum* L., *Carex praecox* Schreb., *Galium boreale* L., *Sanguisorba officinalis* L., *Selinum dubium* (Schkuhr) Leute, *Silaum silaus* (L.) Schinz & Thell., *Thalictrum flavum* L., *Veronica maritima* L. and *Viola stagnina* Kit. ex Schult. Other frequently occurring species are *Anthoxanthum odoratum* L., *Daucus carota* L., *Euphorbia esula* L., *Galium album* Mill., *Lathyrus pratensis* L., *Leucanthemum ircutianum* Turcz. ex DC., *Lychnis flos-cuculi* L., *Poa angustifolia* L., *Ranunculus acris* L., *Rumex acetosa* L., *Scorzoneroideis autumnalis* (L.) Moench and *Trifolium pratense* L. The dominant grass was *Alopecurus pratensis*. The nomenclature of vascular plants follows Jäger (2017).

The meadow was traditionally mown twice in the growing season for hay making, and, depending on the growth, additionally grazed by sheep in autumn and not fertilized for at least 30 years. During the experiment, an exceptional flooding event flooded the area for six weeks in summer 2013 and destroyed all vegetation. However, the vegetation recovered within a few weeks and only the less flood-tolerant species *Festuca pratensis* Huds. declined sharply.

Experimental design and sampling

In 2010, the experiment was established in four completely randomized blocks with each block containing five square sample plots (5.0 × 5.0 m) each for one of the five treatments. The meadow was mown twice, with a first cut in early June and a second cut mid-August. The fertilizer treatments were: (i) unfertilized control (N0), (ii) phosphorus and potassium as withdrawn (PK), (iii) nitrogen 60 kg ha⁻¹ year⁻¹ (N60), (iv) nitrogen 60 kg ha⁻¹ year⁻¹ plus phosphorus and potassium as withdrawn (N60PK) and (v) nitrogen 120 kg ha⁻¹ year⁻¹ plus phosphorus and potassium as withdrawn (N120PK). The phosphorus and potassium content of the biomass was determined together with measurement of the forage quality parameters. Both nutrients were applied in the amounts that were removed with the harvest in the previous year, calculated for each treatment. We used mineral fertilizer applied in commercially available granules, nitrogen as urea in two doses before and shortly after the first cut, phosphorus in the form of triple superphosphate and potassium in the form of 60er Kali in one dose before the first cut.

Soil samples were taken at the plot level at a depth of 0–10 cm (mixing of 10 soil core samples per plot), in March 2010 to 2015 and 2017. Overall, the soil had a pH of 4.88 (\pm 0.17) (analyzed

after Deutsches Institut für Normung, 2005), contained 0.47% (± 0.11) total nitrogen (analyzed after Deutsches Institut für Normung, 2001) and 5.73% (± 1.11) total carbon (analyzed after Deutsches Institut für Normung, 1990). Plant-available phosphorus (*double-lactate method*) was measured at a very low level (14.6 mg kg \pm 6.9) and potassium at an optimal level (127.0 mg kg \pm 35.9) (both analyzed after VDLUFA, 1991), classification of nutrient concentration level after LLG, 2019). The soil chemical characteristics before the start of the experiment in 2010 are shown in Table 1. Despite different fertilization treatments, the soil characteristics did not differ between the treatments over time (Figure A1).

We recorded vascular plant species abundance from 2010 to 2018 (except in 2016) using the Londo scale (Londo, 1976) on the 5.0 \times 5.0 m plots annually in mid-May. The estimation of the percentage contribution of *Cirsium arvense* L. to the aboveground plant biomass (yield proportion: Voigtländer and Voss, 1979) was made on the 5.0 m \times 5.0 m plots shortly before the first and second cuts.

From 2010 to 2017 (except 2013, 2016), we cut all plants within one randomly selected 1 m² frame in each plot at a height of 3–5 cm shortly before mowing to estimate forage quality. Samples were dried and analyzed in a laboratory by near-infrared reflectance spectroscopy (NIRS) according to standard VDLUFA (2004) methods for the concentrations of crude protein and crude fiber in the dry matter (DM). Metabolizable energy was calculated according to Losand et al. (2007) (with cellulase solubility ELOS and acid detergent fiber ADF determined by NIRS). The NIRS method was used due to the high number of samples (40 per year) to reduce costs.

Table 1. Mean and standard deviation (in brackets) of soil chemical characteristics (sampling depth 0–10 cm) before the experiment started.

Treatment	pH Value	Phosphorus (mg kg)	Potassium (mg kg)	Total carbon (%)	Total nitrogen (%)
N0	4.84 (0.14)	9.50 (2.65)	105.00 (20.90)	5.23 (0.91)	0.46 (0.10)
PK	4.92 (0.14)	13.75 (6.24)	111.50 (21.08)	5.43 (1.23)	0.48 (0.12)
N60	4.84 (0.14)	10.75 (6.24)	121.75 (19.05)	5.80 (1.33)	0.50 (0.13)
N60PK	4.96 (0.05)	13.50 (7.77)	126.25 (18.87)	5.48 (0.71)	0.47 (0.08)
N120PK	4.83 (0.17)	10.50 (0.58)	127.25 (17.91)	5.90 (0.80)	0.50 (0.08)

Species groups

The species group of grasses included all species of the plant families Poaceae, Cyperaceae and Juncaceae. Legumes are species of the plant family Fabaceae. Target species are characteristic species of habitat type 6440 (Schuboth and Frank, 2010) and characteristic species and other low growing forbs of the *Sanguisorbo officinalis*-Silaetum silai (Schubert, 2001). High-competitive target forbs are species of the ecological strategy types C and CS (after Grime et al., 1979).

Statistical analyses

All statistical analyses were performed in R version 4.0.4 (R Development Core Team, 2021).

We modelled the soil variables, the forage quality variables (Q1), the target species number (Q2) and the cover variables (Q3, Q4) as functions of fertilization treatments and time with generalized linear mixed models (GLMMs; Bates et al., 2015). All soil variables, i.e., pH, phosphorus, potassium, total carbon and total nitrogen; and forage quality variables, i.e., crude protein, crude fiber and metabolizable energy, were modelled with identity-link and Gaussian errors. The cover of grasses, legumes and all target forbs, as well as of low- and high-competitive target forbs, were expressed as percentages, that is as strictly bounded but non-binomial data. We therefore logit-transformed these variables (Warton and Hui, 2011) before modelling them with identity-link and Gaussian errors. The target species number was modelled with log-link and Poisson errors.

All models were parameterized using the R package LME4, version 1.1.26 (Bates et al., 2015), accounting for repeated measurements and the spatially nested sampling structure by specifying plot nested within block as random (intercept) effects in the models. For four of the models, i.e., crude protein (first cut), cover of all target forbs, cover of high-competitive target forbs and the target species number, the mixed-effects model was singular. For the first three models, we used a simpler model with only one random effect with accounting for the repeated measurements but not for the target species number; the model was still singular. Therefore, we used generalized linear model without random effects for target species number. Thus, all these three models therefore needed to be interpreted more conservatively. We simplified the models as much as possible to find the minimal adequate model by using a backward selection procedure with likelihood ratio tests (Crawley, 2013).

Results

Forage quality

At the beginning of the experiment in 2010, crude fiber concentration in all treatments was higher for the first cut than for the second cut. Crude protein concentrations and the values for metabolizable energy showed the opposite (Table A1). For all forage quality parameters, the range of variation occurring during the study period was always higher for the second cut than for the first cut (Table 2). The raw data are provided in Table S1.

Crude protein showed significant differences between the three treatment groups obtained as minimal adequate model, N0 & PK, N60 & N60PK and 120PK (Figure 1), for both the first and second cuts, and these groups also had a different development over time (Table 3, Figure 1). The crude protein concentration of the treatment group N0 & PK remained at similar levels throughout the study period at both cuts, while the nitrogen-fertilized treatment groups, N60 & N60PK and N120PK, changed significantly more over time. For the first cut, the crude protein concentration increased from the third year of the experiment and was particularly high in the years 2014 and 2015, after the summer flood in 2013. The largest increase was observed for the N120PK treatment in the 2014–2015 period (Figure 1). For the second cut, crude protein

contents of the nitrogen-fertilized treatments peaked in 2014, after the summer flood of 2013, before decreasing continuously in the following years. In particular, the N120PK treatment had a strong decrease in these years (Figure 1).

Table 2. Forage quality shown as minimum and maximum yearly means of the six sampling years for the first and second cuts. The raw data are provided as a Supplementary File (Table S1).

		NO	PK	N60	N60PK	N120PK
Crude fiber (g kg DM ⁻¹)	First cut	257–295	259–289	257–293	258–307	268–308
	Second cut	242–291	241–297	228–288	237–293	250–320
Crude protein (g kg DM ⁻¹)	First cut	90–110	92–101	96–121	94–126	104–131
	Second cut	102–124	94–120	105–132	105–142	98–150
Metabolizable energy (MJ kg DM ⁻¹)	First cut	8.5–9.5	8.5–9.5	8.7–9.5	8.4–9.4	8.4–9.3
	Second cut	8.1–9.6	7.9–10.1	8.1–9.9	7.8–10.0	7.6–9.4

Table 3. Effects of fertilizer and year on the crude protein of the first cut in early June and the second cut in mid-August according to the minimal adequate model. Both models had three levels for the fertilizer, for the first cut four levels for year, and for the second cut five levels for year (Figure 1).

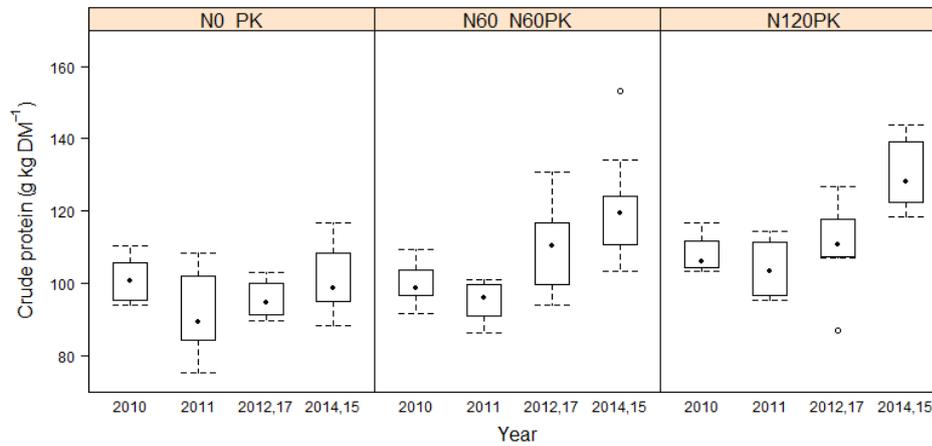
	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
First cut						
Fertilizer	2385.9	1192.9	2	19.6	15.1	<0.001
Year	5736.8	1912.3	3	91.0	24.1	<0.001
Fertilizer × Year	1947.3	324.6	6	91.0	4.1	0.001
Second cut						
Fertilizer	2538.6	1269.3	2	15.7	10.6	0.001
Year	9408.6	2352.2	4	87.3	19.6	<0.001
Fertilizer × Year	5255.9	657.0	8	87.3	5.5	<0.001

For the first cut, crude fiber (Table 4, Figure 2a) and metabolizable energy (Table 5, Figure 3a) did not develop differently over time in the fertilizer treatments. In contrast, for the second cut, the two treatment groups, NO & PK & N60 & N60PK and N120PK, developed significantly differently over time for crude fiber (Table 4) and metabolizable energy (Table 5). For crude fiber, both treatment groups increased from 2010 to the 2011, 2012 and 2014 period before they decreased in 2015 and then increased again in 2017, and in particular in the N120PK treatment (Figure 2b). Metabolizable energy decreased from 2010 to 2011 before increasing in the 2012 to 2015 period (Figure 3b). In 2014, the year after the summer flood, metabolizable energy showed the lowest concentration. While metabolizable energy in the NO & PK & N60 & N60PK treatment group increased again at the end of the study period in 2017, the N120PK treatment differed and returned to the low 2014 level (Figure 3b).

Summarizing for the second cut, at the end of the study period in 2017, the forage value of the N120PK treatment was the lowest due to low crude protein and metabolizable energy concentrations and the highest crude fiber concentration. This development was

accompanied by the strongest increase in the yield proportion of *Cirsium arvense* in 2017 (Figure A2).

(a)



(b)

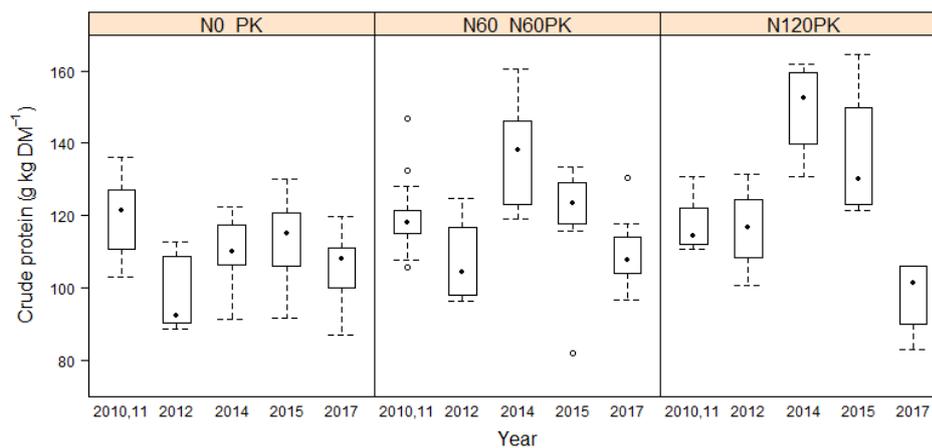


Figure 1. The minimal adequate model for the effect of fertilization treatment and time on the concentration of crude protein (g kg DM⁻¹) for the first (a) and second (b) cuts with different fertilization treatments between 2010 and 2017 (except 2013 and 2016). Level needed for female sheep (70–100 kg): 72–73 g kg DM⁻¹, for ewes (70–100 kg) rearing a single lamb: 122–143 g kg DM⁻¹, for rams 100–120 kg: 75 g kg DM⁻¹, for rams in the breeding season: 140 g kg DM⁻¹ (Lfl, 2019).

Table 4. Effects of fertilizer and year on the crude fiber of the first cut in early June and the second cut in mid-August according to the minimal adequate model. Both models had two levels for the fertilizer and four levels for year (Figure 2).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
First cut						
Fertilizer	2348.9	2348.9	1	15	17.0	<0.001
Year	21,407.3	7135.8	3	97	51.7	<0.001
Second cut						
Fertilizer	1702.5	1702.5	1	23.9	5.9	0.023
Year	24,466.3	8155.4	3	94.4	28.3	<0.001
Fertilizer × Year	6627.1	2209.0	3	94.4	7.7	<0.001

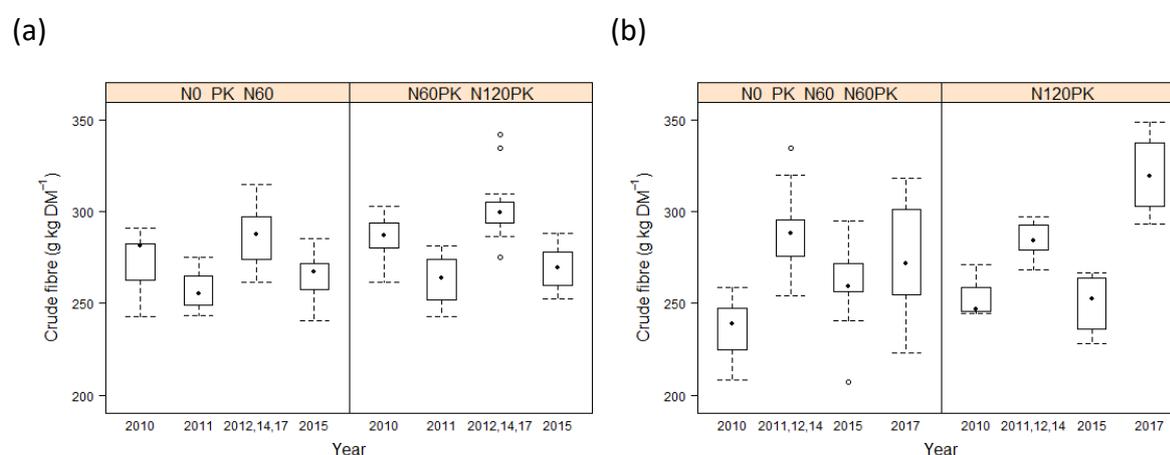


Figure 2. The minimal adequate model for the effect of fertilization treatment and time on the concentration of crude fiber (g kg DM⁻¹) for the first (a) and second (b) cuts with different fertilization treatments between 2010 and 2017 (except 2013 and 2016).

Table 5. Effects of fertilizer and year on the metabolizable energy of the first cut in early June and the second cut in mid-August according to the minimal adequate model. Both models had two levels for the fertilizer and five levels for year (Figure 3).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
First cut						
Fertilizer	0.6	0.6	1	15	8.8	0.01
Year	10.6	2.7	4	96	35.8	<0.001
Second cut						
Fertilizer	1.8	1.8	1	18.6	12.6	0.022
Year	28.0	7.0	4	92.2	48.2	<0.001
Fertilizer × Year	2.9	0.7	4	92.2	5.0	0.001

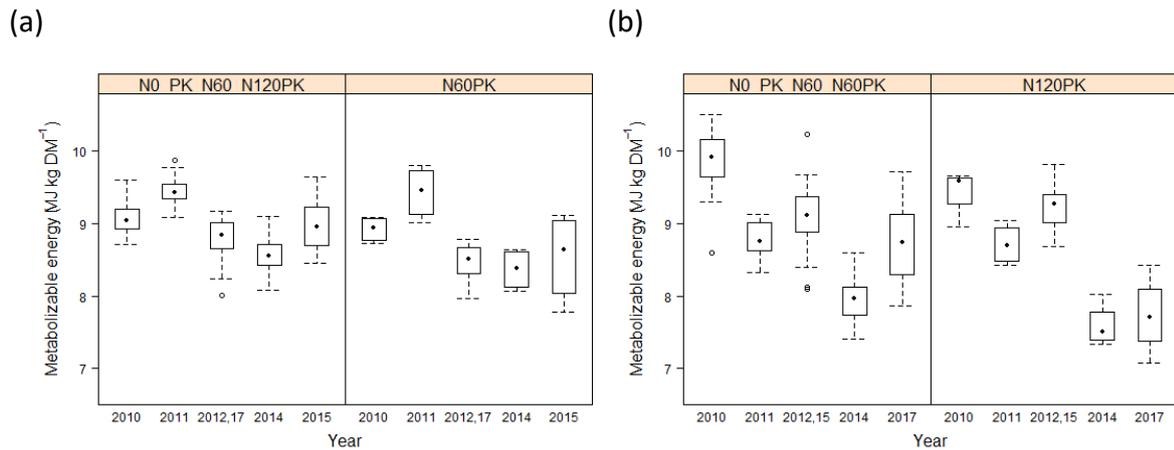


Figure 3. The minimal adequate model for the effect of fertilization treatment and time on the metabolizable energy (MJ kg DM⁻¹) for the first (a) and second (b) cuts with different fertilization treatments between 2010 and 2017 (except 2013 and 2016). Level needed for female sheep (70–100 kg): 8.7–8.8 MJ kg DM⁻¹, for ewes (70–100 kg) rearing a single lamb: 10.4–10.7 MJ kg DM⁻¹, for rams 100–120 kg: 9.4 MJ kg DM⁻¹, for rams in the breeding season: 10.0 MJ kg DM⁻¹ (Lfl, 2019).

Species assembly

Development of target species number

Almost two-thirds of the species were target species, of which 28 were forbs (including four legumes) and eleven were grasses (Table A2). In the observation period, mean target species number did not differ significantly between treatments or years, and neither did the treatments differ over time (Figure 4). The target species persisted despite high nitrogen application over the 8-year study period.

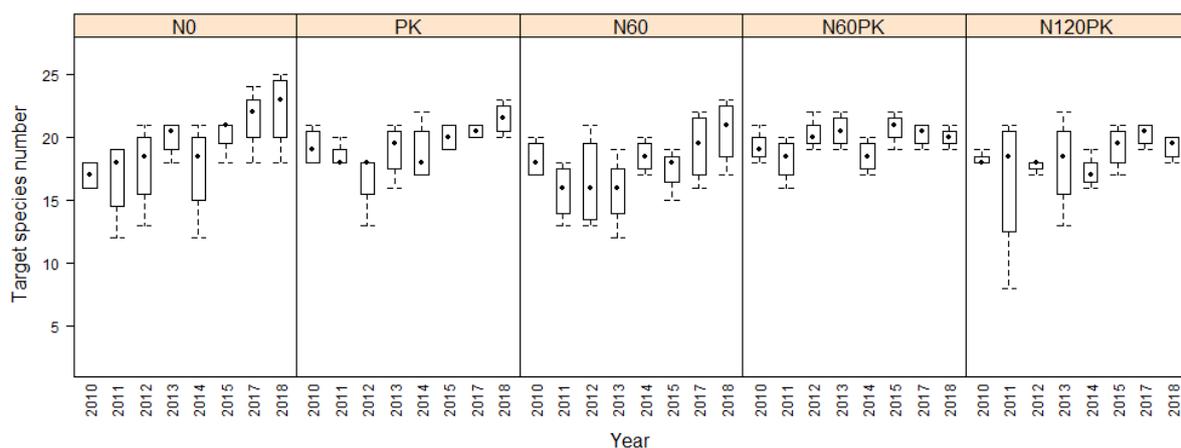


Figure 4. Development of the number of target species with different fertilization treatments between 2010 and 2018 (except 2016). There were no significant differences between treatments or years according to the generalized linear model (GLM).

Development of grass cover

The grass cover developed differently over time for the three treatment groups NO & PK, N60 and N60PK & N120PK (Table 6). For the NO & PK and the N60 group it decreased considerably over time from 2014 onwards and stabilized at a low level in the years 2017–2018 (Figure 5). The N60PK & N120PK treatments were less affected by the flooding, showing a smaller decline in grass cover. After the flood in 2013, *Festuca pratensis* declined sharply and remained at very low levels in all plots, regardless of whether fertilizer was applied (Figure A3). By 2017–2018, grass cover in nitrogen-fertilized plots recovered almost to the baseline level as *Alopecurus pratensis* and, especially for N120PK, *Elymus repens* (L.) Gould increased in cover (Figure A3).

Table 6. Effects of fertilizer and year on the cumulative cover of grasses according to the minimal adequate model. The model had three levels for the fertilizer and three levels for year (Figure 5).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
Fertilizer	1.0	0.5	2	15.3	6.5	0.001
Year	19.7	9.9	2	134.0	134.7	<0.001
Fertilizer × Year	4.3	1.1	4	134.0	14.7	<0.001

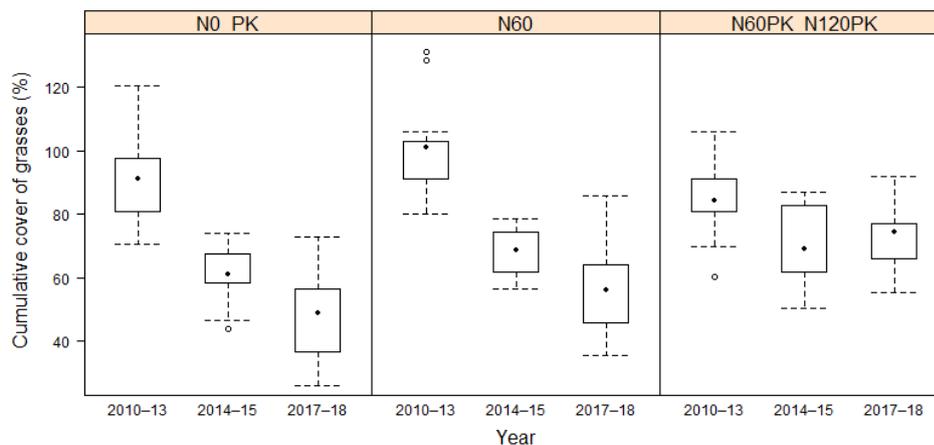


Figure 5. The minimal adequate model for the effect of fertilization treatment and time on the cumulative cover of grasses with different fertilization treatments between 2010 and 2018 (except 2016).

Development of legume cover

In 2010 before application of the treatments, the mean cover of legumes ranged between 15.0 (\pm 5.1) and 22.3 (\pm 8.4) and was formed by *Lathyrus pratensis*, *Lotus corniculatus*, *Trifolium pratense*, *T. repens*, *T. dubium*, *Vicia sepium*, *V. cracca* and *V. tetrasperma*. The treatment groups, i.e., NO & PK, N60 & N120PK and N60PK developed significantly differently over time (Table 7), the former group with higher cover of legumes (Figure 6). From 2010 to

2011–2013 legume cover decreased considerably in all treatments and had almost disappeared in 2013. After the flood in 2013, legumes started to recover and increased continuously the following years. By the end of the study period in 2018, legume coverage in the N0 & PK treatments was approaching baseline. Whereas in the nitrogen fertilization treatments, legume cover was still at a much lower level than their 2010 baseline.

Table 7. Effects of fertilizer and year on the cumulative cover of legumes according to the minimal adequate model. The model had three levels for the fertilizer and five levels for year (Figure 6).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
Fertilizer	4.3	2.1	2	17.0	6.2	0.009
Year	58.0	14.5	4	128.0	42.3	<0.001
Fertilizer × Year	10.7	1.3	8	128.0	3.8	<0.001

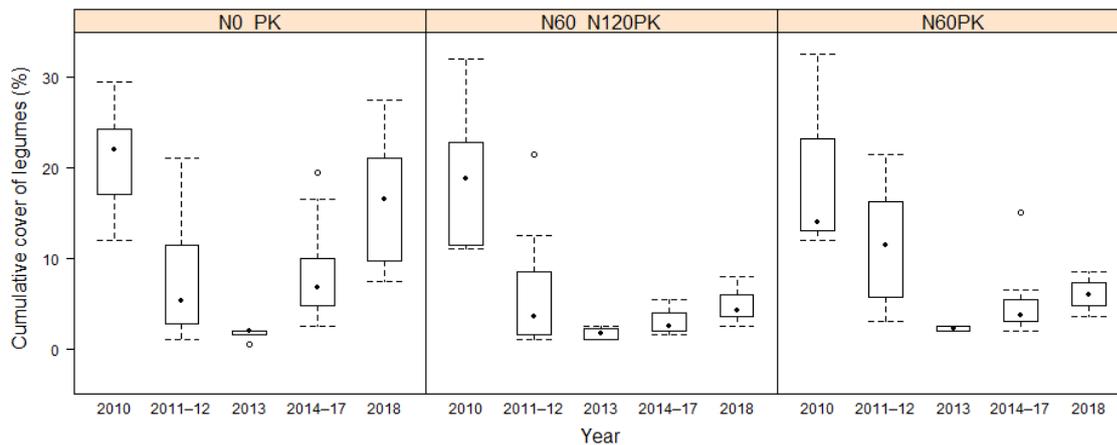


Figure 6. The minimal adequate model for the effect of fertilization treatment and time on the cumulative cover of legumes with different fertilization treatments between 2010 and 2018 (except 2016).

Development of target forbs cover

The cover of all target forbs and the cover of low-competitive forbs (Table A2) did not differ with respect to fertilization treatments, only for years (Tables A3 and A4, Figures A4 and A5). For the high-competitive target forbs, the treatment groups N0 & PK & N60PK and N60 & N120PK developed differently over time (Table 8), the latter group with lower cover of high-competitive target forbs (Figure 7). The cover decreased significantly from 2010 to 2011–2013, before dropping significantly again in 2014, the year after the summer flooding, before significantly increasing the following years (Figure 7). In particular, the increase was consistent and large for the N0, PK and N60PK treatments that almost reached their 2010 baseline level. *Cnidium dubium* and *Ranunculus acris* were mainly responsible for this development (Figure A6).

Table 8. Effects of fertilizer and year on the cumulative cover of high-competitive target forbs according to the minimal adequate model. The model had two levels for the fertilizer and four levels for year (Figure 7).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
Fertilizer	1.1	1.1	1	19.6	5.4	0.031
Year	33.4	11.1	3	134.0	52.9	<0.001
Fertilizer × Year	3.5	1.2	3	134.0	5.6	0.001

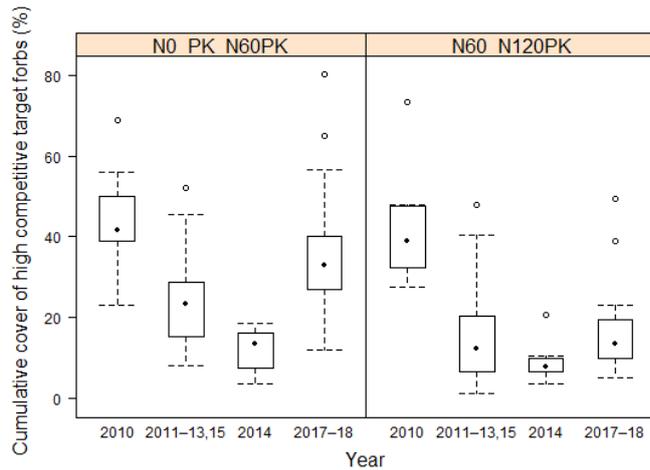


Figure 7. The minimal adequate model for the effect of fertilization treatment and time on the cumulative cover of high-competitive target forbs with different fertilization treatments between 2010 and 2018 (except 2016).

Discussion

Effect of fertilization on forage quality parameters

In the Dessau grassland experiment, the low forage quality of the alluvial grassland, which was prevalent at the beginning, could hardly be improved during the study period with any of the applied fertilizer treatments. For sheep feeding, the crude protein concentration and metabolizable energy concentration of both cuts were adequate for low-performance phases (female sheep without lamb, rams not in breeding season) but did not meet the needs of higher performance phases (lactating ewes, rams in the breeding season) only exceptionally. Our results are in line with other authors, who found only few effects on forage nutrient concentrations in alluvial grassland when nitrogen up to 200 kg ha⁻¹ year⁻¹ was applied (Vargová et al., 2012) and in mountain grasslands when nitrogen 85 kg, phosphorus oxide 80 kg and potassium oxide 240 kg ha⁻¹ year⁻¹ were applied (Ineichen et al., 2020). The fertilization experiment by Dindová et al. (2019) showed a significant increase in crude protein concentration on a submontane Arrhenatherion grassland only when at least 200 kg ha⁻¹ year⁻¹ of nitrogen was supplied.

The 8-year study period may not have been long enough to trigger vegetation restructuring in the different fertilizer treatments. Schellberg et al. (1999) stated that changes in botanical

species composition in a mountain hay meadow fertilizer experiment improved forage quality by the increase of tall growing grasses only in a long-term process.

The significant changes between the years for all investigated parameters at both cuts indicate a typical year-to-year variability, which was also described by other authors (Pavlů et al., 2006; Larter and Nagy, 2001), but seems to have been particularly affected by the summer flooding event. Exclusively after the summer flood, the tested nitrogen fertilizer treatments had such an increasing one- to two-year effect on crude protein concentration that higher requirements in sheep feeding could be met, while the metabolizable energy concentration responded in the opposite way. Blöschl et al. (2020) predicted an increase of exceptional flooding events for the future and therefore, further research is needed to evaluate short- and long-term effects of flooding on forage quality parameters.

The decrease in forage quality for the second cut in the high-nitrogen-fertilized N120PK plots was visible at the end of the study period in the low crude protein concentration, low metabolizable energy and high crude fiber concentration. This could be related to the increase of *Cirsium arvense*, whose crude protein level decreases significantly in later stages of development in summer and fall (Peters et al., 2010). Nitrogen fertilization promotes ruderal grassland forbs with low feeding values (Wesche et al., 2012; Galka et al., 2005). This is consistent with many twice-mowed, more heavily fertilized grasslands of the Dessau Elbe floodplain. The increase of ruderalization indicators such as *Cirsium arvense*, *Elymus repens* and *Tanacetum vulgare* L. is a phenomenon that has been increasingly observed there in recent years (Pannach, 2021).

Effect of fertilization on species assembly

Grass and legume performance and legume cycle

Interestingly, the effect of the nitrogen fertilization became apparent in the experiment only after the summer flood in 2013, but without the expected increase in grass cover as other authors stated (Honsova et al., 2007; Hejcman et al., 2007, Hejcman et al., 2014; Kacorzyk and Głąb, 2017). An increase in grass cover can be achieved with even low to moderate nitrogen application of 35–50 kg ha⁻¹ year⁻¹ (Honsova et al., 2007; Kidd et al., 2017). However, the effect of nitrogen application can be influenced by water availability (Witwicki et al., 2016) or limitation by phosphorus (Güsewell, 2005; Oelmann et al., 2009). Possibly, the low phosphorus content in the soil combined with the summer drying of the study site prevented an increase in grass cover. The tall grass *Alopecurus pratensis* was responsible for maintaining the grass cover, a result which is in agreement with those from the Jizera Mountains grassland experiment (Pavlů et al., 2012). *Elymus repens*, which benefits from nitrogen fertilization (Ringselle et al., 2020), performed the same function in the N120PK treatment.

Compared to the Černíkovice grassland experiment (Honsova et al., 2007), the legume cover was quite high at the beginning of the Dessau grassland experiment. It can be assumed that the vegetation was at the top of the curve of the legume cycle, as a strong decrease occurred in the following year, as also documented by Schwinning and Parsons (1996a; 1996b) in grazing systems. In the year after the summer flood, all plots suffered a great loss of legumes

until their almost complete disappearance. Afterwards, their cover increased again only on the plots not fertilized with nitrogen and approached the initial state after eight years. This period corresponds to the legume cycle duration in permanent grasslands (Herben et al., 2017). The persistently low cover of legumes in plots with nitrogen application is consistent with the results from other long-term experiments, not only on alluvial grasslands (Honsova et al., 2007; Hejcman et al., 2007; Kidd et al., 2017; Pavlů et al., 2011; Pavlů et al., 2012). Our study illustrates for alluvial grasslands that the legume cycle could be maintained only for the non-nitrogen-fertilized plots, as already described for montane grasslands (Herben et al., 2017).

The fact that no difference in legume cover was found between the unfertilized control and PK fertilization treatments contradicts the findings for montane grasslands, where legumes benefited from PK fertilization compared to unfertilized controls (Holúbek and Petrovič, 2011). These results are however in line with the Černíkovice alluvial grassland experiment, where legumes achieved the highest cover in the control and PK treatments (Honsova et al., 2007).

Legumes can have a significant influence on the forage quality and elasticity of forage utilization by maintaining overall sward digestibility over a longer period (Hopkins and Holz, 2006), increasing protein in fodder (Weggler et al., 2019) or increasing the mineral content (Opitz v. Boberfeld and Laser, 1999). In addition, they can play an important role for securing the feed supply for livestock with regard to future climatic conditions. Legumes are well adapted to higher temperatures and can also use water reserves in deeper soil layers through their deep root system (Lüscher et al., 2014).

Target species number and target forbs cover

Contrary to the assumption that alluvial grassland target species number decreases under nitrogen fertilization according to species richness (Honsova et al., 2007; Raus et al., 2014), there was no decline during the 8-year study period. This is in accordance with the Steinach grassland experiment, the results of which concluded that species composition in productive alluvial grasslands is substantially less affected by fertilizer application than in low-productivity grasslands (Hejcman et al., 2014). Flooding events act as a major influencing factor by overruling the fertilization effects on species richness (Beltman et al., 2007). Although the study area was no longer flooded annually in the spring during the study period, it seems that the exceptional summer flood of 2013 was sufficient to produce this effect. In addition to flooding events, the low soil phosphorus level in the studied alluvial grassland could also be responsible for the persistence of target species. Several authors (Tallowin and Jefferson, 2008; Kirkham et al., 2014; Van Dobben et al., 2017) found that low soil phosphorus availability seems to be a key factor in maintaining high species richness. Moreover, in the Dessau grassland experiment, no soil-acidifying nitrogen fertilizer was applied which, according to Hejcman et al. (2014), can also have a positive effect on maintaining the target species number.

The assumption that nitrogen fertilization leads to a decrease in cover of low-competitive target forbs cannot be confirmed. This can be explained by the fact that the grass cover did

not increase under nitrogen fertilization as observed in other grassland experiments (Honsova et al., 2007; Hejzman et al., 2007; Hejzman et al., 2014; Kacorzyk and Głąb, 2017). Furthermore, the expected increase in light competition and niche dimension reduction (Molina et al., 2021; Dickson et al., 2014) has not occurred in our study. In addition, low-competitive species present on the study site, such as the target forbs *Cardamine pratensis* L., *Prunella vulgaris* L., *Ranunculus auricomus* L. and *Veronica chamaedrys* L., responded neutrally to fertilization as also described by Busch et al. (2019). In contrast, after declining in the first four years of the experiment, the high-competitive target forbs did not reach the 2010 level after the summer flood on the nitrogen-fertilized plots, which can be attributed to the fact that even high-competitive species can be negatively affected by nitrogen fertilization (Busch et al., 2019). On the plots without nitrogen fertilization, however, some species (e.g., *Selinum dubium* and *Ranunculus acris*) clearly benefited from the summer flood by considerably increasing their cover. Interestingly, the negative effect of moderate nitrogen fertilization (N60) was compensated for by additional P and K fertilization (N60PK). This is confirmed by Kidd et al. (2017), whose study showed that nitrogen fertilization alone has a stronger negative effect than in combination with phosphorus and potassium.

Conclusions

Regarding forage quality, no significant improvement could be achieved for sheep feeding. Both cuts provided sufficient crude protein concentrations for low-performance phases in all treatments. The concentration was significantly improved by higher nitrogen fertilization, but higher feeding requirements of lamb-rearing ewes or rams in breeding season were only met in individual years. Especially in the second cut, the metabolizable energy concentration decreased during the study period independent of fertilization treatment and in most years not even the requirements of the low-performance phases were met. Concerning species assembly, we found that moderate application of N60PK does not generate irreversible changes in target forbs cover, but hampers the legume cycle of permanent alluvial grassland. N60 without PK fertilization additionally shows clear negative effects on high-competitive target forbs cover and applying N120PK, the originally good conservation status of the alluvial grassland deteriorates due to the strongest increase of the tall grass *Alopecurus pratensis* and the ruderal species *Cirsium arvense* and *Elymus repens*. For N60 and N120PK, irreversible damage in species assembly cannot be ruled out in the case of further application beyond the study period and should therefore not be applied on species-rich alluvial grasslands that have good conservation status.

Supplementary Materials: The following are available online at

<https://www.mdpi.com/article/10.3390/agriculture11040339/s1>, Table S1: Raw data of the forage quality parameters (crude fiber, crude protein, metabolizable energy).

Author Contributions: Conceptualization, S.D., U.G.J. and S.T.; data curation, S.D.; formal analysis, K.R. and S.D.; funding acquisition, S.D. and S.T.; investigation, S.D., U.G.J. and M.H.M.; methodology, S.D. and U.G.J.; project administration, S.D.; writing—original draft, S.D., K.R.,

A.K.; writing–review and editing, S.D., K.R., A.K., S.T. and M.H.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data of forage quality parameters (crude protein, crude fiber and metabolizable energy) are provided as a source data file on <https://opendata.uni-halle.de>, accessed on 28 March 2021. All other data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix

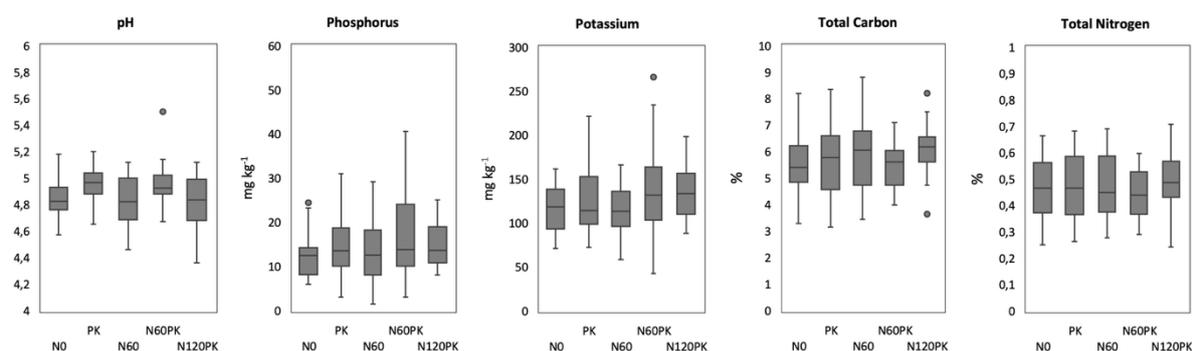


Figure A1. Soil chemical characteristics of fertilization treatments during the study period (soil sampling in 2010–2015, 2017). All variables did not significantly differ between treatments according to the generalized linear mixed model (GLMM).

Table A1. Mean values and standard deviation (in brackets) of crude fiber (g kg DM^{-1}), crude protein (g kg DM^{-1}) and metabolizable energy (MJ kg DM^{-1}).

Year	2010		2011		2012		2014		2015		2017	
cut	first	second										
Crude fiber (g kg DM^{-1})												
NO	268 (18.8)	242 (17.8)	257 (9.2)	291 (7.8)	295 (8.1)	278 (19.3)	292 (4.5)	281 (15.7)	267 (13.0)	267 (17.4)	280 (20.9)	266 (36.5)
PK	285 (4.5)	241 (5.3)	259 (10.1)	290 (9.8)	289 (13.1)	292 (4.1)	284 (23.4)	297 (35.2)	262 (14.7)	254 (32.6)	278 (9.2)	275 (30.5)
N60	269 (12.1)	227 (17.9)	257 (13.9)	287 (12.6)	288 (11)	288 (9.2)	293 (14.8)	279 (12.6)	268 (15.1)	268 (18.3)	284 (17.8)	270 (27.9)
N60PK	282 (15.4)	237 (14.1)	258 (18.3)	286 (9.1)	306 (24.1)	284 (12.0)	300 (4.5)	286 (19.8)	271 (10.0)	258 (13.8)	305 (4.7)	293 (20.2)
N120PK	289 (9.7)	254 (14.7)	268 (9.6)	284 (5.9)	297 (5.4)	286 (10.9)	293 (12.9)	284 (11.6)	268 (15.7)	250 (17.6)	308 (19.9)	320 (23.6)
Crude protein (g kg DM^{-1})												
NO	101 (6.1)	124 (4.5)	90 (15.1)	121 (16.5)	96 (5.9)	102 (10.9)	110 (8.5)	113 (6.3)	100 (5.1)	116 (3.9)	99 (4.2)	107 (11.1)
PK	102 (6.7)	116 (7.6)	94 (9.3)	120 (13.7)	92 (1.2)	94 (8.9)	97 (5.1)	107 (12.6)	99 (8.9)	110 (17.7)	96 (4.9)	104 (11.6)
N60	99 (7.5)	115 (3.1)	96 (4.7)	124 (16.4)	111 (12.7)	105 (8.1)	121 (5.3)	132 (11.5)	118 (9.1)	124 (7.5)	117 (10.5)	115 (11.5)
N60PK	101 (3.5)	118 (9.2)	94 (6.2)	121 (7.6)	113 (14.9)	110 (13.8)	126 (22.1)	142 (17.2)	114 (8.5)	115 (22.4)	101 (6.5)	105 (6.4)
N120PK	108 (6.0)	120 (4.6)	104 (8.9)	116 (9.6)	104 (11.3)	116 (12.6)	130 (8.9)	150 (13.6)	131 (11.7)	137 (19.6)	118 (8.3)	98 (10.9)
Metabolizable energy (MJ kg DM^{-1})												
NO	9.2 (0.3)	9.6 (0.8)	9.5 (0.1)	8.7 (0.3)	8.7 (0.2)	9.3 (0.3)	8.5 (0.1)	8.1 (0.5)	8.8 (0.4)	9.2 (0.4)	9.0 (0.2)	9.0 (0.6)
PK	9.0 (0.1)	10.1 (0.3)	9.5 (0.2)	8.8 (0.2)	8.6 (0.3)	9.0 (0.2)	8.5 (0.5)	7.9 (0.3)	9.1 (0.4)	9.1 (0.8)	8.8 (0.3)	8.9 (0.4)
N60	9.3 (0.2)	9.9 (0.5)	9.5 (0.3)	8.9 (0.2)	9.0 (0.2)	9.3 (0.3)	8.7 (0.4)	8.1 (0.4)	8.9 (0.4)	8.9 (0.5)	8.9 (0.2)	8.6 (0.5)
N60PK	8.9 (0.2)	10.0 (0.2)	9.4 (0.4)	8.9 (0.2)	8.5 (0.4)	9.3 (0.3)	8.4 (0.3)	7.8 (0.2)	8.5 (0.6)	8.9 (0.6)	8.4 (0.2)	8.4 (0.4)
N120PK	8.9 (0.2)	9.4 (0.4)	9.3 (0.2)	8.7 (0.3)	8.9 (0.2)	9.1 (0.3)	8.6 (0.1)	7.6 (0.3)	9.0 (0.4)	9.4 (0.4)	8.4 (0.3)	7.7 (0.6)

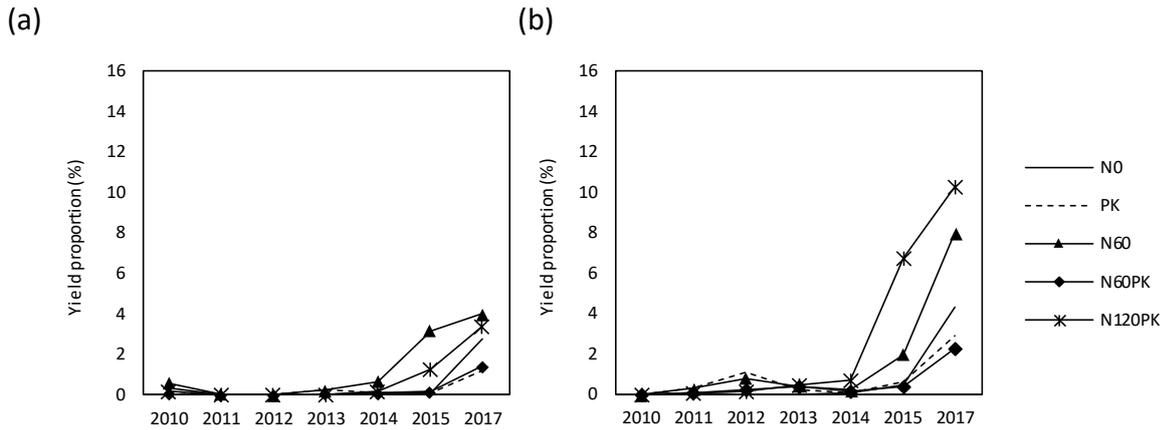


Figure A2. Percentage contribution of *Cirsium arvense* to the aboveground plant biomass in the first (a) and second (b) cuts with different fertilization treatments between 2010 and 2017 (except 2016). Two years after the extreme summer flood in 2013, the treatments N60 and N120PK most strongly increased.

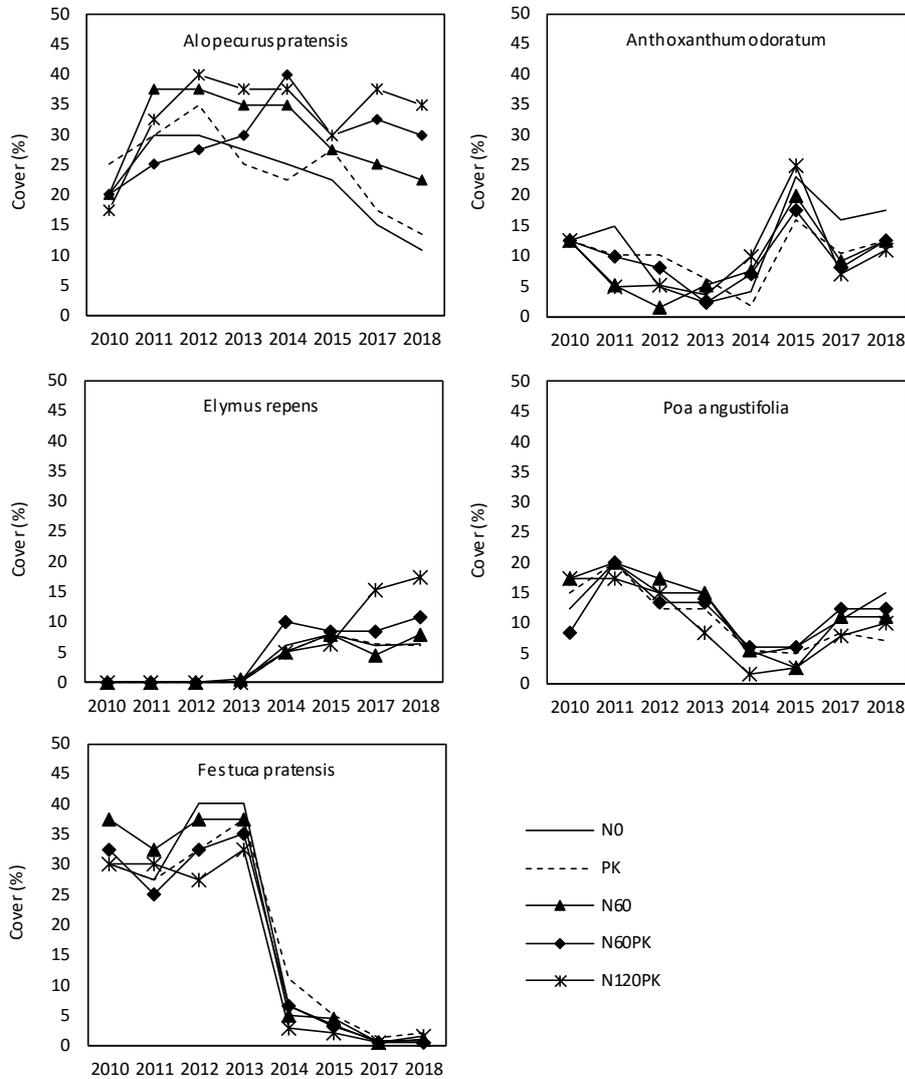


Figure A3. Development of percentage cover of the five most important grasses with different fertilization treatments between 2010 and 2018 (except 2016).

Table A2. Target species (characteristic species of habitat type 6440 (Schuboth and Frank, 2010), characteristic species and low-growing forbs of the *Sanguisorbo officinalis*-*Silaetum silai* (Schubert, 2001). High-competitive target forbs (hc) = ecological strategy types C and CS (after Grime et al., 1988), all other species belong to the group of low-competitive target forbs.

Target forbs (including legumes)	<i>Prunella vulgaris</i>	<i>Viola stagnina</i>
<i>Achillea millefolium</i> (hc)	<i>Ranunculus acris</i> (hc)	Target grasses
<i>Allium angulosum</i>	<i>Ranunculus auricomus</i>	<i>Agrostis capillaris</i>
<i>Cardamine pratensis</i>	<i>Ranunculus polyanthemus</i> (hc)	<i>Alopecurus pratensis</i>
<i>Centaurea jacea</i> (hc)	<i>Rumex acetosa</i> (hc)	<i>Anthoxanthum odoratum</i>
<i>Daucus carota</i>	<i>Sanguisorba officinalis</i> (hc)	<i>Carex acuta</i>
<i>Euphorbia esula</i>	<i>Selinum dubium</i> (hc)	<i>Carex disticha</i>
<i>Galium album</i> (hc)	<i>Senecio aquaticus</i>	<i>Carex nigra</i>
<i>Galium boreale</i>	<i>Silaum silaus</i> (hc)	<i>Carex praecox</i>
<i>Inula salicina</i>	<i>Thalictrum flavum</i> (hc)	<i>Deschampsia cespitosa</i>
<i>Lathyrus pratensis</i> (hc)	<i>Trifolium pratense</i> (hc)	<i>Luzula campestris</i>
<i>Leucanthemum ircutianum</i> (hc)	<i>Veronica chamaedrys</i>	<i>Poa angustifolia</i>
<i>Lotus corniculatus</i>	<i>Veronica maritima</i> (hc)	<i>Poa trivialis</i>
<i>Lychnis flos-cuculi</i>	<i>Vicia sepium</i> (hc)	

Table A3. Effects of year on the cumulative cover of target forbs according to the minimal adequate model. The model had three levels for year (Figure A4).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
Year	25.8	12.9	2	138	62.5	<0.001

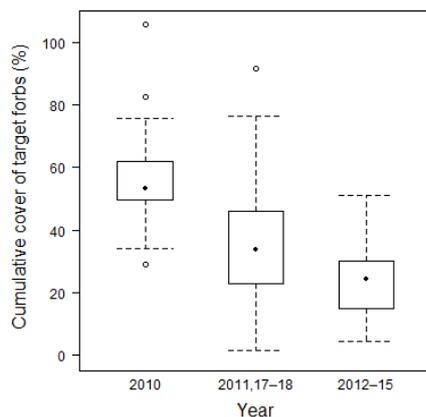


Figure A4. The minimal adequate model for the effect of time on the cumulative cover of target forbs with different fertilization treatments between 2010 and 2018 (except 2016).

Table A4. Effects of year on the cumulative cover of low-competitive target forbs according to the minimal adequate model. The model had four levels for year (Figure A5).

	Sum Sq	Mean Sq	NumDF	DenDF	F value	<i>p</i>
Year	26.1	8.7	3	137	29.2	<0.001

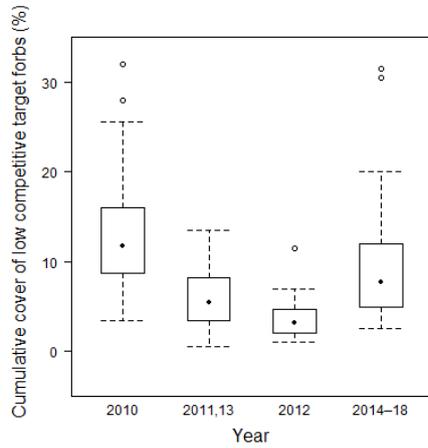


Figure A5. The minimal adequate model for the effect of time on the cumulative cover of low-competitive target forbs with different fertilization treatments between 2010 and 2018 (except 2016).

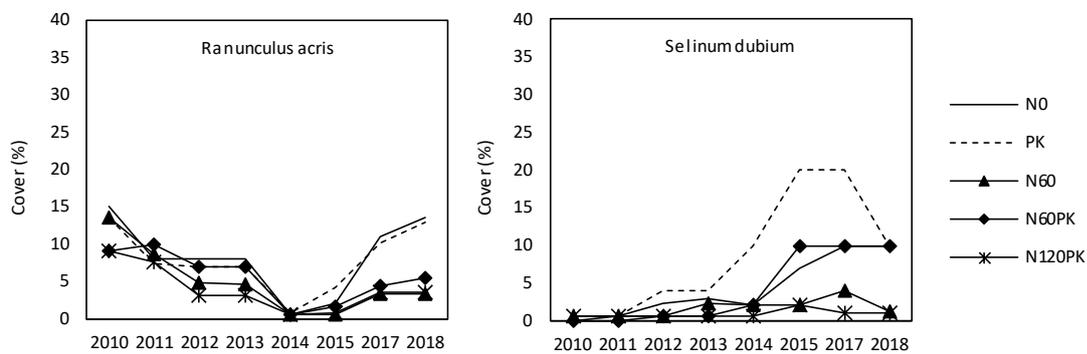


Figure A6. Development of percentage cover of the high-competitive target forbs *Ranunculus acris* and *Selinum dubium* with different fertilization treatments between 2010 and 2018 (except 2016).

Re-introduction of target species into degraded lowland hay meadows: How to manage the crucial first year?

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Abstract

In grassland restoration, the first year after species re-introduction by sowing in former species poor grasslands is a crucial period for the restoration progress. Despite the preparation of the restoration site by ploughing or grubbing, the establishment window is usually open for only a short time period and germination as well as establishment of the sown target species is often hampered by dense vegetation stands and related low above-ground light-availability. However, concepts how to manage freshly sown sites differ widely. In the Elbe lowland plain (Saxony-Anhalt, Germany), we tested the effects of three different cutting treatments (cut once, twice, three times), three nitrogen fertilization treatments (120 kg N ha⁻¹ yr⁻¹, 60 kg N ha⁻¹ yr⁻¹, without fertilization), rolling versus no rolling as well as species-trait affiliation on the establishment of sown target species in a species-poor, grass-dominated hay meadow using a split-split-plot design. Eighteen target species were sown into ploughed and grubbed plots in autumn. In the following year, individuals of each sown species were counted in microplots before the first and after the last cutting date. The treatment effects as well as the species-trait affiliation were tested using generalized linear mixed models and principal component analysis.

Cutting three times significantly enhanced the number of established target species compared to cutting once showing the importance of biomass removal after species re-introduction into productive hay meadows. Compared to control plots, the 120 kg N ha⁻¹ yr⁻¹ fertilization led to a lower number of established target species as well as individuals, whereas moderate fertilization did not hamper the establishment success significantly. Rolling did not show a significant effect. In addition, species traits, such as the potential to build large hemirosettes close to the ground, specific leaf area, and species height are good predictors for the establishment success under the different treatments.

Keywords: Cutting treatments; Fertilization treatments; Grassland restoration: Seeding; Seedling establishment; Species traits

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Introduction

Species-rich mesophilic grasslands are considered as endangered habitats throughout Europe thus identified as an essential objective of general interest, for example, within Natura 2000 network (Council Directive 92/43/EEC). Major threats are intensification or conversion to arable land on the one hand and abandonment of management on the other hand (Bakker and Berendse, 1999; Dengler et al., 2014; Lindborg and Eriksson, 2004; Walker et al., 2004). Lowland hay meadows (habitat type 6510) have especially strongly declined since 1950 in Germany (Briemle et al., 1999), but also in other European countries (e.g. Öster et al., 2009b; Walker et al., 2004). Therefore, they are a focus of restoration projects throughout Europe (e.g. Lepš et al., 2007 for European grasslands, Germany: Buchwald et al., 2007; Conrad and Tischew, 2011, UK: Edwards et al., 2007; Pywell et al., 2002; Walker et al., 2004; Norway: Rydgren et al., 2010; Sweden: Öster et al., 2009b). In grassland restoration, the first year after species introduction seems to be the most crucial period. Young seedlings are extremely sensitive to different biotic as well as abiotic factors resulting in a high loss rate (Fenner, 1987, Grubb, 1977). However, as shown by Öster et al. (2009b), a high first-year recruitment of sown species is very important for long-term establishment and therefore for the restoration outcome. It is thus a question how to manage this crucial first year to achieve high establishment rates of introduced species. Particularly if a major part of farmed grassland is embedded into restoration schemes, restoration projects are often confronted with farmers demands. Because lowland hay meadows represent major forage sources for livestock feeding, farmers stipulate that the restoration sites are sufficiently productive and provide hay with a good forage quality already in the restoration phase. This would imply to fertilize restoration sites (mainly with nitrogen), cut them twice a year as well as roll them using large machinery, like under traditional grassland management practices. The question is, how would this affect the establishment of introduced target species? On the one hand many grassland restoration projects cut their restoration sites only once a year (Hölzel and Otte, 2003; Jongepierová et al., 2007; Török et al., 2010; van der Putten et al., 2000) or even refrain from cutting in the early restoration period (Nordbakken et al., 2010, Rydgren et al., 2010). A relatively low biomass production is given as one argument for this management (Hölzel and Otte, 2003). Other arguments may be the fear of increasing soil compactness by cutting with large machinery (Schäffer et al., 2007), which can reduce seedling establishment (Török et al., 2011) or the disturbances caused by cutting. On the other hand, some restoration studies have demonstrated that frequent cutting already in the first year can have positive effects on seedling establishment (Hofmann and Isselstein, 2004; Lawson et al., 2004). In particular, on sites with high soil nutrient contents seedlings of less competitive species can be suppressed by an increase in aboveground biomass and therefore increased light limitation for understory species (Borer et al., 2014; Hautier et al., 2009). Thus, frequent cutting may be a useful measure to reduce negative competition effects particularly in the first crucial year of establishment and on more productive sites (Borer et al., 2014; Lawson et al., 2004). Furthermore, effects of fertilization on seedling establishment might also be different depending on the intensity of biomass removal, i.e. cutting time and frequency. Therefore, testing the effects of different mowing regimes in combination with different nitrogen fertilization treatments is highly relevant for restoration practice, but has rarely been tested

(Foster et al., 2009; Jones and Hayes, 1999; Smith et al., 2003). Apart from cutting and fertilization, rolling represents a further common management measure in Europe particularly practiced on former fen sites which are currently used as hay meadows. Rolling is mostly practiced in spring for soil consolidation and frost crack closing. There is little knowledge on the effects of rolling on the establishment of freshly sown species (e.g. Harper et al., 1965). It is a question whether rolling damages seedlings and if the soil consolidation reduces suitable micro sites for seed germination. In order to allow for generalization independent of site-specific species, species traits may be good predictors of establishment success under different management practices (Kahmen and Poschlod, 2008; Pywell et al., 2003). This approach was already successfully applied in several studies on grassland restoration (Andrade et al., 2014; Bissels et al., 2006; Öster et al., 2009a), but not yet used for a systematic analysis of integrated fertilization and cutting treatments. In the present study, a field experiment was conducted to test under practice-oriented on-site conditions the effects of relevant management treatments in different combinations on the performance of target species in the crucial first year after sowing, especially on the number of target species and individuals as well as composition of target species. Since small-scale heterogeneity of soil parameters are supposed to be a common feature of large-scale restoration sites (Baer et al., 2004; Maestre et al., 2003), we also included selected soil parameters into our analysis. We hypothesized that (1) a high cutting frequency in the first year after sowing is important for the establishment success of the sown target species on productive grassland sites, (2) high nitrogen fertilization inhibits target species establishment but moderate nitrogen fertilization might not impede sown target species, and (3) rolling damages seedlings and thus reduces the establishment rate. We further hypothesized that (4) specific species traits are good predictors for species-specific establishment success under the different treatments.

Materials and methods

Study site

The study site was located in the Wulfener Bruch, which is part of a large lowland plain area of the Elbe River in the center of the German federal state of Saxony-Anhalt (11°58' E, 51°50' N) with an altitude of about 52 m above sea level. The area is characterized by a continental climate with mean annual precipitation of about 500 mm and mean annual air temperature of 9°C (climatologic station: Köthen, period: 1961–1990, DWD, 2015). The study site was a former half-bog, but, as in many other regions, the site was long-since drained and used as a hay meadow. The soils are gley soils with high organic matter content (14.3%) and therefore of high fertility. The total nitrogen content of the soil is about $0.7 \pm 0.3\%$, available phosphorus (DL extraction) 11.6 ± 7.5 mg per 100 g soil and pH (0.01 M CaCl₂) of 6.5 ± 0.7 . The high nutrient level is due to intensive use during the times of the former GDR (high fertilization with slurry, several cuts per year). After German reunification, the meadow has been extensively managed (no fertilization, only one cut per year). This former management resulted in species poor stands (18.3 species per 16 m²) with a clear dominance of grasses and a dense vegetation structure, as well as high litter accumulation. The meadow can be

characterized as a species-poor lowland hay meadow (Arrhenatherion, Habitats Directive code 6510) which shows some features of an alluvial meadow (Cnidion, code 6440).

Study design

We used a split-split-plot design with four blocks (replications) to study the effects of different treatments on the number and individuals as well as composition of target species in the first year after sowing (Table 1). The cutting treatment was the main treatment of each block, split by the fertilization treatment (split-plot treatment) which was additionally split by the rolling treatment (split-split-plot treatment). All possible combinations of these single treatments resulted in 18 different management treatments. For each of the 18 treatments and their replications a sample plot with a size of 4 m × 4 m was installed (altogether 72). Interspaces between sample plots were about 12 m to avoid reciprocal interference of the different fertilization treatments and to facilitate the use of large machinery for mowing and rolling. The cutting height was about 10 cm and cuttings were subsequently removed within one week. The nitrogen fertilizer (urea) was spread by hand only within the 4 m × 4 m sample plots. Rolling was carried out using machinery typically used by the local farmers for this purpose (manufacturer Güttler GmbH Germany, prismatic roll, width: 6 m, mass: about 3 t). All treatments represent typical management practices for this region. Cutting once represents the management practiced over the last 20 years at the study site, but came under criticism because of the unsatisfactory results in terms of the loss of many typical meadow species, particularly low-competitive forbs. Cutting twice is nowadays applied at nature conservation grasslands and cutting three times is mostly practiced at sites where farmers need high amounts of forage for cattle. With regard to the fertilization treatments, no fertilizers are used at nature conservation grassland sites where the soil should be impoverished or where atmospheric nitrogen input is high, while 60 kg N ha⁻¹ yr⁻¹ are often used in already developed species-rich lowland hay meadows and alluvial meadows where atmospheric nitrogen input is low. Fertilization with 120 kg N ha⁻¹ yr⁻¹ is a typical practice if a high hay quality is required. Rolling is typically used at drained half-bog sites for soil consolidation and frost crack closing as explained above. All sample plots were ploughed and grubbed before sowing at the beginning of September 2011 and were afterwards sown with 18 target species (Table 2, about 635 seeds m⁻², 1.11 g m⁻²). Target species in this context are species characteristic for lowland hay meadows or alluvial meadows that were missing or present in only very low abundances on our study site. Most of the species are adapted to at least moderately nutrient-rich site conditions with Ellenberg *N*-values ≥ 4, with the exception of *Silaum silaus*, *Selinum carvifolia* and *Galium verum* (*N*-value = 3, Ellenberg et al., 1991). Seeds were of regional origin and propagation, as recommended for example by Kiehl et al. (2014) and Tischew et al. (2011). To facilitate an even sowing pattern seeds were mixed with soymeal.

Table 1 Experimental treatments.

Code	Treatment and dates 2012
Cutting treatments	
Ct 1	One cut June 18th
Ct 2	First cut May 19th and second cut August 13th
Ct 3	First cut May 19th, second cut July 10th, and third cut September 3rd
Fertilization treatments	
N0	No fertilization
N60	Fertilization with 60 kg N ha ⁻¹ yr ⁻¹
N120PK	Fertilization with 120 kg N ha ⁻¹ yr ⁻¹
Rolling treatments	
-r	No rolling
+r	Rolled once in April

Soil survey

In February/March 2012, soil samples were taken from every 4 m × 4 m sample plot. In each sample plot, we took eight single samples to a depth of 0–10 cm using a Pürckhauers soil core sampler and pooled them to a mixed sample. To analyze pH, 10 g dry soil of every sample was dissolved in 25 ml 0.01 M CaCl₂. The pH was measured after 30 min using a Mettler-Toledo pH-electrode (Mettler-Toledo, Gießen/Germany). The total N- and C-contents (%) of 200 mg dry soil were determined at 950°C using a TruSpec CN-analyzer (manufacturer LECO, Mönchengladbach/Germany). To analyze the contents of plant-available phosphorus (mg P/100 g soil) and plant-available potassium (mg K/100 g soil) the double-lactate-digestion-method (DL-method) was applied. Phosphorus was then measured photometrically using a Spekol 1100 (manufacturer Carl Zeiss, Jena/Germany) and potassium was measured by atom absorption spectrometry (AES) with a ContraAA 700 (manufacturer Analytic Jena AG, Jena/Germany).

Vegetation survey

On each 4 m × 4 m sample plot one microplot of 0.5 m × 0.5 m size was installed for the vegetation surveys in the lower left corner. Before the first cut in 2012, individuals of sown species were counted on each of the 72 microplots (4 replicates for each of the 18 treatments). The counting was repeated after the third cut in autumn 2012.

Data analysis

We calculated generalized linear mixed models (GLMM) using IBM SPSS Statistics 22.0 (IBM Corporation, Chicago Illinois, USA) to test the effects of different management treatments on the establishment of target species. Cutting treatment, fertilization treatment and rolling treatment as well as the interactions of the management treatments (cutting*fertilization,

cutting*rolling, fertilization*rolling) and the pH of the soil in spring 2012 (to consider differences in soil characteristics) were included as fixed factors. Block was the random factor. GLMM with a Poisson-distribution and identity-link were calculated for the two dependent variables, number of target species and number of target species individuals in autumn 2012. Furthermore, GLMM with the spring data were analyzed to test for differences between the microplots before the different management treatments started. Post hoc tests were conducted to compare means by multiple tests after Bonferroni adjustment of 95% confidence intervals. A principal component analysis (PCA) was performed to arrange the microplots according to the target species composition (species by sample matrix with the number of individuals of each target species per microplot). Information about the environment, i.e., management treatments (cutting, fertilization, rolling), soil chemical properties of the sample plots (compare Section 2.3) and selected species traits (compare Table 2) was used for post hoc correlation analyses as an interpretative tool to explain this arrangement. With respect to species traits the number of target species individuals with heavy seeds (TSW ≥ 2 g), large specific leaf area (SLA > 20 mm² mg⁻¹), large potential height (PH > 100 cm) and the ability to form large hemirosettes (HR) were used. The analyses were performed using PC-ORD 6.0 (McCune and Mefford, 2011).

Table 2 Sown target species with codes later used in results, sown quantities, and selected species traits: TSW = thousand-seed weight (specific for sown seeds), SLA = specific leaf area (LEDA Traitbase; Kleyer et al., 2008; na = not available), PH = potential height (Jäger and Rothmaler, 2011), large HR = large hemirosette close to the ground (BIOLFLOR; Klotz et al., 2002 and own on-site observations, + = yes, – = no).

Code	Plant species	Seed mixture		Plant traits used in analysis			
		seeds m ⁻²	g m ⁻²	TSW [g]	SLA [mm ⁻² mg ⁻¹]	PH [m]	large HR
Cpa	<i>Campanula patula</i>	100	0.004	0.04	34.89	0.6	–
Cpr	<i>Cardamine pratensis</i>	100	0.057	0.57	25.13	0.6	–
Cca	<i>Carum carvi</i>	20	0.060	3.00	24.78	0.8	–
Cja	<i>Centaurea jacea</i>	20	0.042	2.10	18.31	0.6	+
Col	<i>Cirsium oleraceum</i>	20	0.050	2.50	25.74	1.5	+
Cbi	<i>Crepis biennis</i>	15	0.012	0.80	na	1.2	+
Dca	<i>Daucus carota</i>	50	0.050	1.00	21.70	1.0	+
Gal	<i>Galium album</i>	30	0.018	0.60	24.09	1.0	–
Gve	<i>Galium verum</i>	20	0.010	0.50	18.39	0.7	–
Gpr	<i>Geranium pratense</i>	20	0.120	6.00	20.00	0.6	+
Kar	<i>Knautia arvensis</i>	10	0.040	4.00	18.72	0.8	+
Lpr	<i>Lathyrus pratensis</i>	10	0.110	11.00	26.21	1.0	–
Pma	<i>Pimpinella major</i>	30	0.051	1.70	13.25	1.0	–
Rac	<i>Rumex acetosa</i>	20	0.011	0.55	23.08	1.0	–
Sof	<i>Sanguisorba officinalis</i>	50	0.100	2.00	15.06	1.5	+
Sca	<i>Selinum carvifolia</i>	50	0.050	1.00	17.98	0.9	–
Ssi	<i>Silaum silaus</i>	30	0.075	2.50	na	1.0	–
Tpr	<i>Tragopogon pratensis</i>	20	0.140	7.00	na	0.6	–

Results

Species establishment

By autumn 2012, 17 of 18 sown species were established on the study site. But, all 18 target species could be detected at least once at the first and/or the second counting on one of the microplots. On average $4.5 \pm 2.8\%$ of sown seeds m^{-2} germinated and established individuals in autumn 2012.

Effects of management on number of target species and individuals

In spring 2012, before management started, no significant differences in the number of established sown target species as well as the number of established individuals of target species between the different treatments were observed, which indicates a homogeneous initial state. On average, 3.4 target species and 5.6 individuals were established per microplot in spring 2012. In autumn 2012, after management was finished, the number of established target species and individuals differed significantly between the different cutting and fertilization treatments (Table 3). Target species benefited from frequent cutting. The numbers of established sown target species and individuals were significantly higher on microplots cut three times compared to microplots cut only once (Figure 1). In addition, even microplots cut twice showed significantly lower numbers of established target species individuals compared to microplots cut three times. Nitrogen fertilization with $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ led to both a significantly lower number of established sown target species as well as individuals compared to unfertilized microplots (Figure 1). In contrast, microplots fertilized with $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ did not have significantly lower numbers of established sown target species and individuals compared to unfertilized microplots. Moreover, the number of target species individuals on microplots fertilized with $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ was significantly higher than on microplots fertilized with $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Rolling as well as pH had no significant effect on target species establishment (Table 3). However, we found a significant interaction of fertilization and cutting on the number of target species individuals. Furthermore, the interaction of fertilization and rolling also significantly affected both the numbers of established target species and individuals.

Effects of management on species composition and species traits

The PCA confirms the negative effect of the high fertilization treatment of $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ superposing all other effects (Figure 2). The largest distance exists between the centroids of the Ct1 N120 treatment (lowest biomass removal + highest nitrogen input) and the Ct3 N0 treatment (highest biomass removal + lowest nitrogen input). In post hoc correlation analysis, none of the soil chemical parameters but three of four species traits were correlated with PCA-axis. Species, which are able to build large hemirosettes close to the ground, like *Centaurea jacea*, *Daucus carota* and *Geranium pratense*, profit from no or only moderate fertilization and can cope with frequent cutting. On the other hand, species which have a high specific leaf area and/or which can potentially reach a height of at least 1 m, like *Galium album*, are not restricted to specific management treatments.

Table 3 The effects of the fixed factors cutting, fertilization, rolling, cutting*fertilization, cutting*rolling and fertilization*rolling as well as soil pH on the numbers of established sown target species and individuals. Statistics were calculated using generalized linear mixed models (GLMM). Significant differences ($p < 0.05$) are indicated with bold face. Groups for cutting and fertilization are shown in Figure 1.

	Number of target species			Number of target species individuals		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
cutting	2	3.748	0.030	2	7.056	0.002
fertilization	2	3.706	0.031	2	11.033	0.000
rolling	1	0.089	0.767	1	0.023	0.879
cutting*fertilization	4	0.177	0.950	4	2.691	0.040
cutting*rolling	2	0.734	0.484	2	2.398	0.100
fertilization*rolling	2	5.300	0.008	2	8.861	0.000
pH 2012	1	3.200	0.079	1	3.078	0.085

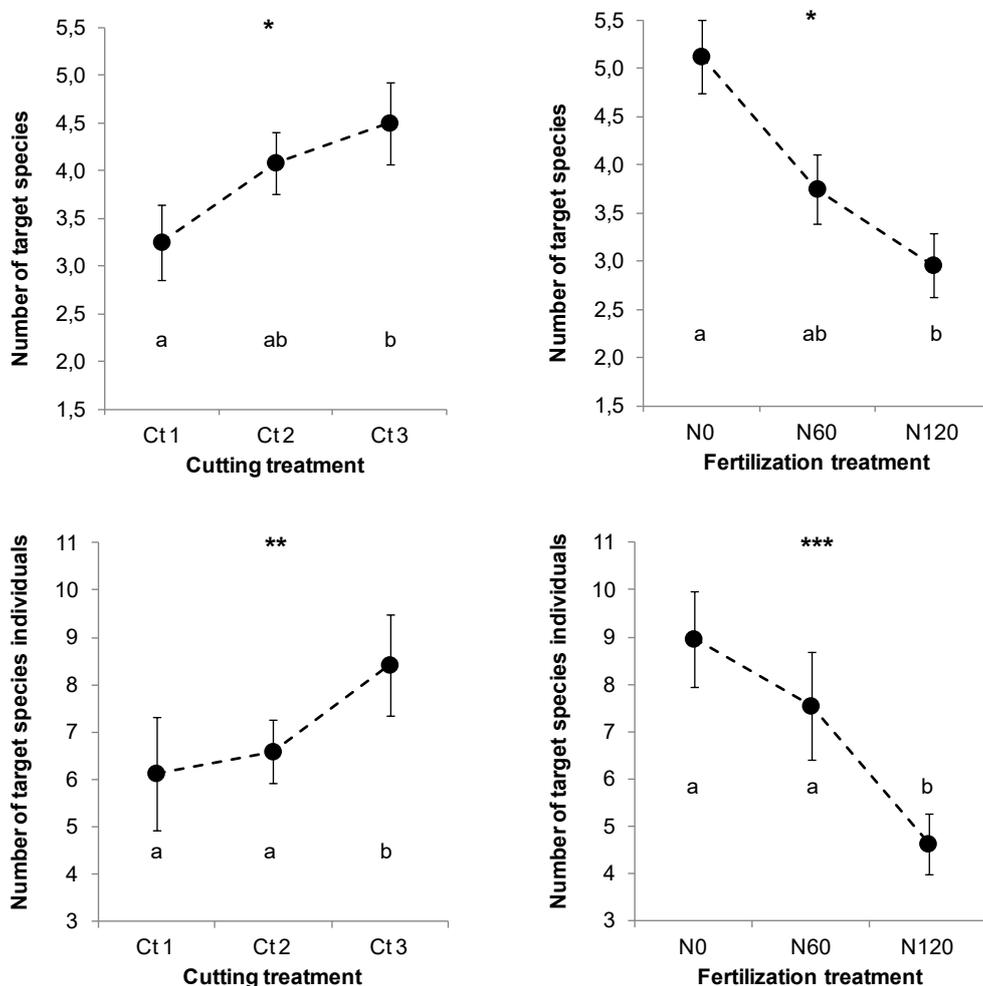


Fig. 1 The effects of the cutting and fertilization treatments on the numbers of established sown target species and individuals per microplot (0.25 m²) in autumn 2012. Statistics were calculated using generalized linear mixed models (GLMM). Significant differences ($p < 0.05$) are indicated by different letters. Shown are means ($n = 24$) with standard error. n.s. not significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

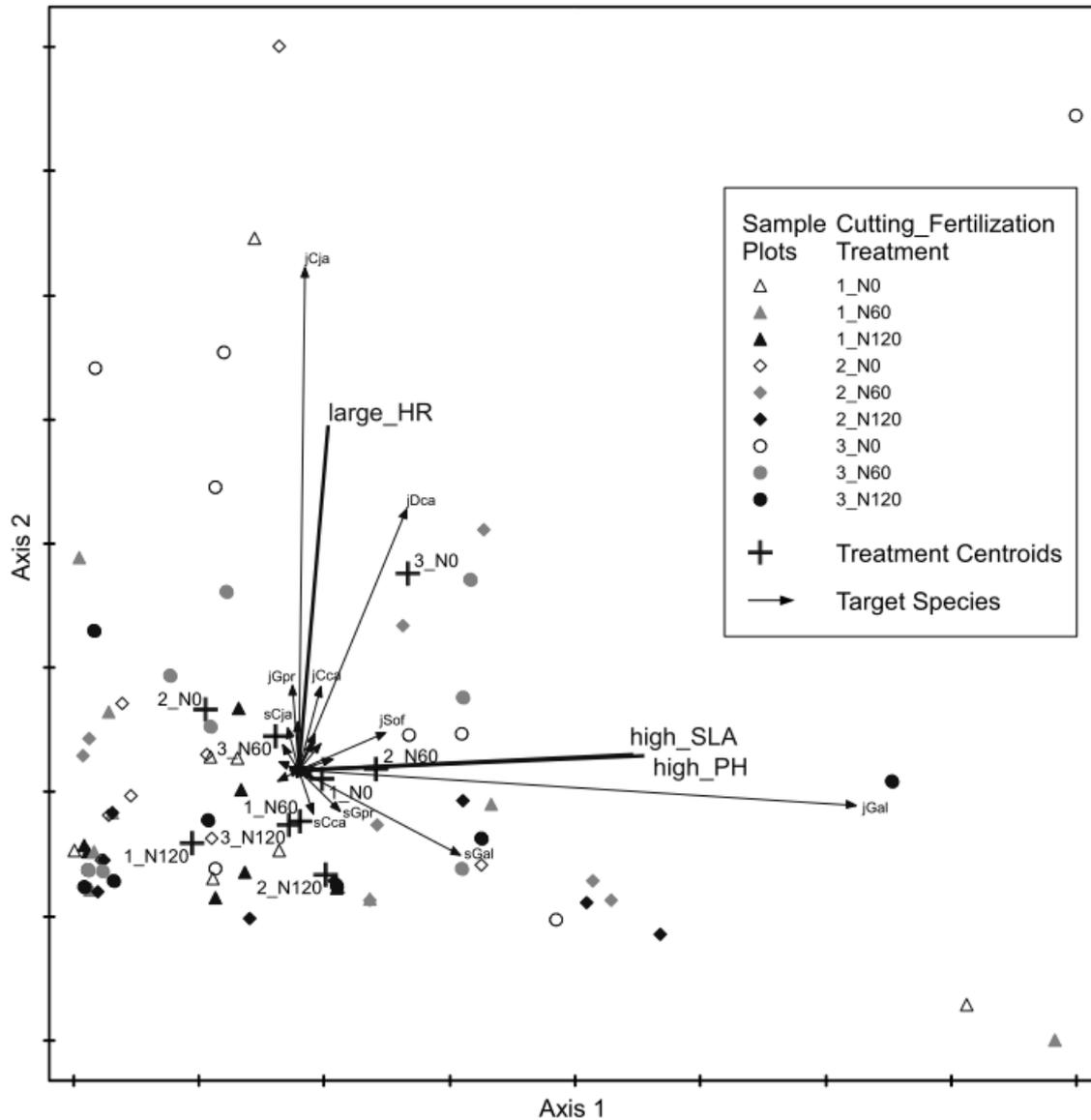


Fig. 2 PCA ordination diagram showing the distribution of all microplots based on the established individuals of every sown target species (j = juveniles, s = seedlings, for species abbreviations see Table 2) and post hoc correlations of each axis with species traits ($r > 0.8$ large_HR = large hemirosettes close to the ground, high_SLA = specific leaf area $>2 \text{ mm}^2 \text{ mg}^{-1}$, high_PH = potential height of at least 100 cm, compare Table 2). Microplots are labeled according to the cutting*fertilization treatment (compare Table 1) and centroids of all treatment groups are shown. Explained variance: axis 1: 27.4%, axis 2: 17.6%.

Discussion

In support of hypothesis 1, we found that a high cutting frequency (cutting three times) in the first year after sowing was important for the establishment success of the sown target species. This is in accordance with other studies (for example Hofmann and Isselstein; 2004, Lawson et al., 2004). The absence of a shading canopy in frequently cut plots is probably the main reason for this result (Isselstein et al., 2002), because competition for light is one decisive factor for the formation of plant species assemblages and affects the species richness of plant communities (Dybziński and Tilman, 2007; Harpole and Tilman, 2007; Hautier et al., 2009). Thus, canopy removal decreases competition (Borer et al., 2014). The advantages of suitable initial site conditions for the survival rates of introduced endemic species, for instance lower competition with other plant species, were also shown by Colas et al. (2008). Furthermore, litter reduction as an effect of frequent cutting maintains gaps for seedling establishment (Török et al., 2011) and may thus have increased the number of established target species and individuals in the present study. The benefits of frequent cutting, especially in decreasing competition for light and litter accumulation, obviously outbalanced potential threats such as mechanical destruction in the course of cutting. However, other studies also achieved good results with only one cut in the first year indicating the importance of initial site conditions. For example, if sites formerly used as arable land have been restored (for example Andrade et al., 2014; Hölzel, 2005; Török et al., 2012), competition by grasses might be less important than on former species poor grasslands dominated by competitive grasses. The presence of more short-lived species on ex-arable land promotes the establishment of target species whilst grasses on former species poor grasslands might outcompete target species (Donath et al., 2007). Further reasons for good results with only one cut in the first year might be the different nutrient conditions of restoration sites. In many cases, ex-arable sites have low nitrogen contents (Gough and Marrs, 1990), which reduces competition even if phosphorous and potassium supplies are high (Pywell et al., 2002). Furthermore, topsoil removal as a site preparation treatment for restoration measures can also reduce the productivity of the restoration site to such an extent that biomass production, and therefore competition, is very low. In such cases, the cutting treatment can be reduced or even omitted during the first year (Andrade et al., 2014; Hölzel and Otte, 2003). We obtained a significantly lower total target species number and total number of established individuals on the intensely fertilized plots ($120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) compared to unfertilized plots, findings which support the first part of hypothesis 2. This negative effect of fertilization on restoration success is in accordance with other studies (for example Foster et al., 2009; Jones and Hayes, 1999; Smith et al., 2000). Since most of the introduced species are adapted to at least moderately nutrient-rich site conditions the reasons might be the same as shown for frequent cutting. Nitrogen application increases aboveground biomass resulting in increased light limitation for understory species, which are then outcompeted (Borer et al., 2014). A moderate fertilization with $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ did not significantly reduce the number of established target species and individuals in the present study, thus supporting the second part of hypothesis 2. This finding suggests that moderate fertilization did not lead to a significant lowering of the competitive ability of the sown species, which is important for restoration practice, because it allows for a moderate fertilization if an adequate biomass removal by cutting will be applied. Therefore, the respective meadows can

provide hay with good fodder quality already during the restoration phase, which can increase farmers' acceptance of restoration actions.

Rolling had no effect on the number of established target species and individuals in the present study, thus hypothesis 3 cannot be supported. This may be due to the prismatic surface of the roll resulting in a patchy distribution of pressure. Closing frost cracks and structuring the soil surface may have even improved seedling conditions and thereby compensated for potential losses of seedlings by mechanical damage. The significant interaction of fertilization and rolling is hardly interpretable in ecological terms, whereas the significant effect of the interaction between cutting and fertilization on the establishment of target species individuals supports the afore mentioned main effects of cutting and fertilization. The conditions for the establishment of individuals diminish with higher biomass accumulation and nutrient supply, thus an adequate biomass removal by cutting is indispensable especially on fertilized sites. In order to establish a large number of target species and individuals in the first year, we recommend a management treatment of cutting three times and to use no or only moderate amounts of nitrogen fertilizer. At the species level, we found differences in the establishment success, which can be related to specific species traits thus supporting hypothesis 4. As already shown above, the mowing treatments affected seedling establishment in general, but the species responded differently to the mowing treatments, which is in line with Bissels et al. (2006). In the present study, species which are able to build large hemirosettes close to the ground could cope particularly well with frequent cutting as has been already assumed by Kahmen and Poschlod (2008). *Centaurea jacea*, *Daucus carota* and *Geranium pratense* are typical examples for this group of species. After cutting, they can quickly re-build dense hemirosettes and therefore permanently occupy the respective space. These species also profit from no or only moderate fertilization, which may be an effect of less dense stands and higher light availability near to the ground. On the other hand, species having a high specific leaf area (SLA) and/or potentially reaching a height of at least 1 m were not restricted to specific cutting or fertilization treatments, which is also in line with Kahmen and Poschlod (2008). Having a large SLA and height enables these species to cope with different cutting and fertilization treatments. It can be assumed that species with such traits, for example *Galium album*, can on the one hand better compensate for reduced light-availability in dense stands caused, for example, by insufficient cutting, then species with a lower SLA and potential height. On the other hand, a high SLA, representing the relative growth rate of a species (Wilson et al., 1999), can also promote these species when a site is frequently cut. Therefore, a high SLA cannot be used as a predictor for species cutting response, as found by Díaz et al. (2001) for different intensities of biomass removal. Furthermore, a high SLA is said to be positively related to nutrient availability (Wilson et al., 1999). But, in accordance with Duru et al. (2014) and Andrade et al. (2014), who studied sites with different nutrient availability, we did not find any effects of different fertilization treatments on the establishment success of species with a high SLA, although our field observations indicated such a relation. We therefore suggest to use individual (site-depending) SLA instead of mean SLA from a database in future studies. Altogether, integrating a species-trait approach into the procedure of the seed mixture composition may enhance restoration success. On the one hand, the management after sowing can be adapted toward

a more frequent cutting if species with specific traits (such as large hemirosettes close to the ground) do not show the expected establishment success. On the other hand, it seems sensible to enhance the share of species robust to different management regimes if a frequent cutting during the first establishment year is not feasible.

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Effects of fertilizer levels and drought conditions on species assembly and biomass production in the restoration of a mesic temperate grassland on ex-arable land

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Abstract

The restoration of degraded arable land to species-rich and functional grasslands by sowing native has been tested successfully, while studies on restoration considering land use interest and climate change challenges are underrepresented. In this five-year study, we focused on the process of restoring grassland biodiversity and biomass production under different fertilizer levels in the face of several years of under-averaged precipitation. In 2017, we sowed a species and forb-rich native seed mixture to establish a submontane Arrhenatherion grassland. We applied fertilizer treatments (0, 60, 120 kg N ha⁻¹ yr⁻¹, combined with and without P and K fertilizing) in order to meet local farmers' demands on biomass for hay production with nature conservation goals that aim to promote a highly species-rich and functional grassland community.

Our results show that sowing a high-diverse and forbs-rich mixture not only leads to a high species richness, but also to usable aboveground biomass production for animal feeding, even with below-average precipitation. However, the slight decline in species number and cover of sown forbs following the dry period in the first year after sowing indicates the sensitivity of less drought-resistant forbs. Due to the priority effects of sown species, no undesirable species have invaded the sward. The nitrogen treatments shifted the grass-forb ratio, with grasses dominating in the nitrogen enrichment treatments due to their increased competition ability, while forbs dominating in the non-nitrogen enrichment treatments. Biomass production was higher at the first cut than at the second, and non-nitrogen fertilized treatments had a lower biomass production compared to nitrogen fertilized treatments.

Both grasses and forbs contributed to drought resilience related to biomass production, but forbs contributed relatively more in the first cut under moderate or no nitrogen fertilization and in the second cut only without nitrogen application. Biomass production was strongly

determined by year, and thus precipitation. Under drought conditions, species-rich stands produced sufficient biomass even without nitrogen fertilization. In order to establish and maintain species and forb-rich grasslands on ex-arable land, nitrogen fertilization should be moderate at most. Six of the 44 sown species, namely *Arrhenatherum elatius*, *Alopecurus pratensis*, *Dactylis glomerata*, *Poa pratensis*, *Centaurea jacea*, and *Trifolium pratense*, contributed significantly to the biomass and could act as matrix species in climate-adapted high-diverse native seed mixtures for our study region.

Keywords: Arable land; Grassland restoration; Seeding, Native species; Climate change; Drought; Fertilizer levels; Biomass production

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Introduction

Grasslands play an important role as part of agricultural production systems in Europe (Isselstein et al., 2005; Peyraud et al., 2016). They provide multiple ecosystem functions and services (Bengtsson et al., 2019; Manning et al., 2018; Garland et al., 2020; Zhao et al., 2020), such as feed base for livestock (Boval and Dixon, 2012; Berauer et al., 2020), pollination services (Orford et al., 2016), carbon storage (Bai and Cotrufo, 2022) and have an important contribution to biodiversity (Dengler et al., 2014). The drastic loss of semi-natural grasslands in Europe during recent decades (van Dijk, 1991; Wesche et al., 2012; Diekmann et al., 2019) has led to the development of various techniques for the restoration of grassland communities (Kirmer et al., 2012a; Blakesley and Buckley, 2016). A natural and low cost way is spontaneous recolonization (Prach and Hobbs, 2008), which can occur in the short term from persistent seeds in the soil seed bank (von Blanckenhagen and Poschlod, 2005) and in the long run from large target species populations in the immediate vicinity (Prach et al., 2021). However, if seeds are not available due to soil seed bank and dispersal limitations, this presents the main barrier to spontaneous grassland restoration (Öster et al., 2009; Török et al., 2018). Pywell et al. (2002) also identified the presence of seeds of target species as a key factor for the development of species-rich meadow communities. To overcome these limitations, active introduction of target species during restoration is required (Bakker and Berendse, 1999; Walker et al., 2004). The sowing of native seeds from regional propagation is one of the most commonly used method in Europe to restore species-rich semi-natural grassland on ex-arable land (Kiehl et al., 2010; Kirmer et al., 2012a), where the soil seed bank tends to be dominated by ruderals, whereas typical grassland species are usually absent (Walker et al., 2004). Seeding creates a priority effect for the target species (Weidlich et al., 2021) and suppresses undesirable species, like weeds and invasive plant species (Kiss et al., 2022; Halassy et al., 2023). To increase community resilience, Kiss et al. (2018) recommend prioritizing the

introduction of native matrix species that can tolerate the predicted climatic changes. This study focuses on the restoration and biomass production of a re-created species-rich mesic grassland in the face of changing climate conditions, which are predicted to differentially impact grassland species abundance and productivity (Andrews et al., 2007). In addition to our objective to restore a species-rich grassland on ex-arable land, we were also keen to test restoration coupled with yield-enhancing fertilizer applications that result in a grassland system that can be integrated into sustainable value chains through hay production for fodder, as well as also being resilient during periods of drought. The use of native seed mixtures in the restoration of arable land to grassland can lead to the rapid establishment of meadow species producing vegetation suitable for haymaking (Mitchley et al., 2012). To maintain biomass production, grassland has traditionally been fertilized to balance nutrient removal (Kapfer, 2010). In contrast, the higher use of nutrient concentrations, especially nitrogen, is a common practice in grassland systems (Oenema et al., 2012; Einarsson et al., 2021), and conflicts with biodiversity conservation goals (Boch et al., 2021; Ranta et al., 2021). In the context of different land use priorities, it is important to address the challenge of reconciling farmers' demands on biomass for hay production with the nature conservation goal to restore species-rich grasslands. The restoration of grasslands from previously arable lands has been the subject of numerous studies focused on various factors, i.e. active versus passive restoration (Prach et al., 2014), low versus high-diverse seed mixtures (Pywell et al., 2002; Lepš et al., 2007), various directly harvested seed material (Albert et al., 2019), time and management (John et al., 2016; Garrouj et al., 2019), techniques and costs (Török et al., 2011), underlying restoration successes or failures. In the context of restoring species-rich grasslands, the role of fertilization (e.g. Pecháčková et al., 2010) or the impact of drought (e.g. Cole et al., 2019) has been studied only in existing grasslands. Studies on fertilization in relation to species richness and the resilience of biomass production in the course of grassland restoration by seeding are lacking so far. To fill this knowledge gap, we studied the effect of different fertilizer levels in a grassland restoration project conducted on an ex-arable land. We focused on the following questions:

- (1) How successfully do sown species establish under different fertilizer applications?
- (2) Do species number and cover of functional plant group respond similarly to different fertilizer applications?
- (3) Can productivity resilience be increased by fertilization?
- (4) Which species of the regional assemblage contribute to biomass production even in dry years and should therefore be included as matrix species in seed mixtures?

We discuss our findings from the perspective of providing recommendations for future approaches to restoring grassland communities that consider farmer demands for biomass production and, following Lindenmayer (2020), link the ecological concepts and underlying mechanisms.

Materials and methods

Study area

The Hayn grassland experiment is located in the southern Harz region near Hayn in Saxony-Anhalt, Germany (N 51°57′29.93″, E 11°08′14.28″, 441 m above sea level). The percentage of grassland on the agricultural land in Saxony-Anhalt is only 15%, which is the lowest of all German federal states (BMEL, 2022). While in the montane region of the Harz mountain meadows still dominate the landscape with about 1650 ha (Bruehlheide et al., 1997), the plateau areas in the submontane region, which were cleared for agricultural use in the early Middle Ages, are mainly used as cropland (about 30%) and are hardly structured (Funkel and Szekely, 2011). The experiment is part of the experimental station of the state agency for agriculture and horticulture Saxony-Anhalt and was used for field trials in crop production until 2016. The climate is subcontinental with a mean annual precipitation of 742 mm and a mean annual temperature of 7.3 °C (long-term mean 1992–2021, LLG, 2022). In 2017, at the start of the experiment, precipitation was in line with the long-term mean. The years 2018–2022 were characterized by an annual precipitation between 541 and 696 mm (Figure A1). In the growing season from April to September 2018–2022, precipitation amounted to 161–349 mm (Figure A1), which was 10.0–58.4% lower when compared to the long-term mean (1992–2016: 387 mm). The monthly deficit fluctuated between the years and was most extreme in July 2018, April 2020, and March 2022 (Figure A2). In contrast to precipitation, temperature showed increased values in 2018–2022. The long-term mean 1992–2016 was exceeded by 0.8–2.5 degrees (Figure A3). The monthly temperature was exceeded by 75% of the values (Figure A4). Before the experiment started, the moderately acidic loam soil (classification follows Ad-hoc-AG Boden, 2005) was characterized by medium phosphorus and potassium levels (both class C, classification follows LLG, 2019).

Establishment of the grassland

In 2017, we sowed a regionally certified species-rich native seed mixture on a 0.5 ha restoration site. Following Kirmer and Tischew (2014), Arrhenatherion was selected as the target vegetation type. We aimed to establish a submontane mesic grassland of the plant association *Alchemillo vulgaris*-*Arrhenatheretum elatioris* (Oberd. 1957) Sougn. Et Limb. 1963 (Schubert, 2001), which belongs to the Natura 2000 habitat type 6510 (lowland hay meadows, European Commission, 2013) and is of interest for hay production for suckler cow husbandry. The mixture contained 44 native species, composed of 12 grasses and 32 forbs, including five legumes (Table A.1). Seeds were purchased from a regional wild plant propagator. We added three annual cultivar species as a nurse crop to ensure the establishment of native species by creating improved site conditions. While the cultivars germinate and grow quickly, they protect the soil from desiccation and erosion (Anderson, 2004). In September 2017, we mulched the cultivars at a height of 15–20 cm to hinder their development and not to impede the sown species.

Experimental design and sampling

In 2018, the experiment was established in a fully randomized design with 17 management treatments in six replications on 102 square plots (5.0 m × 5.0 m). This study focuses on the twice mown treatment with a first cut in mid-June and a second cut at the beginning of September. Six fertilizing treatments were tested (Table 1). We used mineral fertilizer in commercially available granules, N as urea in two applications before and shortly after the first cut, P in the form of triple superphosphate 46 and K in the form of K₂O 60 in one application before the first cut. Both P and K were applied in the amounts removed by the previous year's harvest, which was calculated separately for each treatment as it is common practice in agricultural meadow management. In the five-year experiment from 2018 to 2022, the percentage of vascular plant species cover was recorded annually at the end of May on 4.0 m × 4.0 m plots, and the percentage contribution of each species in the aboveground plant biomass (yield proportion: Voigtländer and Voss, 1979) was estimated on the same plot just before the first and second cut. The nomenclature of vascular plants follows Jäger (2017). To calculate dry matter yield and determine biomass P and K contents for the determination of nutrient removal, all plants in a randomly selected 1 m² frame in each plot were cut at a height of 3–5 cm just before mowing. Biomass samples were dried and analyzed in a laboratory according to standard methods (VDLUFA, 2004). The dry matter yield of each species was calculated from the total dry matter yield and the estimated yield proportion. Soil samples were collected at the plot level at a depth of 0–10 cm (mixture of 10 soil core samples per plot) each March from 2018 to 2022. Soil pH was analyzed after DIN (2005), total nitrogen after DIN (2001), total carbon after DIN (1990), and phosphorus (Calcium-Acetate-Lactate extraction method) and potassium after VDLUFA (1991), the classification of nutrient concentration level follows LLG (2019).

Table 1

Experimental fertilizer treatments.

Code	Treatment
N0	No fertilization (control)
PK	Fertilization with P and K as withdrawn
N60	Fertilization with 60 kg N ha ⁻¹ yr ⁻¹
N60PK	Fertilization with 60 kg N ha ⁻¹ yr ⁻¹ plus P and K as withdrawn
N120	Fertilization with 120 kg N ha ⁻¹ yr ⁻¹
N120PK	Fertilization with 120 kg N ha ⁻¹ yr ⁻¹ plus P and K as withdrawn

Functional groups and other species groups

The functional group of grasses included all species of the plant families Poaceae, Cyperaceae and Juncaceae. The functional group of forbs contained all other species. Sown species were defined as all native species of the seed mixture; whereas sown nurse crop cultivars were excluded. Apart from the sown species, spontaneous species encompass species from the soil seed bank or seed rain. They were grouped as grassland species if listed in Ellenberg's

sociological group 5.3 (Festuco–Brometea) and 5.4 (Molinio–Arrhenatheretea) (Ellenberg et al., 1991; Klotz and Kühn, 2002) or as a characteristic species of Natura 2000 habitat type 6510 (Schuboth and Frank, 2010).

Statistical analyses

To statistically analyze whether the species number, cumulative cover or dry matter yield of sown or spontaneously established species, forbs or grasses (see Table 1 for list of response variables) varied by fertilizer treatment over several years, we fitted linear mixed models (LMM) using lme4 1.1–31 (Bates et al., 2015). By adding plot-ID as a random variable in the models, we accounted for temporal pseudo-replication arising from repeated measurements in the same plots. For all analyses, we included year (5 levels), fertilizer treatment (6 levels) and their interaction as fixed factors in the models as the potential explanatory variables. Multi-model selection based on the Akaike Information Criterion (AIC) was used to identify the best model (MuMIn 1.47.1, Bartoń, 2022). Comparisons between pairs of elements were made on the basis of Tukey's contrasts using emmeans 1.8.4–1 (Lenth, 2023) and are presented only as supplementary material (see Tables A.4–A.28). To achieve normally distributed residuals and avoid heteroscedasticity, the number of sown and spontaneous species were log-transformed and the cover of sown and spontaneous species as well as the dry matter yield of sown forbs (first and second cut) and sown grasses second cut were logit-transformed prior to analyses (see Table 1). Assumptions were checked using DHARMA 0.4.6. (Hartig, 2022). Figures were created using ggplot2 3.4.0 (Wickham, 2016) and dplyr 1.1.0 (Wickham et al., 2023). All statistical analyses were performed in R version 4.2.2 (R Core Team, 2022).

Results

Soil chemical properties

Before the start of the experiment, the soil had a pH of 5.28, contained 0.21% total nitrogen and 2.11% total carbon. Phosphorus (50.37 mg kg⁻¹) and potassium (164.97 mg kg⁻¹) were measured at a moderate level, and magnesium (60.88 mg kg⁻¹) at a low level (Table A.2). The pH value tended slightly toward acidification in all treatments, and the total nitrogen and total carbon content showed a trend toward slightly increasing values (Figure A.5a–c). The content of phosphorus increased over time for all treatments even when not applied within the fertilization treatments, while potassium showed this trend only for the treatments where potassium was applied (Figure A.5d–e). Magnesium first showed a continuous increase in all treatments and decreased to or even below the 2018 level in the last year of observation (Figure A.5f).

Plant species number and establishment rate

During the five-year observation period, species richness decreased continuously for all fertilizer treatments, mainly caused by the significant decline of spontaneously established species, most of which were not typical grassland species (Figure 1, Table 2, Figure 2). In

contrast to spontaneous species, the sown grass and forb species numbers slightly but significantly declined only from 2018 to 2019 (Figure 1, Table A.4–A.6). N0, PK, N60 and N60PK treatments increased sown forb number again ranging from 19.5 to 20.8 by 2022, and N120 and N120PK treatments stabilized by 2022 on lower levels of 17.7–18.5. Five years after sowing, the mean establishment rate of sown species was about 66.9% ($\pm 5.7\%$) and was slightly higher in the lower fertilizer treatments, but did not differ significantly among fertilizer treatments (Table A.3).

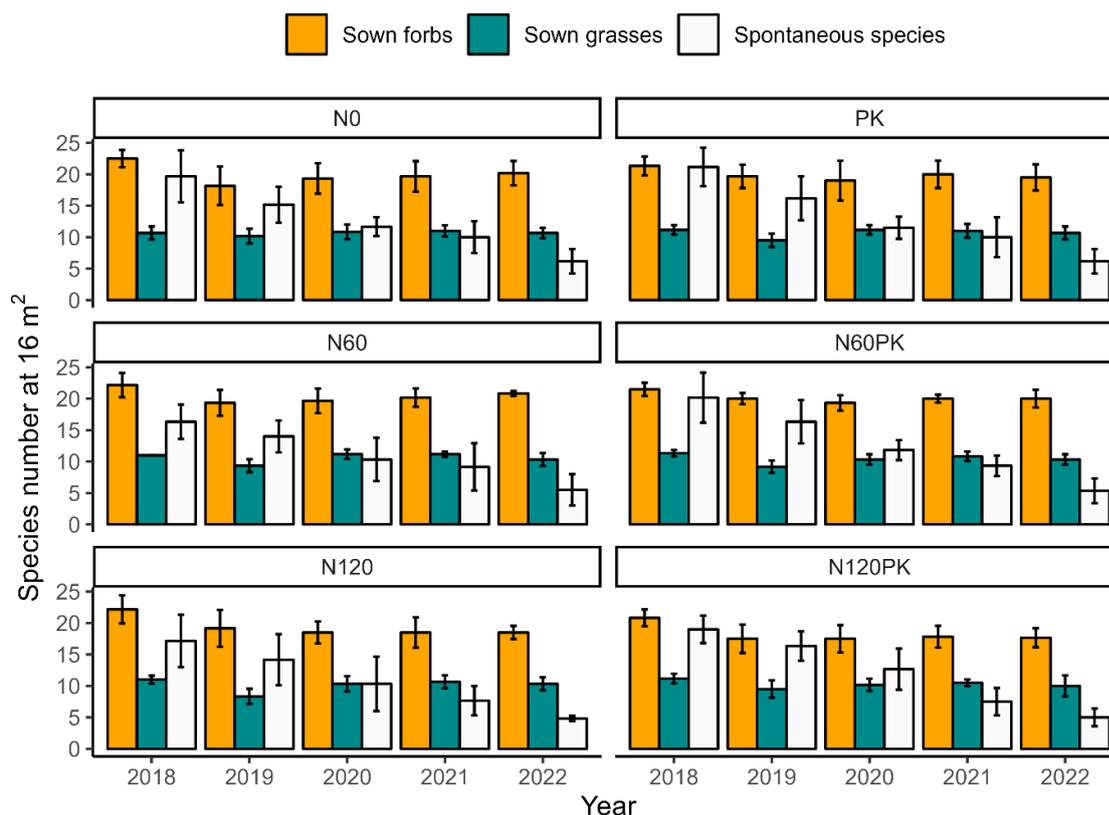


Fig. 1. Mean species number at 16 m² plot of sown grasses, sown forbs and spontaneously established plant species on different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

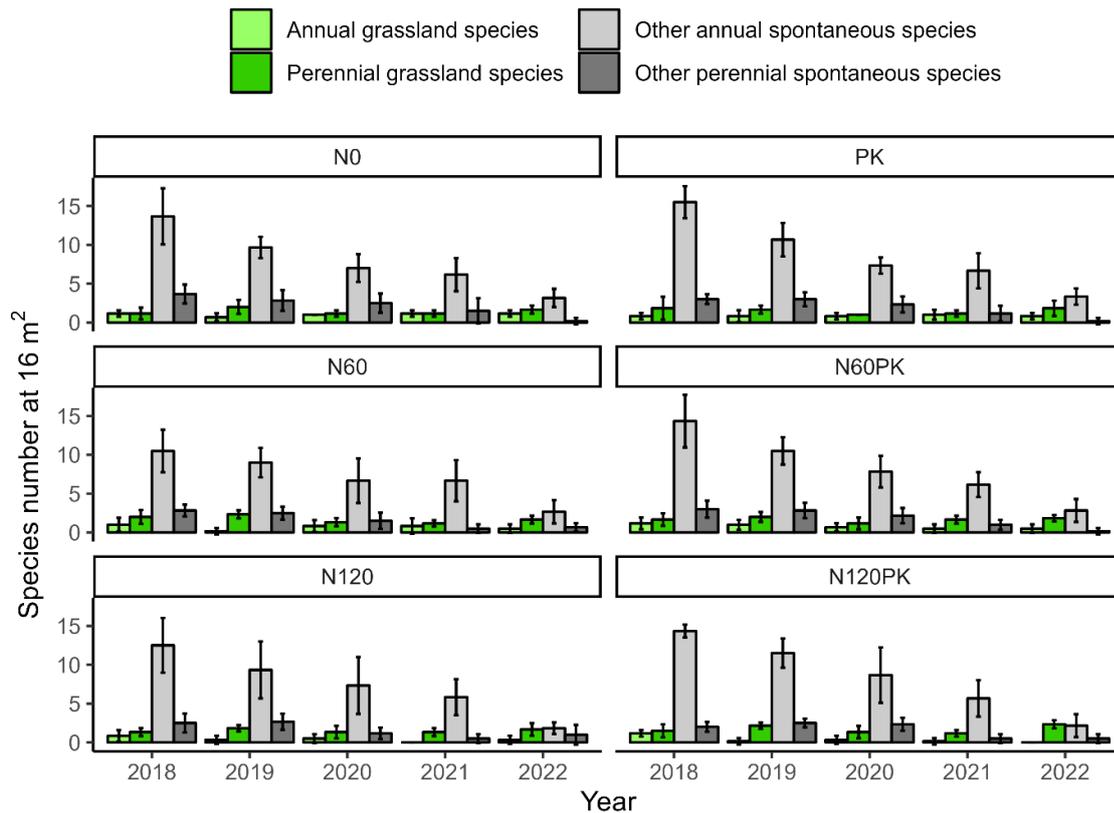


Fig. 2. Mean species number at 16 m² plot of spontaneous grassland species and other spontaneous species in different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

Cover of sown grasses, sown forbs, and spontaneous species

Sown species cover increased significantly from 2018–2022 (Figure 3, Table 2). Compared to the control (N0), nitrogen application resulted in significantly higher cover of sown species in 2020 and 2021 regardless of fertilizer level (Table A.10). However, in 2022, this effect was no longer detectable (Table A.10).

In the first year after seeding, the cover of sown grasses and sown forbs was similar for all treatments (Figure 3). While the cover of the sown grasses initially increased in all fertilizer treatments from 2018 to 2019, it benefited significantly from fertilization since 2020 (Figure 3, Table A.11, A.13). This trend continued through 2022 and resulted in higher cover of sown grasses as nitrogen intake increased. The addition of the nutrients P and K did not result in differences within a nitrogen fertilizer level (Table A.13).

In contrast to the sown grasses, sown forbs significantly decreased in cover from 2018 to 2019 in all fertilizer treatments (Figure 3, Table A.14, A.16). At the end of the observation period, sown forb cover of non-nitrogen fertilized treatments (N0, PK) was significantly higher compared to all other treatments (Figure 3, Table A.16), mainly caused by the increase of legumes, especially *Trifolium pratense*, and *Centaurea jacea*.

In 2022, the sown grasses with the highest cover were *Arrhenatherum elatius*, *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Poa pratensis*, and *Dactylis glomerata*, and the sown forbs with the highest cover were *Achillea millefolium*, *Centaurea jacea*, *Trifolium pratense*, and *Trifolium repens* (Table A.29).

The cover of spontaneously established species decreased significantly from 2020 onwards and was almost non-existent by the end of the observation period in all fertilizer treatments (Figure 3, A.6, Table 2, A.18).

Table 2

Effects of LMM testing of fertilizer (six-level factor), and year (five-level factor) on species number and the cumulative cover of sown grasses, sown forbs, and spontaneous species, and total dry matter yield of sown grasses and sown forbs of the first and second cuts. Results of the pairwise comparisons can be found in Table A.4–A.28.

Response variables	Fertilizer		Year		Fertilizer x year	
	F _{df}	p	F _{df}	p	F _{df}	p
Species number						
Sown species*	.	.	37.559 _{4, 140}	<0.001	.	.
Sown grasses	.	.	18.056 _{4, 140}	<0.001	.	.
Sown forbs	1.623 _{5, 30}	0.184	26.833 _{4, 120}	<0.001	0.947 _{20, 120}	0.529
Spontaneous species*	.	.	141.510 _{4, 140}	<0.001	.	.
Cumulative cover						
Sown species	8.232 _{5, 36}	<0.001	113.054 _{4, 144}	<0.001	2.328 _{20, 144}	0.002
Sown grasses	30.502 _{5, 36}	<0.001	252.450 _{4, 144}	<0.001	12.220 _{20, 144}	<0.001
Sown forbs**	4.165 _{5, 36}	0.004	24.200 _{4, 144}	<0.001	7.759 _{20, 144}	<0.001
Spontaneous species**	0.970 _{5, 36}	0.448	512.577 _{4, 144}	<0.001	4.145 _{20, 144}	<0.001
Dry matter yield						
Total	8.066 _{5, 36}	<0.001	305.775 _{4, 144}	<0.001	.	.
Sown grasses 1 st cut	21.448 _{5, 36}	<0.001	262.332 _{4, 144}	<0.001	.	.
Sown forbs 1 st cut**	0.882 _{5, 36}	0.502	485.223 _{4, 144}	<0.001	5.525 _{20, 144}	<0.001
Sown grasses 2 nd cut**	9.609 _{5, 36}	<0.001	81.398 _{4, 144}	<0.001	2.396 _{20, 144}	0.001
Sown forbs 2 nd cut**	.	.	30.817 _{4, 144}	<0.001	.	.

* log-transformed, ** logit-transformed

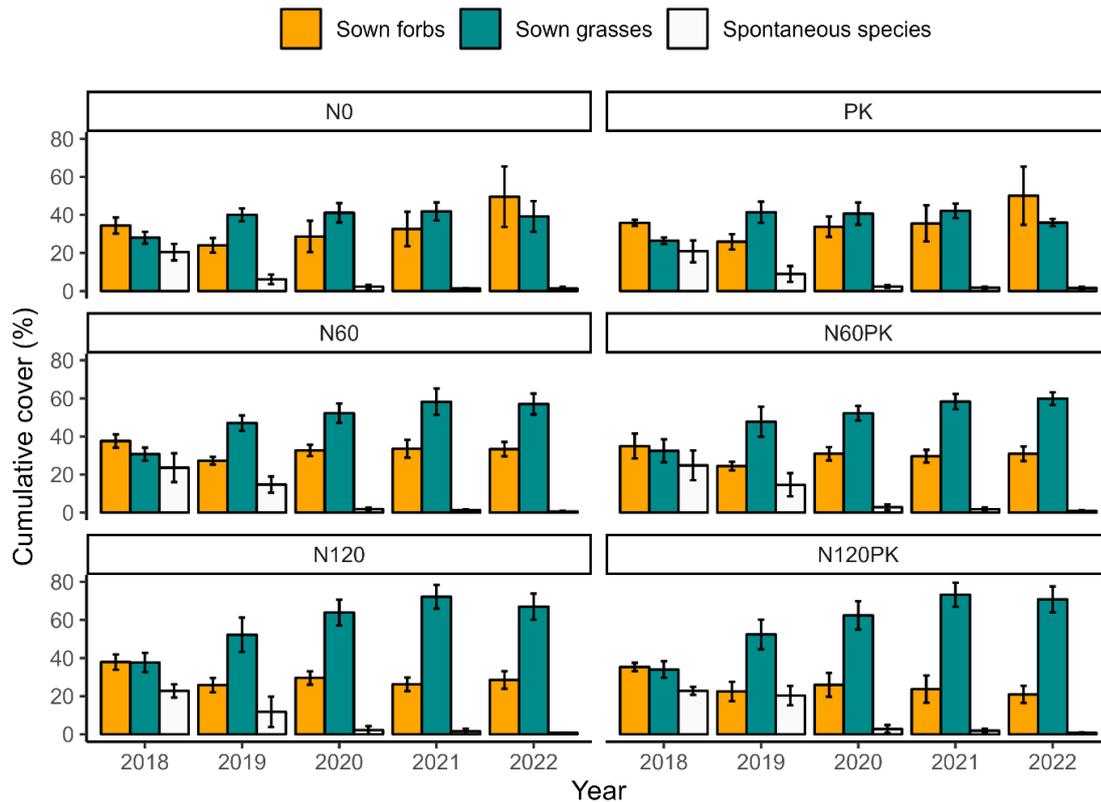


Fig. 3. Mean cumulative cover of sown grasses, sown forbs and spontaneously established species in different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

Dry matter yield of sown grasses and sown forbs

In the first year after seeding, dry matter yield (DMY) was significantly higher compared to all other years, then decreased in subsequent years and showed lowest values in 2020 (Figure 4, Table A.19). The non-nitrogen fertilized treatments (N0, PK) had a lower DMY compared to nitrogen fertilized treatments, but only differences to N120 and N120PK and the difference between PK and N60PK were significant (Figure 4, Table A.20). DMY was strongly determined by year (Table A.19), and thus precipitation. In the last observation year, the nitrogen-fertilized treatments (N60, N60PK, N120, N120PK), in contrast to the non-nitrogen-fertilized (N0, PK), could not reach the previous year's level. In all five years of observation, the DMY of sown grasses of the first cut was higher than that of the second cut (Figure 5). Sown grasses dominated the DMY of the first cut for all treatments, but showed significant differences for all years (Figure 5, Table 2, Table A.21). After a sharp decline from 2018 to 2020, DMY rebounded in 2021 and remained in the same range in 2022, but did not return to 2019 levels. N0 and PK showed significantly lower DMY of sown grasses than nitrogen fertilized treatments, while N60 and N60PK had lower DMY than N120 (Table A.22). The sown forbs responded to the first cut over time in the same way as the grasses (Figure 5, Table 2, Table A.23), but treatments N0, PK, N60, and N60PK exceeded the 2019 DMY by the end of the observation period in 2022. DMY of the second cut declined equally for both functional groups

from 2018 to 2019 and remained below $10 \text{ dt ha}^{-1} \text{ yr}^{-1}$ in 2019, 2020, and 2022. The functional groups responded oppositely in 2021, with significantly higher DMY for the sown grasses in the nitrogen fertilized treatments N60PK, N120, and N120PK (Table A.27), but higher DMY for the sown forbs in the N0 and PK treatments. Only six of the 44 sown species yielded more than $5 \text{ dt ha}^{-1} \text{ yr}^{-1}$ from the second year after sowing. Of these, *Arrhenatherum elatius* was the most productive species at the first cut, with its DMY increasing again after a sharp decline in 2020 and clearly benefiting from nitrogen fertilization (Figure 6). For the second cut, the DMY dropped sharply and did not peak until 2021. Over time, *Alopecurus pratensis*, *Dactylis glomerata*, and *Poa pratensis* showed a similar response, but at a lower DMY level (Figure 6). In contrast to the grasses, *Centaurea jacea* had a higher DMY on the second cut in 2019–2021 and tended to benefit when no nitrogen was applied (Figure 6). *Trifolium pratense* achieved significant DMY in the first cut, but only in the first year after sowing, while the treatments N0 and PK, which were not fertilized with nitrogen, reached maximum values in 2022 in the first cut and 2021 in the second cut (Figure 6).

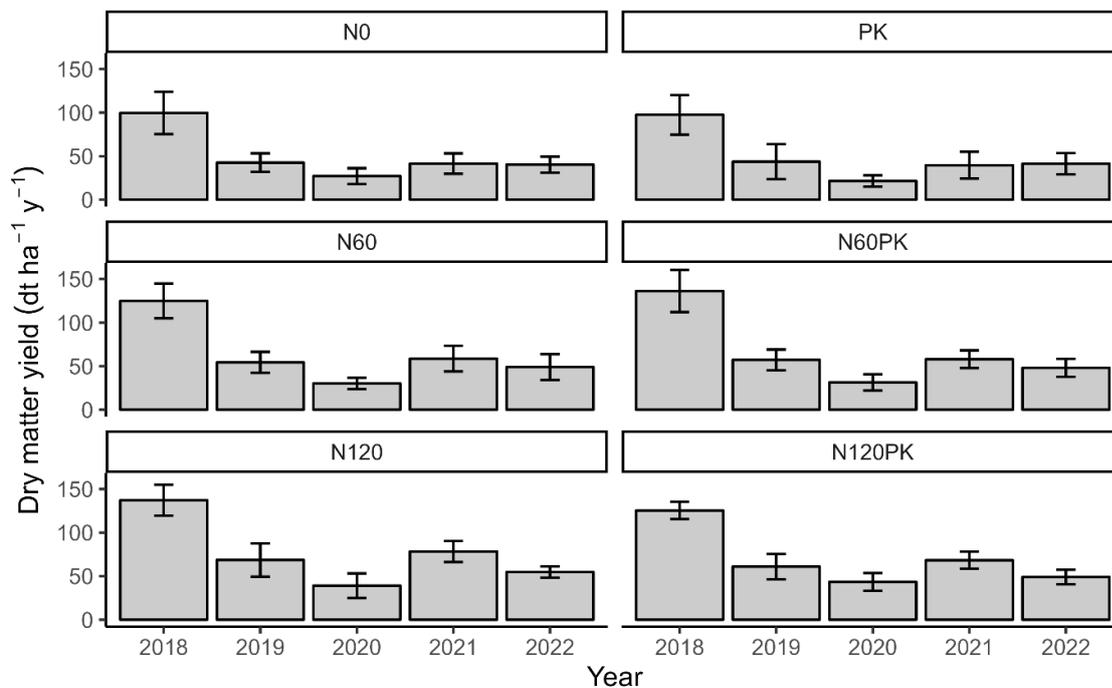


Fig. 4. Mean total dry matter yield in different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

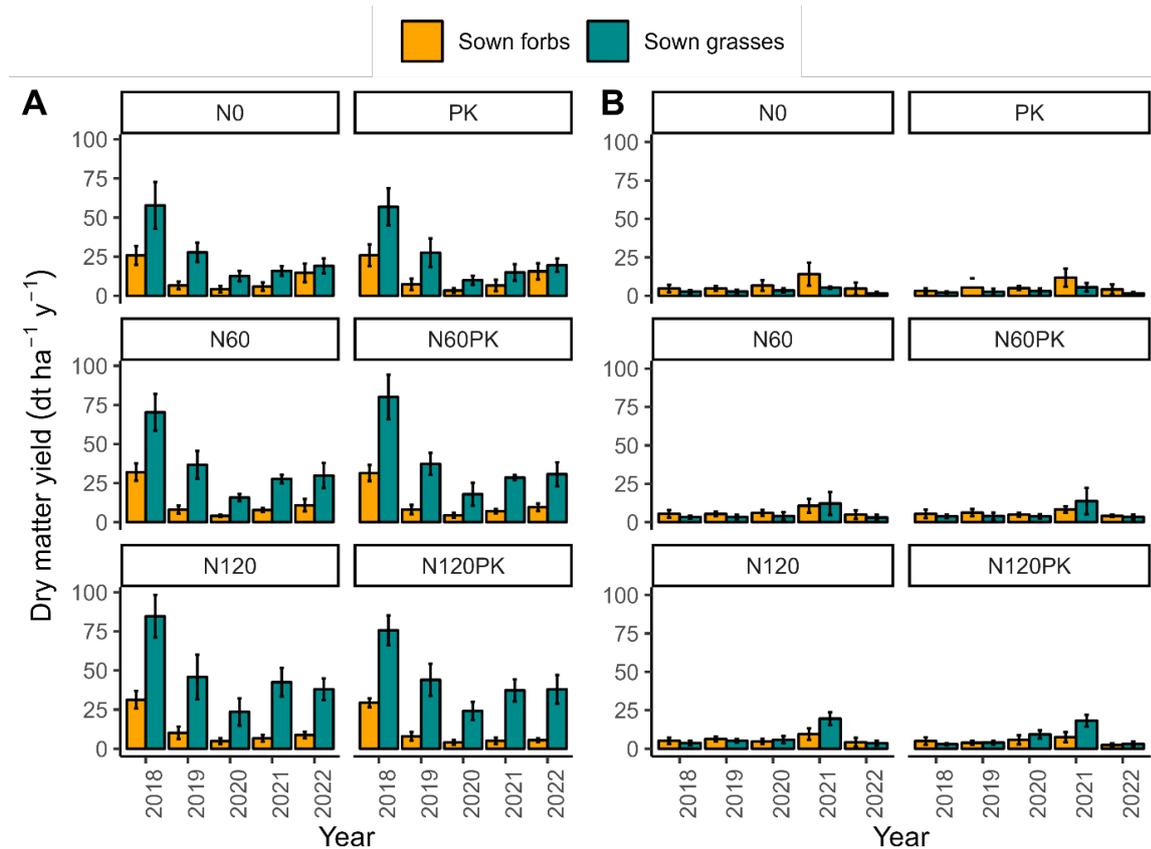


Fig. 5. Mean dry matter yield of sown grasses and sown forbs for the first cut (A) and second cut (B) in different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022. Whiskers refer to standard deviation. Yields of spontaneous species were between 8.6 and 15.5 dt ha⁻¹ y⁻¹ in 2018, and between 0.01 and 0.68 dt ha⁻¹ y⁻¹ during the rest of observation period and are not shown.

Discussion

Establishment success of native seed mixture and effect of fertilization

With 44 plant species, the native seed mixture used can be classified as highly diverse (Kirmer et al., 2012b; Klaus et al., 2017; Schaub et al., 2021). The present vegetation is dominated by sown species with its species number at a level typical for mesic grassland on ex-arable land following the seeding of a highly diverse seed mixture (Stevenson et al., 1995; Prach et al., 2014). Only five species did not establish (*Alchemilla vulgaris*, *Anthriscus sylvestris*, *Campanula rotundifolia*, *Heracleum sphondylium*, *Hypericum maculatum*). The species assembly was dominated by target species of the Arrhenatherion over the entire study period, and undesirable species could not immigrate, indicating a priority effect of sown target species (Weidlich et al., 2021). However, as the study site is isolated and surrounded by arable fields, no target species other than those sown emerged. Hence, sowing species-rich mixtures is particularly important in areas where target species do not occur in the surrounding or in the soil seed bank and cannot migrate due to a lack of habitat connectivity (Török and Helm, 2017; Lyons et al., 2023). The seed germinated successfully in all experimental plots, reaching a high establishment rate already in the first year. Most probably, the following reasons were

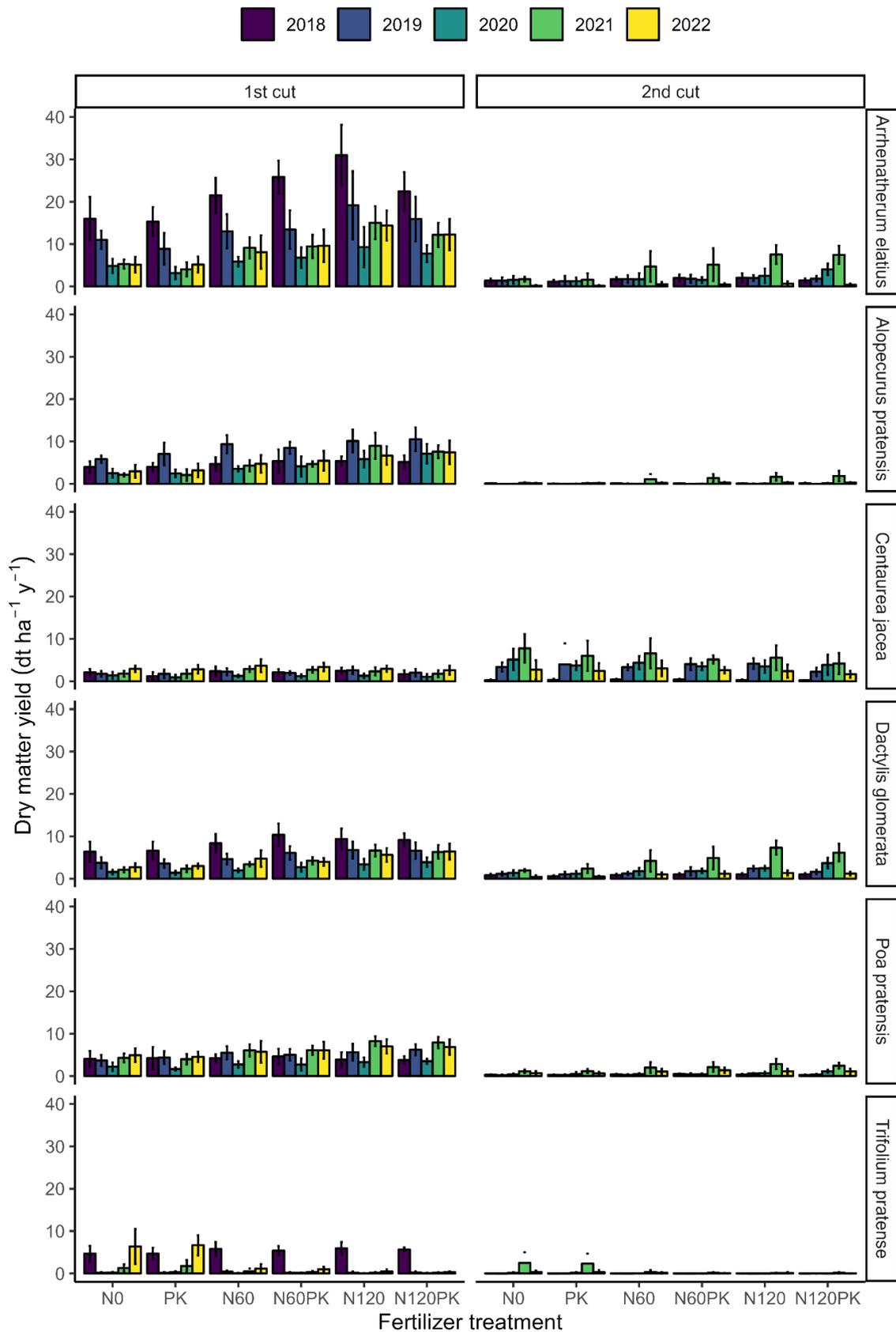


Fig. 6. Dry matter yield of the first and second cuts in different fertilizer treatments (abbreviations see Table 1) between 2018 and 2022 for the six species with $> 5 \text{ dt ha}^{-1} \text{ y}^{-1}$ in 2019–2022. Whiskers refer to standard deviation.

responsible. The average rainfall in 2017, as well as near-average rainfall in March and April 2018 promoted germination and early establishment. In addition, the sown nurse crop obviously had direct facilitative effects by providing shade and enhancing soil moisture (Callaway, 2007), which is important for seedlings when environmental conditions are severe (Wright et al., 2014). However, as all treatments were subject to the same conditions, the facilitative effect, which might be important driver of community assembly (Weidlich et al., 2021), could not be systematically tested. The need of facilitators, particularly in changing climate conditions, should be investigated in future grassland studies. Mainly due to decreasing frequencies of mesic and wet grassland species (Table A.30), the number of sown species decreased slightly from the first to the second year after sowing, most probably caused by the severe drought in 2018. Tilman and El Haddi (1992) as well as Ghermandi and Gonzalez (2009) also found that drought can negatively affect species richness. In the Hayn grassland experiment, despite continued below-average rainfall, sown species richness stabilized or even increased in subsequent years. Surprisingly, fertilization had no significant effect on the number of successfully established sown species, which according to Rajaniemi (2008) would have been expected due to competition effects, even when considering the functional groups separately. This is in contrast to John et al. (2016) who found that nitrogen fertilization impaired first-year-success of their sown target species, when restoring a degraded lowland hay meadow after sward disturbance. While the NutNet study indicated species loss at NPK fertilization rates of $100 \text{ kg ha}^{-1} \text{ y}^{-1}$ per nutrient (Borer et al., 2014; Ladouceur et al., 2022), persistence of target species has been demonstrated for ancient alluvial grasslands at fertilizer rates up to $120 \text{ kg N ha}^{-1} \text{ y}^{-1}$ regardless of P and K application (Dullau et al., 2021). Since high potassium contents are compatible with high values of diversity, but the phosphorus content in the soil is not in an optimal range for species-rich stands (Janssens et al., 1998), an important condition for the maintenance of species richness is not present, and the effects of increasing grass cover on nitrogen fertilized plots must be observed in the following years. Competition is a key factor affecting the performance and co-existence of species and is influenced by environmental conditions and their variations (Johnson et al., 2008). Nitrogen addition significantly increases the aboveground biomass of grasses (You et al., 2017), and thus competition ability (Michalet et al., 2022), in our study namely *Arrhenatherum elatius*, *Alopecurus pratensis*, *Dactylis glomerata* and *Poa pratensis*. However, it should be noted that grass species can react with a different competition intensity (DiTomasso and Aarssen, 1991). Starting in the third year after sowing, fertilization significantly affected functional group coverage. The observed increased promotion of grass cover by increasing nitrogen application is consistent with the results of several studies on ancient grasslands (Kacorzyk and Głęb, 2017; Dindová et al., 2019; Ranta et al., 2021). Contrary to the findings of Hejzman et al. (2014), *Anthoxanthum odoratum* and *Festuca rubra*, which are typical species of nutrient-poor and summer-dry sites (Schubert, 2001; Jäger, 2017), did not respond negatively to fertilization in our study, which could be due to the precipitation deficit that prevailed throughout the study period. Although water deficiency reduces nitrogen uptake (Arfin Khan et al., 2014), precipitation appears to have been sufficient to absorb the applied nitrogen and, combined with the high drought recovery potential of temperate grasses (Hahn et al., 2021), provide increasing cover. The negative response of the

sown forb cover from the first to the second year after sowing is related to the largest annual precipitation deficit of the observation period and highlights their sensitivity in the early stages of vegetation development. As already confirmed for calcareous grassland (Bobbink et al., 1988), mountain grassland (Pecháčková et al., 2010), and alluvial grassland (Hejcman et al., 2014; Dullau et al., 2021), forb cover benefited from the absence of nitrogen supply, resulting in a cover balance between grasses and forbs due to reduced grass competition. In this context, it is also worth considering the overall increased phosphorus content in the soil, which can be attributed to the precipitation deficit (Delgado-Baquerizo et al., 2013; Mariotte et al., 2020). Interestingly, increases in phosphorus occurred even when no phosphorus was fertilized. Sylvain and Wall (2011) noted that plant and soil biome diversity are closely linked, and it is conceivable that a more diverse soil biome may release nutrients, although this needs further investigation. The interaction of omitted nitrogen fertilization and improved phosphorus availability promoted the sown legumes (see Figure 6 *Trifolium pratense*), thus initiating the legume cycle known from grasslands (Schwinning and Parsons, 1996, Herben et al., 2017). The observed steady decline in the number and cover of spontaneous species, including mainly weedy, short-lived species, represents a typical development under mowing (Török et al., 2010; Kaulfuß et al., 2022). The fact that some annual spontaneous species (among them *Veronica arvensis*, *Trifolium dubium*, *Vicia hirsuta*, and *V. segetalis*) regularly persisted in the stand even in the fifth year after sowing may be attributed to the early stage of vegetation development, the poorly developed litter layer (Figure A.7), and to drought leading to gaps in the stand.

Effect of fertilization on aboveground biomass production

The high DMY determined in the first year after seeding equaled or even exceeded the values known for fertilized Arrhenatherion grassland (overview in Tischew and Hölzel, 2019). This had already been observed for highly diverse seed mixtures on former arable land by Lepš et al. (2007). The precipitation in spring 2018 appears to have been sufficient for the high DMY at first cutting. In addition, plowing directly before sowing in summer 2017 released nutrients present in the soil (Lal et al., 2007) and were not removed by mulching the nurse crop. Following Donath et al. (2021) and Hölzel and Tischew (2019), in subsequent years, except 2020, DMY production was still at expected levels despite the drought. Overall, the DMY of the first cut was important for hay production, whereas a balanced distribution of DMY is more characteristic of twice mown meadows with average precipitation (Boob et al., 2019). A significant increase of the first cut's DMY could only be significantly improved by the highest nitrogen fertilizer rates (N120, N120PK).

Since the focus was not only to establish a species-rich grassland, but also to provide aboveground biomass for animal feed, a large number of grasses were included in the mixture. These, in agreement with You et al. (2017), were able to slightly increase DMY with increasing levels of nitrogen application. Van Sundert et al. (2021) found in the European-wide NutNet experiment that nutrient addition amplified detrimental drought effects on community aboveground biomass production, mainly caused by the negative effect on graminoids. The

dominance of drought-sensitive grasses over forbs should therefore be avoided, which should already be taken into account when the seed mixture is composed.

In our experiment, the perennial grasses *Alopecurus pratensis*, *Dactylis glomerata*, *Poa pratensis*, and especially *Arrhenatherum elatius*, were critical to the DMY in the first cut. Since *A. elatius* is a widespread and agriculturally important European grass species (Michalski et al., 2010) and characteristic of the Arrhenatherion (Schubert, 2001), its suitability for use in seed mixtures under drought conditions is of particular interest. In a rainfall reduction experiment, *A. elatius* subjected to drought had more tillering and more biomass per individual than the non-stressed ones (Otieno et al., 2012) and reached a higher reproductive fitness when exposed to drought compared to control (Gellesch et al., 2017). Hoover et al. (2014) also found increased aboveground net primary production of grasses one year after a two-year extreme drought period, which was associated with a 60% increase in plant density.

Van Sundert et al. (2021) noticed that drought has no effect on overall forb biomass production. By contrast, in our experiment we observed that the forb yield increased in dry years. In this respect, it is interesting to consider the two cuts separately. The non-nitrogen fertilized (N0, PK) to moderately nitrogen-fertilized (N60, N60PK) treatments showed an increase in DMY in the important first cut despite drought. Interestingly, *Trifolium pratense* benefited only in the non-nitrogen fertilized treatments, where it contributed substantially to the DMY increase of forbs in the fifth year of observation. This confirms the evidence of Spehn et al. (2002) that good phosphorus availability in the soil facilitates the legume effect on biomass production, but in agreement with Dullau et al. (2021) only when no nitrogen is fertilized. Because *T. pratense* efficiently fixes atmospheric nitrogen (Fustec et al., 2010), it could increase biomass production in the future without artificial nitrogen fertilization.

Conclusion

The present results of our five-year study showed that the restoration of submontane species-rich grassland by seeding native species on ex-arable land is possible even under below-average precipitation and independent of the tested fertilizer levels. Our results provide evidence that nitrogen application increases biomass provision as a delivering service, but does not affect productivity recovery in several years of drought. Since nitrogen fertilization shifts the vegetation composition in favor of grasses, and a balanced grass-forb cover ratio and legume cycle cannot be established, it is necessary to weigh which ecosystem services are of primary interest. Due to the significant increasing dominance of grasses in the treatments with the highest nitrogen fertilization of $120 \text{ kg ha}^{-1} \text{ y}^{-1}$, it is questionable how long the richness of the sown species can be maintained under this treatment beyond the five-year study period. To reconcile farmers' demands on biomass for hay production with the nature conservation goals, we recommend the moderate nitrogen fertilization of $60 \text{ kg ha}^{-1} \text{ y}^{-1}$ as a maximum. Importantly, our results revealed that six plant species characteristic of the Arrhenatherion, including the four grasses *Alopecurus pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata* and *Poa pratensis*, the forb *Centaurea jacea* and the legume *Trifolium pratense*, appear to be suitable for sustaining productivity over several drought years. They

should therefore always be included in high-diverse native seed mixtures. However, the effect of *Trifolium pratense* will be constrained if nitrogen application is intended. We assume that, for different subclimatic regions, other species have to be defined as matrix species for seed mixtures. Therefore, we recommend expanding research in grassland restoration in this regard. As many other species have also contributed to biomass production under drought conditions and it is also important to provide diverse pollen and nectar services, species-rich seed mixtures should be used when restoring grassland.

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CRedit authorship contribution statement

S.D. and S.T. designed the study; S.D. planned and undertook the fieldwork, M.H.M. was involved in the fieldwork; M.H.M. organized data entry and undertook data management; S.D. and A.S. analyzed the data, under supervision of S.T. and A.K.; S.D. wrote the manuscript with contributions by all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.gecco.2023.e02730.

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Appendix

Only selected figures and tables are shown. All supplementary materials can be downloaded at <https://ars.els-cdn.com/content/image/1-s2.0-S2351989423003657-mmc1.docx>

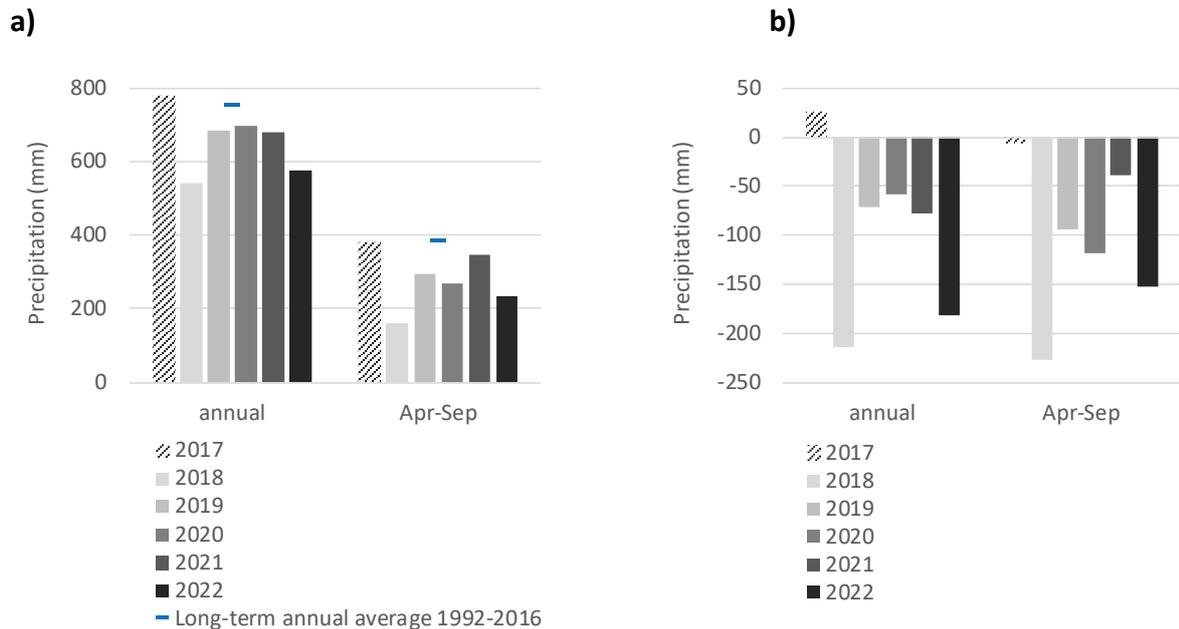


Fig. A.1 Annual and April–September precipitation for 2017–2022 (a) and relative to the long-term average for the period 1992–2016 (b). April to September is defined as the growing season for the experiment site. All data were measured with the weather station of the state agency for agriculture and horticulture Saxony-Anhalt in Hayn. The annual deviation was +3.3% in 2017, -28.3% in 2018, -9.4% in 2019, -7.8% in 2020, -10.3% in 2021, and -24.0% in 2022. The April–September deficit was -1.6% in 2017, -58.4% in 2018, -24.1% in 2019, -30.4% in 2020, -10.0% in 2021, and -39.4% in 2022.

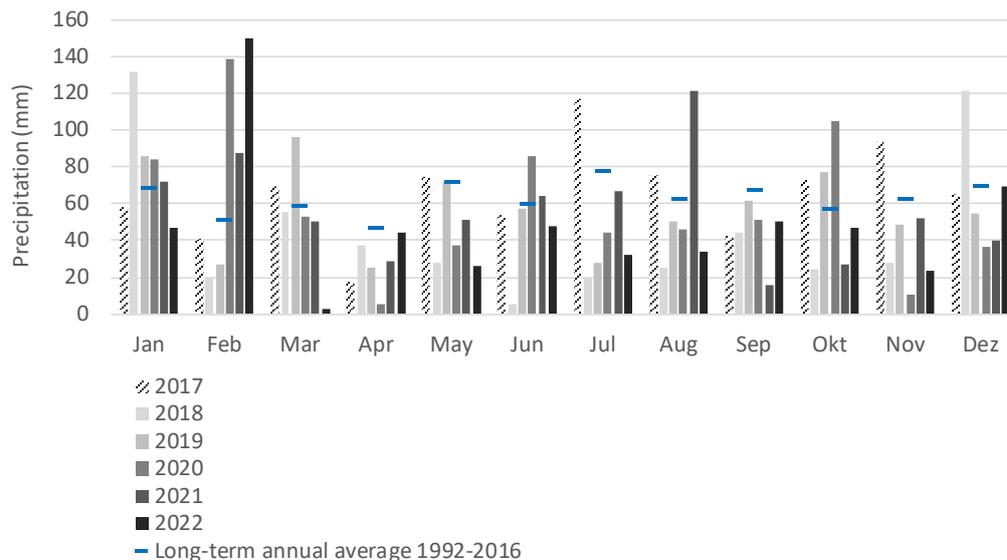


Fig. A.2. Monthly precipitation for the years 2017–2022 and long-term annual average for the period 1992–2016. All data were measured with the weather station of the state agency for agriculture and horticulture Saxony-Anhalt in Hayn.

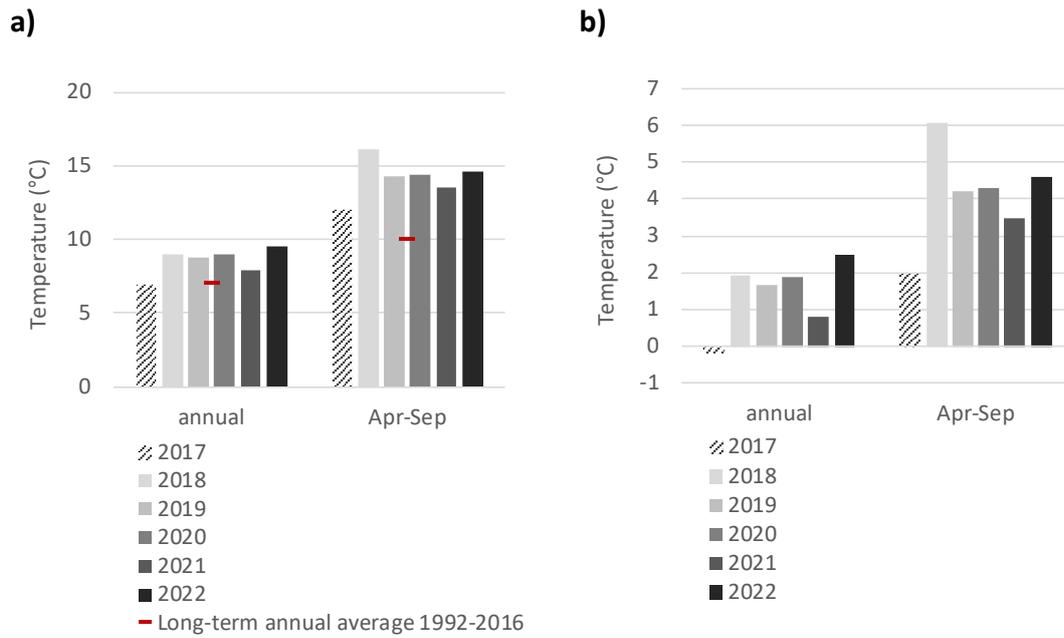


Fig. A.3 Mean annual and April–September temperature for the years 2017–2022 (a) and relative to the long-term average for the period 1992–2016 (b). April to September is defined as the growing season for the experiment site. All data were measured with the weather station of the state agency for agriculture and horticulture Saxony-Anhalt in Hayn.

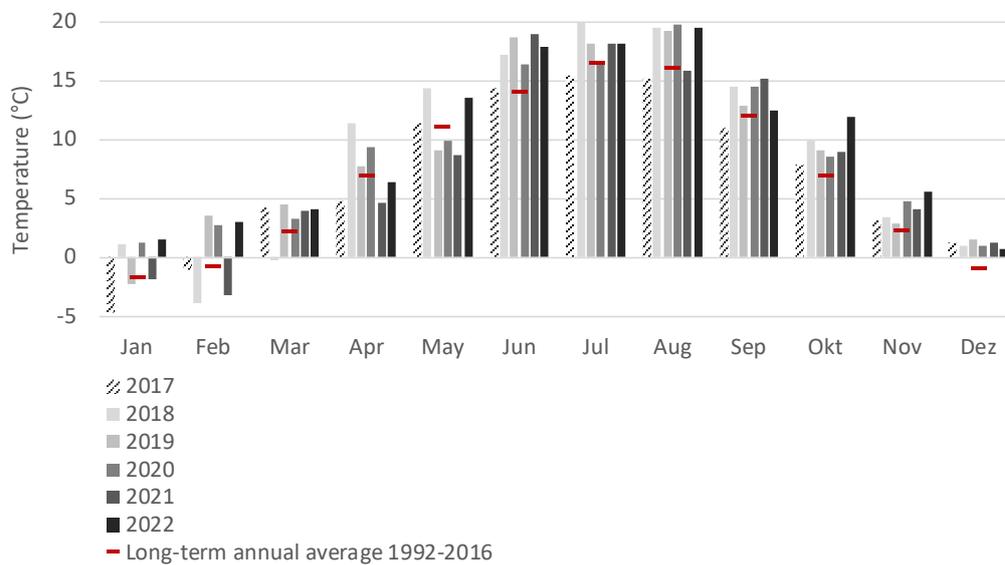


Fig. A.4 Monthly temperature for the years 2017–2022 and long-term annual average for the period 1992–2016. All data were measured with the weather station of the state agency for agriculture and horticulture Saxony-Anhalt in Hayn.

Table A.1

Seed mixture used for the re-creation of a mesic submontane Arrhenatherion grassland.

*Species did not establish until 2022. a = annual, b = biennial, p = perennial.

Species	Life span	Thousand seed weight	Number of seeds per m ²	g per m ²	Proportion by weight
Native species					
Grasses					40.7%
<i>Alopecurus pratensis</i> L.	p	0.750	60	0.0450	2.4%
<i>Anthoxanthum odoratum</i> L.	p	0.600	100	0.0600	3.2%
<i>Arrhenatherum elatius</i> (L.) J. Presl et C. Presl	p	2.700	60	0.1620	8.7%
<i>Cynosurus cristatus</i> L.	p	0.500	50	0.0250	1.3%
<i>Dactylis glomerata</i> Horv.	p	1.000	25	0.0250	1.3%
<i>Festuca pratensis</i> Huds.	p	0.800	60	0.0480	2.6%
<i>Festuca rubra</i> L.	p	0.800	150	0.1200	6.4%
<i>Helictotrichon pubescens</i> (Huds.) Pilg.	p	2.100	60	0.1260	6.7%
<i>Holcus lanatus</i> L.	p	0.400	30	0.0120	0.6%
<i>Luzula campestris</i> (L.) DC.	p	0.730	100	0.0730	3.9%
<i>Poa pratensis</i> L.	p	0.320	100	0.0320	1.7%
<i>Trisetum flavescens</i> (L.) P. Beauv.	p	0.260	130	0.0338	1.8%
Forbs					41.5%
<i>Achillea millefolium</i> L.	p	0.200	80	0.0160	0.9%
<i>Alchemilla vulgaris</i> L. s. str.*	p	0.500	80	0.0400	2.1%
<i>Anthriscus sylvestris</i> L.*	p	4.000	5	0.0200	1.1%
<i>Campanula patula</i> L.	p	0.040	150	0.0060	0.3%
<i>Campanula rotundifolia</i> L.*	p	0.060	150	0.0090	0.5%
<i>Cardamine pratensis</i> L. s. str.	p	0.570	100	0.0570	3.0%
<i>Centaurea jacea</i> L. s. str.	p	2.100	25	0.0525	2.8%
<i>Cerastium holosteoides</i> Fr.	p	0.100	50	0.0050	0.3%
<i>Crepis biennis</i> L.	b	0.800	20	0.0160	0.9%
<i>Daucus carota</i> L.	p	1.000	35	0.0350	1.9%
<i>Galium album</i> Mill.	p	0.600	35	0.0210	1.1%
<i>Heracleum sphondylium</i> L.*	p	5.500	4	0.0220	1.2%
<i>Hypericum maculatum</i> Crantz*	p	0.100	70	0.0070	0.4%
<i>Knautia arvensis</i> (L.) J.M. Coult. S. str.	p	4.700	20	0.0940	5.0%
<i>Leontodon hispidus</i> L.	p	1.400	35	0.0490	2.6%
<i>Leucanthemum vulgare</i> agg.	p	0.400	80	0.0320	1.7%
<i>Lychnis flos-cuculi</i> L.	p	0.150	100	0.0150	0.8%
<i>Pimpinella major</i> (L.) Huds.	P	1.700	20	0.0340	1.8%
<i>Plantago lanceolata</i> L.	p	1.600	20	0.0320	1.7%
<i>Prunella vulgaris</i> L.	p	0.700	40	0.0280	1.5%
<i>Ranunculus acris</i> L.	p	1.600	20	0.0320	1.7%
<i>Rumex acetosa</i> L.	p	0.550	8	0.0044	0.2%
<i>Sanguisorba officinalis</i> L.	p	2.000	40	0.0800	4.3%
<i>Saxifraga granulata</i> L.	p	0.020	120	0.0024	0.1%
<i>Scorzoneroideis autumnalis</i> (L.) Moench	p	0.700	25	0.0175	0.9%
<i>Tragopogon pratensis</i> L. s. str.	b	7.000	5	0.0350	1.9%
<i>Veronica chamaedrys</i> L. s. str.	p	0.250	60	0.0150	0.8%
Legumes					17.8%
<i>Lathyrus pratensis</i> L.	p	11.000	8	0.0880	4.7%
<i>Lotus corniculatus</i> L.	p	1.200	65	0.0780	4.2%

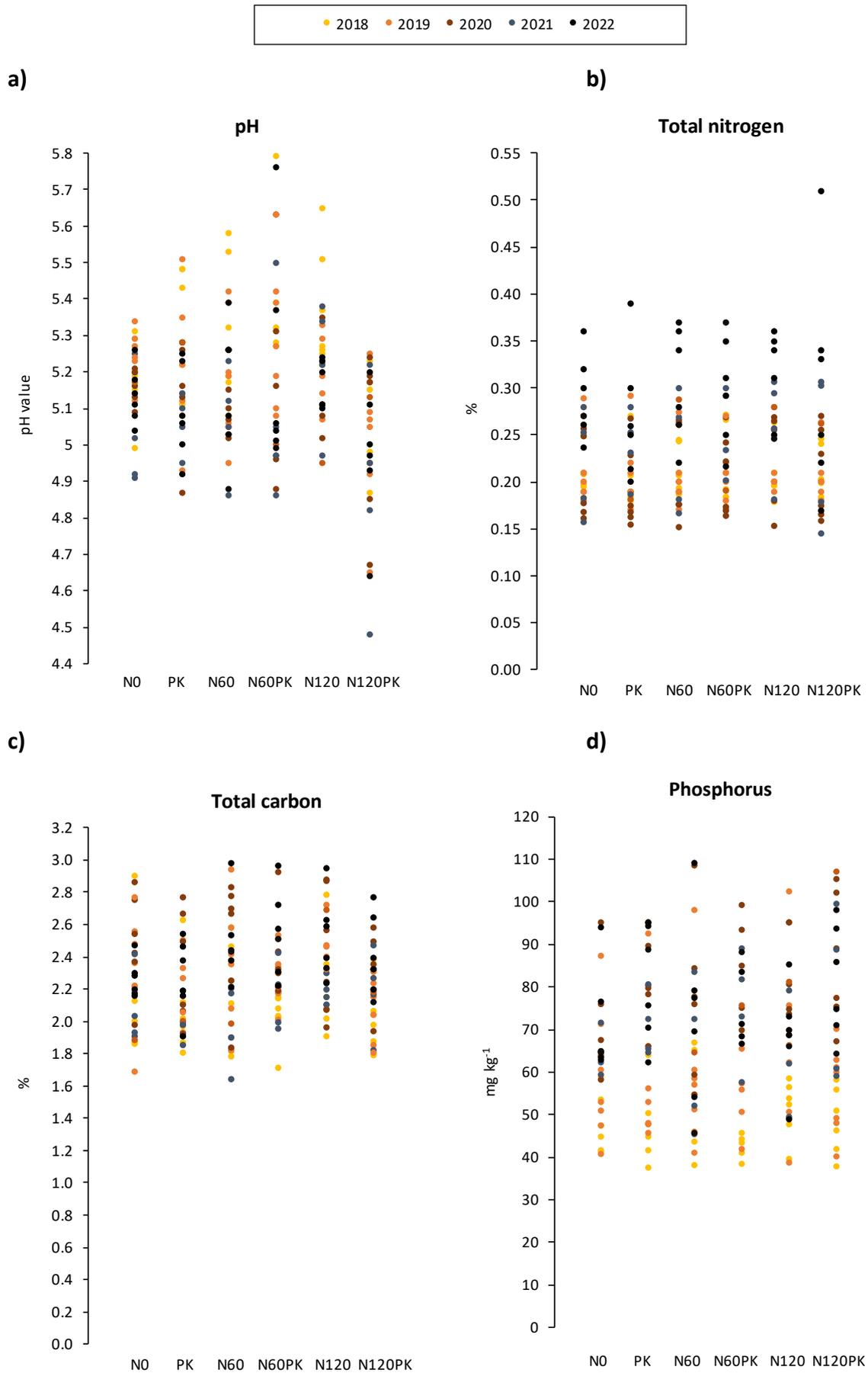
Continuation of Table A.1

Species	Life span	Thousand seed weight	Number of seeds per m ²	g per m ²	Proportion by weight
<i>Trifolium pratense</i> L.	p	1.800	8	0.0144	0.8%
<i>Trifolium repens</i> L.	p	0.690	3	0.0021	0.1%
<i>Vicia sepium</i> L.	p	50.000	3	0.1500	8.0%
Number of species		44			
Number of seeds			2409		
Sowing density g per m ²				1.8711	
Nurse crop with cultivars					
<i>Camelina sativa</i> (L.) Crantz	a	1.000	667	0.6667	33%
<i>Lepidium sativum</i> L.	a	7.000	95	0.6667	33%
<i>Fagopyrum esculentum</i> Moench	a	16.000	42	0.6667	33%
Number of species		3			
Number of seeds			804		
Sowing density g per m ²				2.0000	

Table A.2

Mean soil chemical characteristics (sampling depth 0–10 cm) with standard deviation (in brackets) in March 2018 before the experiment started.

Treatment	pH value	Phosphorus (mg kg ⁻¹)	Potassium (mg kg ⁻¹)	Magnesium (mg kg ⁻¹)	Total carbon (%)	Total nitrogen (%)
N0	5.18 (0.12)	47.80 (6.94)	155.21 (27.78)	58.53 (7.79)	2.15 (0.39)	0.21 (0.03)
PK	5.31 (0.18)	47.64 (9.11)	169.28 (49.67)	64.10 (10.26)	2.07 (0.30)	0.21 (0.04)
N60	5.31 (0.20)	52.28 (11.89)	173.99 (48.58)	58.98 (5.25)	2.17 (0.33)	0.21 (0.02)
N60PK	5.39 (0.20)	54.77 (30.64)	171.03 (35.42)	64.80 (16.63)	2.02 (0.16)	0.21 (0.04)
N120	5.39 (0.16)	51.28 (6.86)	165.98 (44.97)	60.22 (7.13)	2.22 (0.32)	0.21 (0.03)
120PK	5.10 (0.14)	48.45 (7.96)	154.31 (40.15)	58.65 (5.08)	2.02 (0.21)	0.21 (0.03)
Mean	5.28 (0.19)	50.37 (13.80)	164.97 (39.47)	60.88 (9.22)	2.11 (0.28)	0.21 (0.03)



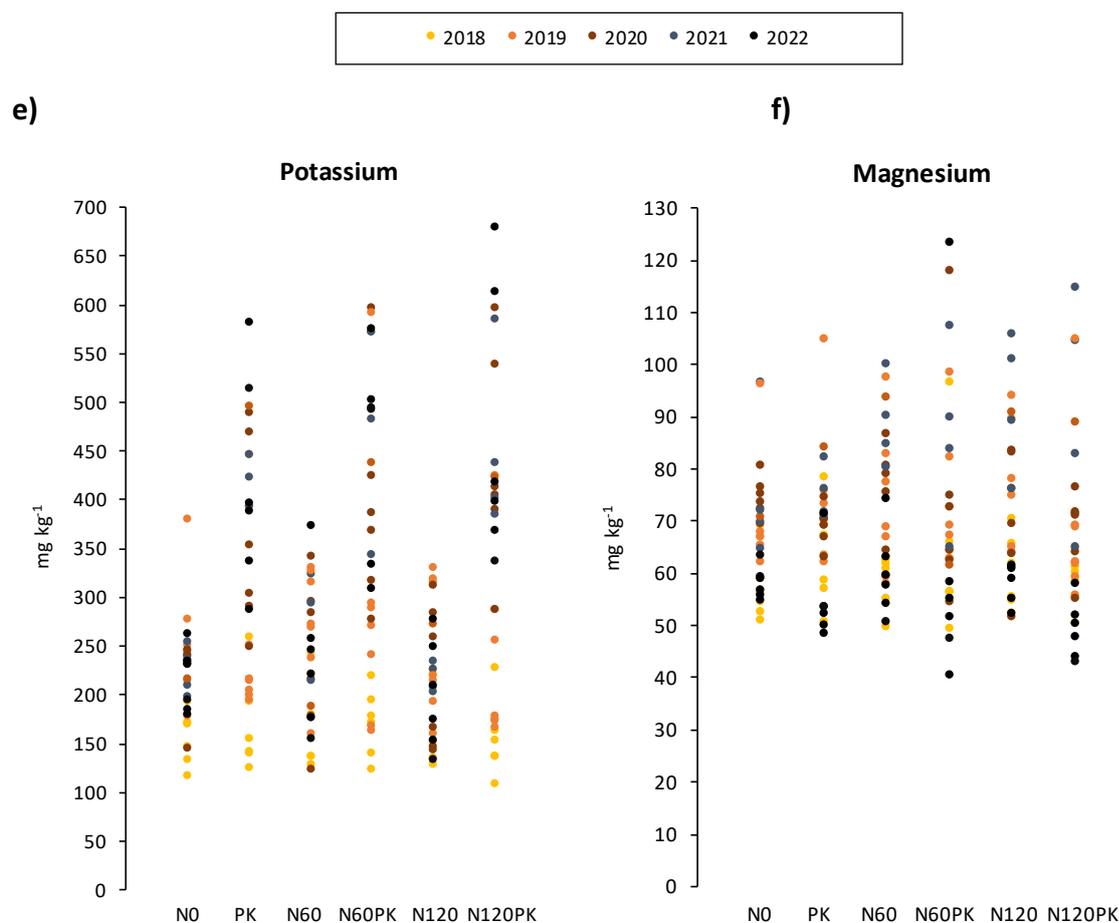


Fig. A.5 Soil chemical characteristics of fertilization treatments (abbreviations see Article: Table 1) during the study period 2018–2022. Phosphorus values without twice mown plot N60PK repetition no. 3.

Table A.3

Mean establishment rate (%) (standard deviation in brackets) of the sown native seed mixture between 2018 and 2022.

Treatment	2018	2019	2020	2021	2022
NO	77.7 (5.3)	65.5 (9.5)	68.6 (7.4)	70.1 (6.7)	69.7 (5.7)
PK	75.4 (2.2)	67.4(5.7)	68.9 (9.1)	69.7 (7.0)	67.8 (6.8)
N60	76.9 (4.4)	67.0 (4.3)	71.2 (5.9)	70.5 (3.2)	70.1 (3.3)
N60PK	76.9 (2.2)	68.6 (3.3)	67.8 (3.9)	69.7 (1.2)	67.8 (3.3)
N120	77.3 (5.7)	63.6 (7.3)	65.9 (5.6)	65.2 (7.1)	64.0 (4.4)
120PK	74.2 (3.1)	63.3 (6.0)	64.0 (4.4)	62.9 (4.5)	61.7 (6.7)

Table A.4 – Table A.28 can be downloaded at <https://ars.els-cdn.com/content/image/1-s2.0-S2351989423003657-mmc1.docx>

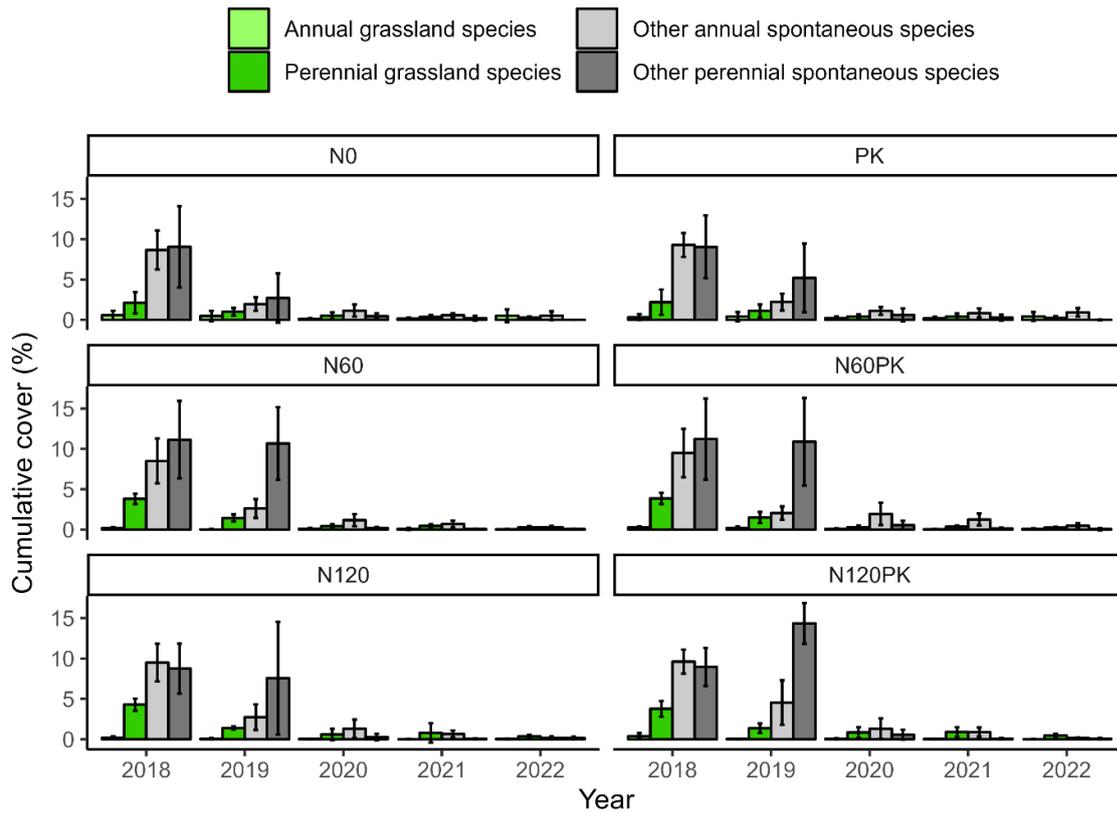


Fig. A.6 Mean cumulative cover of spontaneous grassland species and other spontaneous species in different fertilizer treatments (abbreviations see Article: Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

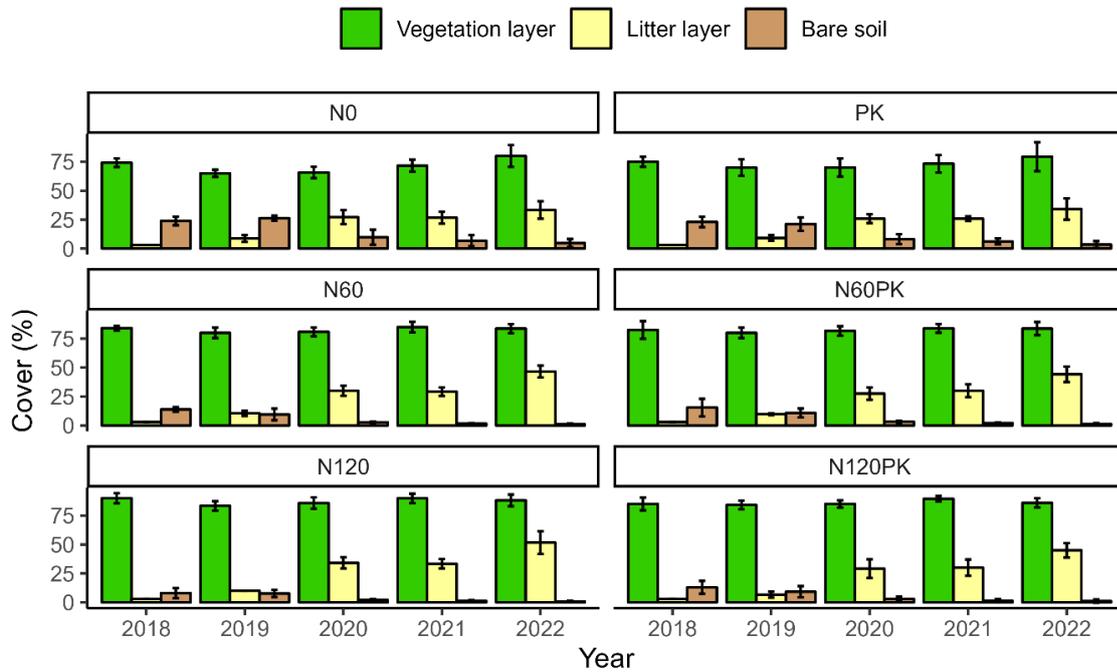


Fig. A.7 Mean cover of the vegetation layer, litter layer and bare soil in different fertilizer treatments (abbreviations see Article: Table 1) between 2018 and 2022. Whiskers refer to standard deviation.

Table A.29

Mean cover (%) of the sown species in the fifth year of the study (2022), shown for the six fertilizer treatments (abbreviations see Article: Table 1). Mean cover from 5% in bold.

Species	NO	PK	N60	N60 PK	N120	N120 PK
Grasses						
<i>Alopecurus pratensis</i> L.	5.3	5.0	7.7	9.8	11.5	13.3
<i>Anthoxanthum odoratum</i> L.	4.3	4.4	11.8	9.5	5.4	8.3
<i>Arrhenatherum elatius</i> (L.) J. Presl et C. Presl	9.5	7.8	13.3	16.7	23.5	21.0
<i>Cynosurus cristatus</i> L.	0.1	0.1	0.1	0.2	0.1	0.1
<i>Dactylis glomerata</i> Horv.	4.3	4.3	6.7	6.2	8.5	9.5
<i>Festuca pratensis</i> Huds.	0.1	<0.1	0.1	0.1	0.1	0.1
<i>Festuca rubra</i> L.	2.6	2.4	2.7	2.2	2.1	1.6
<i>Helictotrichon pubescens</i> (Huds.) Pilg.	0.2	0.2	0.6	0.4	0.6	0.3
<i>Holcus lanatus</i> L.	0.1	0.1	0.1	0.2	<0.1	0.1
<i>Luzula campestris</i> (L.) DC.	0.1	0.2	0.2	0.1	0.1	0.1
<i>Poa pratensis</i> L.	12.0	11.0	13.3	14.2	14.3	15.7
<i>Trisetum flavescens</i> (L.) P. Beauv.	0.5	0.4	0.5	0.5	0.8	0.8
Forbs						
<i>Achillea millefolium</i> L.	3.5	3.3	3.4	4.6	3.9	5.0
<i>Alchemilla vulgaris</i> L. s. str.
<i>Anthriscus sylvestris</i> L.
<i>Campanula patula</i> L.	0.1	0.1	0.1	0.1	<0.1	.
<i>Campanula rotundifolia</i> L.
<i>Cardamine pratensis</i> L. s. str.
<i>Centaurea jacea</i> L. s. str.	8.2	7.5	9.5	9.0	9.2	7.7
<i>Cerastium holosteoides</i> Fr.	0.1	0.1	0.1	0.1	0.1	0.1
<i>Crepis biennis</i> L.	1.0	0.3	1.3	1.6	2.2	0.8
<i>Daucus carota</i> L.	1.1	0.7	0.9	0.5	0.3	0.3
<i>Galium album</i> Mill.	1.5	1.1	2.0	2.1	3.3	1.0
<i>Heracleum sphondylium</i> L.
<i>Hypericum maculatum</i> Crantz
<i>Knautia arvensis</i> (L.) J.M. Coult. s. str.	1.8	1.7	3.0	2.1	2.5	1.6
<i>Leontodon hispidus</i> L.	0.3	0.5	0.5	0.3	0.3	0.2
<i>Leucanthemum vulgare</i> agg.	1.5	1.3	1.7	1.5	1.8	0.9
<i>Lychnis flos-cuculi</i> L.	<0.1	<0.1	0.1	.	<0.1	.
<i>Pimpinella major</i> (L.) Huds.	.	.	<0.1	.	<0.1	.
<i>Plantago lanceolata</i> L.	0.4	0.4	0.3	0.3	0.2	0.2
<i>Prunella vulgaris</i> L.	1.6	1.1	1.5	0.7	0.3	0.2
<i>Ranunculus acris</i> L.	0.2	0.2	0.4	0.3	0.4	0.2
<i>Rumex acetosa</i> L.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
<i>Sanguisorba officinalis</i> L.
<i>Saxifraga granulata</i> L.	<0.1	<0.1	<0.1	<0.1	.	.
<i>Scorzoneroides autumnalis</i> (L.) Moench	0.3	0.3	0.3	0.2	0.1	0.1
<i>Tragopogon pratensis</i> L. s. str.	<0.1	.	.	.	<0.1	<0.1
<i>Veronica chamaedrys</i> L. s. str.	1.0	0.5	1.0	0.7	0.9	0.3
Legumes						
<i>Lathyrus pratensis</i> L.	1.8	2.7	2.4	1.5	0.8	0.5
<i>Lotus corniculatus</i> L.	0.8	0.9	0.1	0.2	<0.1	<0.1
<i>Trifolium pratense</i> L.	20.0	16.8	3.4	3.1	1.4	1.4
<i>Trifolium repens</i> L.	4.0	10.3	1.2	2.0	0.5	0.3
<i>Vicia sepium</i> L.	0.3	0.5	0.2	0.2	0.1	<0.1

Table A.30

Frequency (%) of the sown and established species between 2018 and 2022, shown for all plots (n = 36).

Species	Ellenberg's indicator value for moisture	2018	2019	2020	2021	2022
Grasses						
<i>Alopecurus pratensis</i> L.	6	100	100	100	100	100
<i>Anthoxanthum odoratum</i> L.	-	100	92	97	100	100
<i>Arrhenatherum elatius</i> (L.) J. Presl et C. Presl	-	100	100	100	100	100
<i>Cynosurus cristatus</i> L.	5	86	58	64	89	94
<i>Dactylis glomerata</i> Horv.	5	100	100	100	100	100
<i>Festuca pratensis</i> Huds.	6	100	100	100	50	42
<i>Festuca rubra</i> L.	6	100	100	97	100	100
<i>Helictotrichon pubescens</i> (Huds.) Pilg.	3	97	39	86	100	100
<i>Holcus lanatus</i> L.	6	100	42	69	72	69
<i>Luzula campestris</i> (L.) DC.	4	22	8	53	75	67
<i>Poa pratensis</i> L.	5	100	100	100	100	100
<i>Trisetum flavescens</i> (L.) P. Beauv.	-	100	94	100	100	67
Forbs						
<i>Achillea millefolium</i> L.	4	100	100	100	100	100
<i>Campanula patula</i> L.	5	94	.	8	28	44
<i>Cardamine pratensis</i> L. s. str.	6	6
<i>Centaurea jacea</i> L. s. str.	-	94	100	100	100	100
<i>Cerastium holosteoides</i> Fr.	5	89	100	100	100	97
<i>Crepis biennis</i> L.	6	100	97	97	100	100
<i>Daucus carota</i> L.	4	100	94	94	100	97
<i>Galium album</i> Mill.	5	100	100	100	100	100
<i>Knautia arvensis</i> (L.) J.M. Coult. s. str.	4	100	100	100	100	100
<i>Leontodon hispidus</i> L.	5	83	94	92	89	86
<i>Leucanthemum vulgare</i> agg.	4	100	100	100	100	100
<i>Lychnis flos-cuculi</i> L.	7	67	17	25	19	19
<i>Pimpinella major</i> (L.) Huds.	5	28	33	25	11	6
<i>Plantago lanceolata</i> L.	-	100	97	100	100	100
<i>Prunella vulgaris</i> L.	5	92	67	81	86	83
<i>Ranunculus acris</i> L.	6	97	92	94	92	97
<i>Rumex acetosa</i> L.	-	97	72	97	28	36
<i>Sanguisorba officinalis</i> L.	6	3
<i>Saxifraga granulata</i> L.	4	.	3	8	39	17
<i>Scorzoneroideis autumnalis</i> (L.) Moench	5	89	100	100	100	89
<i>Tragopogon pratensis</i> L. s. str.	-	97	36	11	3	14
<i>Veronica chamaedrys</i> L. s. str.	5	100	100	100	100	100
Legumes						
<i>Lathyrus pratensis</i> L.	6	64	78	83	94	94
<i>Lotus corniculatus</i> L.	4	100	94	94	86	69
<i>Trifolium pratense</i> L.	5	100	92	97	100	100
<i>Trifolium repens</i> L.	5	97	78	53	72	92
<i>Vicia sepium</i> L.	5	58	36	33	56	61

Chapter IV

Synthesis



Species-rich alluvial grassland in favorable conservation status in the Elbe floodplain near Dessau.

1 Main results and general discussion

Although the grassland habitat types 6440 – Alluvial meadows of river valleys of the *Cnidion dubii* and 6510 – Lowland hay meadows are subject to the conservation status deterioration ban since the commencement of the Habitats Directive in 1992, both show an unfavorable–bad conservation status and a deteriorating trend in Germany. In order to improve this situation, the farmers’ demands must be given greater consideration in grassland conservation and restoration (Hammes et al., 2016; Westerink et al., 2024). To my knowledge, the targeted exploration of thresholds for grassland-use intensity for these two habitat types has not yet been addressed. In light of this gap, I used three typical extensive grassland systems of alluvial and mesic meadows as examples. The first system is a species-rich, never-intensified alluvial grassland with low phosphorus levels (Chapter III Article 1). The results indicate up to which fertilization intensity the favorable conservation status can be maintained. The second system is a species-poor, formerly intensified mesic grassland where the typical plant species did not re-colonize despite extensification, and whose soil phosphorus levels are significantly above target (Chapter III Article 2). 18 site-adapted target forbs, typical of habitat type 6510, were sown after sward disturbance. The results provide evidence for the effects of mowing frequency and fertilization intensity in the initial phase after species introduction on a more nutrient-rich site. The third system is a restored species-rich grassland on ex-arable land, whose soil phosphorus levels are slightly above target (Chapter III Article 3). 44 site-adapted target species, including grasses and forbs typical of habitat type 6510, were sown. The results indicate how different fertilizer levels affect target species performance and biomass production, allowing recommendations for adapting seed mixtures to increase the drought resilience of restored mesic grasslands.

This thesis makes an important contribution to adapting agri-environmental schemes and increasing the acceptance of measures for the conservation and restoration of grassland among farmers. It also provides crucial information on how species-rich restored grassland can continue to serve as fodder for livestock in the face of increasing droughts.

1.1 Evaluating ancient alluvial meadow vegetation in favorable conservation status

1.1.1 Effects of fertilization intensity on species assembly

Target species number and target forbs cover

The number of target species and the cover of target forbs was less influenced by fertilization than expected over the 8-year study period. The target species persisted despite high nitrogen application of 120 kg ha⁻¹ yr⁻¹. Mean target species number did not differ significantly between treatments or years, and neither did the treatments differ over time (Chapter III Article 1: Figure 4). Flooding events act as a major influencing factor by overruling the fertilization effects on species richness (Beltman et al., 2007). Although the study area was no longer flooded annually in the spring during the study period, it seems that the exceptional summer flood in the 4th study year was sufficient to produce this effect. Following Tallowin and Jefferson, (2008); Kirkham et al. (2014) and van Dobben et al. (2017), the low soil

phosphorus level in the studied grassland could also be responsible for the persistence of target species.

The assumption that nitrogen fertilization leads to a decrease in cover of low-competitive target forbs cannot be confirmed. This can be explained by the fact that the grass cover did not increase under nitrogen fertilization as observed in other grassland experiments (Hejcman et al., 2007; Honsova et al., 2007; Hejcman et al., 2014; Kacorzyk and Głąb, 2017). Apparently, the expected increase in light competition and niche dimension reduction (Dickson et al., 2014; Molina et al., 2021) has not occurred in our study. In addition, low-competitive species present on the study site responded neutrally to fertilization as also described by Busch et al. (2019). In contrast, after declining in the first four years of the experiment, the high-competitive target forbs did not reach the initial level after the summer flood on the nitrogen-fertilized plots, which can be attributed to the fact that even high-competitive species can be negatively affected by nitrogen fertilization (Busch et al., 2019). On the plots without nitrogen fertilization, however, some species (e.g., *Selinum dubium* and *Ranunculus acris*) clearly benefited from the summer flood by considerably increasing their cover. Interestingly, the negative effect of moderate nitrogen fertilization (N60) was compensated by additional P and K fertilization (N60PK). This is confirmed by Kidd et al. (2017), whose study showed that nitrogen fertilization alone has a stronger negative effect than in combination with phosphorus and potassium. Nitrogen fertilization promotes grasses in particular (You et al., 2019) and can thus lead to an imbalance in the competitiveness of the functional groups. In addition, legumes and forbs performance is positively influenced by phosphorous and potassium supply (Koufali et al., 2016; Ineichen et al., 2020).

Grass and legume performance and legume cycle

The effect of the nitrogen fertilization became apparent only after the summer flood and showed higher grass cover with nitrogen application, but without the expected increase in grass cover as other authors stated (Hejcman et al., 2007; Honsova et al., 2007; Hejcman et al., 2014; Kacorzyk and Głąb, 2017). However, the effect of nitrogen application can be influenced by water availability (Witwicki et al., 2016) or limitation by phosphorus (Güsewell, 2005; Oelmann et al., 2009). Possibly, the overall low phosphorus content in the soil combined with the summer drought at the study site prevented an increase in grass cover.

Legumes showed a clear response to fertilization. Legume cover was quite high at the beginning of the alluvial grassland experiment, followed by a sharp decline in the following year and their almost complete disappearance after the summer flood. Interestingly, their cover increased again only on plots not fertilized with nitrogen and approached the initial state after eight years. This period corresponds to the legume cycle duration in permanent grasslands (Herben et al., 2017). My study illustrates for alluvial grasslands for the first time that the legume cycle could be maintained only for non-nitrogen-fertilized sites. Due to the lower grass cover, less competitive pressure prevailed for the regeneration of the legumes (Luo et al. 2024) and, according to Ledgard and Steele (1992) and Lambers et al. (2004), their ability to fix nitrogen provided a temporary growth advantage in the prevailing soil of low nitrogen content (Chapter III Article 1: Figure A1). Legumes can have a significant influence on

the forage quality and elasticity of forage utilization by maintaining overall sward digestibility over a longer period (Hopkins and Holz, 2006), increasing protein in fodder (Weggler et al., 2019) or increasing the mineral content (Opitz v. Boberfeld and Laser, 1999). In addition, they can play an important role for securing the feed supply for livestock with regard to future climatic conditions. Legumes are well adapted to higher temperatures and can also use water reserves in deeper soil layers through their deep root system (Lüscher et al., 2014).

1.1.2 Effects of fertilization intensity on forage quality

The low forage quality of the alluvial grassland could hardly be improved during the study period with any of the applied fertilizer treatments. For sheep feeding, the crude protein concentration and metabolizable energy concentration of both cuts were adequate for low-performance phases but met the needs of higher performance phases only exceptionally (Chapter III: Article 1, Figure 1 and 3). This is in line with Vargová et al. (2012), who found only few effects on forage nutrient concentrations in alluvial grassland when nitrogen up to 200 kg ha⁻¹ yr⁻¹ was applied. A significant increase in crude protein concentration appears only when at least 200 kg ha⁻¹ yr⁻¹ of nitrogen are supplied (Dindová et al., 2019).

The significant changes between the years for all investigated parameters at both cuts indicate a typical year-to-year variability, which was also described by other authors (Pavlů et al., 2006; Larter and Nagy, 2001), but seems to have been particularly affected by the summer flooding event. Exclusively after the summer flood, the tested nitrogen fertilizer treatments had such an increasing one- to two-year effect on crude protein concentration that higher requirements in sheep feeding could be met, while the metabolizable energy concentration responded in the opposite way.

The decrease in forage quality for the second cut in the high-nitrogen-fertilized N120PK plots was visible at the end of the study period in the low crude protein concentration, low metabolizable energy and high crude fiber concentration. This could be related to the increase of *Cirsium arvense*, whose crude protein level decreases significantly in later stages of development in summer and fall (Peters et al., 2010). Nitrogen fertilization promotes ruderal grassland forbs with low feeding values (Galka et al., 2005; Wesche et al., 2012). This phenomenon has been increasingly observed on many twice-mowed, more heavily fertilized grasslands of the Dessau Elbe floodplain in recent years (Pannach, 2021).

1.2 Evaluating the initial development phase in mesic grassland restoration

1.2.1 Effects of mowing frequency on sown target species number and individuals

In the crucial first year after sowing, frequent cutting was important for the establishment success. The target species number and individuals clearly benefited from the highest cutting frequency (three times) (Chapter III Article 2: Figure 1). The absence of a shading canopy due to biomass removal is probably the main reason for this result (Isselstein et al., 2002; Borer et al., 2014). Competition for light is one decisive factor for plant species assembly and richness (Dybzinski and Tilman, 2007; Harpole and Tilman, 2007; Hautier et al., 2009). The advantages

of suitable initial site conditions, for instance lower competition with other plant species, were also shown by Colas et al. (2008). Furthermore, litter reduction as an effect of frequent cutting maintains gaps for seedling establishment (Török et al., 2011) and may thus have increased the number of established target species and individuals. The benefits of frequent cutting, especially in decreasing competition for light and litter accumulation, obviously outbalanced potential threats such as mechanical destruction in the course of cutting.

However, other studies also achieved good results with only one cut in the first year indicating the importance of initial site conditions. This applies above all to locations where the competition by grasses might be less important, such as on former arable land (e.g., Hölzel, 2005; Török et al., 2012; Andrade et al., 2014), or on restoration sites that have a low nutrient content (Hölzel and Otte, 2003; Andrade et al., 2014).

1.2.2 Effects of fertilization intensity on sown target species number and individuals

Intense nitrogen fertilization (N120) led to both a significantly lower number of established sown target species as well as individuals compared to unfertilized plots, while moderate nitrogen fertilization (N60) did not show these effects (Chapter III Article 2: Figure 1). Since most of the introduced species are adapted to at least moderately nutrient-rich site conditions, the reasons might be the same as shown for frequent cutting. Nitrogen application increases aboveground biomass resulting in increased light limitation for understory species, which are then outcompeted (Borer et al., 2014). Compared to the floodplain grassland studied (Chapter III Article 1; Chapter IV 1.1.1), where low-competitive forbs were not outcompeted by intense nitrogen application, the higher phosphorus level in the soil play an important role, making the experimental site more productive. Moderate fertilization did not lead to a significant lowering of the competitive ability of the sown species, which is important for restoration practice, because it allows for a moderate fertilization if an adequate biomass removal by cutting will be applied. Therefore, the respective meadows can provide hay already during the restoration phase, which can increase farmers' acceptance of restoration actions.

1.3 Evaluating the medium-term development phase in climate-adapted mesic grassland restoration

1.3.1 Effects of fertilization intensity on sown target species performance

During the medium-term development phase, the species assembly was dominated by the target species of the Arrhenatherion, and undesirable species did not immigrate (Chapter III Article 3). Five years after sowing a highly diverse seed mixture (44 species), the mean establishment rate of sown species was about 66.9% ($\pm 5.7\%$) and was slightly higher in the lower fertilizer treatments, but did not differ significantly among fertilizer treatments (Chapter III Article 3: Table A.3). In contrast to seeding after sward disturbance (Chapter III Article 2), target species number is not affected after seeding on former arable land despite higher nitrogen application. This can be attributed to reduced competition effects, as

spontaneously emerging species were particularly low-competition annuals, which almost disappeared in the 2nd year after sowing (Chapter III Article 3: Figure 2). Furthermore, competition is influenced by environmental conditions and their variations (Johnson et al., 2008). Species persistence under nitrogen fertilization up to $120 \text{ kg ha}^{-1} \text{ yr}^{-1}$ could also be demonstrated for ancient, low-productive floodplain grassland (Chapter III Article 1), where competition was reduced by a low phosphorus content in the soil.

Sown species cover increased continuously in the medium-term development phase (Chapter III Article 3: Figure 3). Compared to the control (N0), nitrogen application resulted in significantly higher cover of sown species from the 3rd year regardless of fertilizer level. However, in the 5th year, this effect was no longer detectable. Differences only become apparent when the functional groups are considered individually. Fertilization resulted in higher cover of sown grasses as nitrogen intake increased, while the addition of the nutrients P and K did not result in differences within a nitrogen fertilizer level. Although water deficiency reduces nitrogen uptake (Arfin Khan et al., 2014), precipitation appears to have been sufficient to absorb the applied nitrogen and, combined with the high drought recovery potential of temperate grasses (Hahn et al., 2021), provide increasing cover. Sown forb cover of non-nitrogen fertilized treatments (N0, PK) was significantly higher compared to all other treatments, mainly caused by the increase of legumes, especially *Trifolium pratense*, and *Centaurea jacea*. As already confirmed for alluvial grassland (Chapter III Article 1), forb cover benefited from the absence of nitrogen supply, resulting in a cover balance between grasses and forbs due to reduced grass competition. The interaction of omitted nitrogen fertilization and improved phosphorus availability promoted the sown legumes (Chapter III Article 3: Figure 6 *Trifolium pratense*), thus initiating the legume cycle known from grasslands (Schwinning and Parsons, 1996; Herben et al., 2017).

1.3.2 Effects of fertilization intensity on aboveground biomass production

Following Hölzel and Tischew (2019) and Donath et al. (2021), dry matter yield (DMY) production was at expected levels despite the drought in almost all years of the medium-term development phase. Only in the first year after sowing the yield was considerably higher, as already observed for highly diverse seed mixtures on former arable land by Lepš et al. (2007). Overall, the DMY of the first cut was important for hay production (Chapter III Article 3: Figure 5), whereas a balanced distribution of DMY is more characteristic of twice mown meadows with average precipitation (Boob et al., 2019). Sown grasses dominated the DMY of the first cut and responded to fertilizer applications as follows: N0 and PK showed lower DMY than nitrogen fertilized treatments, while N60 and N60PK had lower DMY than N120 (Chapter III Article 3, Table A.22). The first cut's DMY could only be significantly improved by the highest nitrogen fertilizer rates (N120, N120PK). Since the focus was not only to establish a species-rich grassland, but also to provide aboveground biomass for animal feeding, a large number of grasses were included in the mixture. These grasses, in agreement with You et al. (2017), were able to slightly increase DMY with increasing levels of nitrogen application. However, nutrient addition can amplify detrimental drought effects on community aboveground biomass production, mainly caused by the negative effect on graminoids (Van Sundert et al.,

2021). The dominance of drought-sensitive grasses, such as *Alopecurus pratensis*, *Festuca pratensis* and *Lolium perenne*, over forbs should therefore be avoided, which should already be considered when the seed mixture is composed.

Van Sundert et al. (2021) noticed that drought has no effect on overall forb biomass production. By contrast, I could observe that the forb DMY increased in the non-nitrogen fertilized (N0, PK) to moderately nitrogen-fertilized (N60, N60PK) treatments in the important first cut despite drought, which is one of the key results in terms of climate adaptation.

1.4 Key species in mesic grassland restoration for securing biomass production under drought conditions

Prolonged drought severely impacts grasslands' ecosystem service provision and grassland recovery (Prangel et al., 2024). Kiss et al. (2018) recommend prioritizing the introduction of native matrix species that can tolerate the predicted climatic changes. In the Hayn grassland experiment, six of the 44 sown species yielded more than 5 dt ha⁻¹ yr⁻¹ from the second year after sowing: the perennial grasses *Alopecurus pratensis*, *Dactylis glomerata*, *Poa pratensis*, and *Arrhenatherum elatius*, the forb *Centaurea jacea*, and the legume *Trifolium pratense*. The grasses, especially *Arrhenatherum elatius*, were critical to the dry matter yield (DMY) in the first cut, clearly benefiting from fertilization. Since *A. elatius* is a widespread and agriculturally important European grass species (Michalski et al., 2010) and characteristic of the Arrhenatherion (Schubert, 2001), its suitability for use in seed mixtures under drought conditions is of particular interest. The species reacts to drought stress by increasing tillering, biomass and reproductive fitness (Otieno et al., 2012; Gellesch et al., 2017).

In contrast to the grasses, *Centaurea jacea* had a higher DMY on the second cut in several years and tended to benefit when no nitrogen was applied. Initially, *Trifolium pratense* achieved significant DMY in the first cut of the first year after sowing, while the treatments without nitrogen fertilization reached maximum values at the end of the observation period. This confirms the evidence of Spehn et al. (2002) that good phosphorus availability in the soil facilitates the legume effect on biomass production, but in agreement with the results represented in Chapter III Article 1 only when no nitrogen is fertilized. Because *T. pratense* efficiently fixes atmospheric nitrogen (Fustec et al., 2010), it could increase biomass production in the future without artificial nitrogen fertilization.

2 Conclusions and implications for species-rich grassland conservation and restoration

The grassland-use intensity is a decisive factor for the species assembly and, in combination with the site conditions, determines whether a favorable conservation status can be maintained or restored. Based on the results from the three grassland field experiments in Saxony-Anhalt, the following implications for practice and land use policy can be given:

- Trade-off between farmers' and conservationists' interests in grassland-use intensity

The compromise between grassland farmers and conservationists is characterized by maximizing the quality and quantity of fodder while maintaining a favorable conservation status, characterized by a typical plant species assembly. This can be achieved by applying $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ of nitrogen in combination with phosphorus and potassium. However, in terms of fodder, this only applies to yield and not to quality. Combining nitrogen fertilization with additional applications of phosphorus and potassium plays a key role in maintaining species assembly on low-productive sites and also has a decisive influence on maintaining the legume cycle. With a nitrogen application of only $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ without phosphorus and potassium or even $120 \text{ kg nitrogen ha}^{-1} \text{ yr}^{-1}$, irreversible damage in species assembly cannot be ruled out; these fertilizer variants should therefore not be adopted.

- Evidence-based thresholds for authorities for the further development of management-based agri-environmental schemes

Based on the above, when designing management-based agri-environmental schemes for alluvial and mesic grasslands, authorities should limit the amount of fertilizer to a moderate level of $60 \text{ kg nitrogen ha}^{-1} \text{ yr}^{-1}$ and provide extra salary for the additional application of phosphorus and potassium to under-supplied sites. In addition, the specification of mowing frequency does appear to be necessary. Although no or only moderate fertilization limits the mowing frequency for economic reasons, underuse must be avoided. The aim should be to mow twice, with the second of these mowing events being possible as late grazing. Flexible mowing dates may contribute to an increase in acceptance among farmers and to a higher level of structural diversity in the landscape, which is also important for grassland fauna (e.g., birds, insects). Furthermore, flexible mowing regimes are also necessary in the first year after restoration actions. In particular, on nutrient-rich sites, more frequent mowing in the first year after restoration ensures the restoration success. These implications can also be used as a guide for management under result-based payment schemes, for a more flexible, innovative and performance-oriented approach (Hagemann et al., 2025).

- Recommendations for practitioners for the intensity of grassland use in post-restoration management phases

When restoring species-poor grassland, a higher cutting frequency (cutting three times) in the first year after sowing ensures the establishment success of the sown target species. As moderate fertilization did not result in a significant reduction of the competitive strength of the sown species, fertilization of $60 \text{ kg ha}^{-1} \text{ yr}^{-1}$ should be allowed if an adequate biomass removal is achieved by mowing.

When sowing species-rich grassland on former arable land, there is initially little competition from non-sown species compared to present grassland vegetation. The seed mixture in the grassland experiment established successfully and the establishment rate was hardly affected in the medium-term development phase, despite nitrogen fertilization of up to $120 \text{ kg ha}^{-1} \text{ yr}^{-1}$. However, nitrogen fertilization shifted the vegetation composition in favor

of grasses, and a balanced grass-forb cover ratio and legume cycle could not be established. The recommendation for a maximum nitrogen application of 60 kg ha⁻¹ yr⁻¹ outlined above should therefore be followed to ensure the development of a habitat-typical species assembly.

- Drought-tolerant species for seed mixtures that should be used in species-rich grassland restoration to strengthen climate resilience and future research needs

Six plant species characteristic of the Arrhenatherion, including the four grasses *Alopecurus pratensis*, *Arrhenatherum elatius*, *Dactylis glomerata* and *Poa pratensis*, the forb *Centaurea jacea* and the legume *Trifolium pratense*, appear to be suitable for sustaining productivity over several drought years. They should therefore always be included as matrix species in high-diverse native seed mixtures when arable fields are converted to species-rich grasslands in the southern Harz or climatically similar regions. For different subclimatic regions, other species have to be defined. However, when using nitrogen fertilizer, it must be considered that the contribution of *Trifolium pratense* to plant biomass is significantly reduced. It can be assumed that this also applies to other native legume species. Identifying matrix species for securing yields after restoration is a pioneering approach that also takes farmers' demands into account. Future restoration research should devote more attention to this topic in order to provide practitioners with a selection of potential matrix species for climate-adapted grasslands. This will ensure the on-site production of forage during periods of drought and strengthen the regional value chain. However, as many other species contributed to biomass production under drought conditions and it is also important to provide diverse pollen and nectar services, species-rich seed mixtures should be used when restoring grassland.

3 References

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Overview of the tools used in this dissertation

Tool	Version / link	Utilization
Word	Microsoft Office LTSC Professional Plus 2021	Creation of the text document including the tables
Excel	Microsoft Office LTSC Professional Plus 2021	Creation of graphs (Chapter 3: Supplement of Article 1, Article 2, Supplement of Article 3), creation of tables for the provision of essential research data
Power Point	Microsoft Office LTSC Professional Plus 2021	Creation of the schemes and the map illustration (Chapter 2: Figure 1, 2, 3)
ArcGIS Pro	Desktop version https://www.esri.de/de-de/arcgis/produkte/arcgis-pro/uebersicht	Creation of the maps as the basis for Figure 3 in Chapter 2
SPSS	IBM SPSS Statistics 22.0 (IBM Corporation, Chicago Illinois, USA)	Calculation of statistical tests and models (Chapter 3: Article 2)
PC-ORD	PC-ORD 6.0 (McCune and Mefford, 2011)	Calculation of the principal component analysis and creation of the figure (Chapter 3: Article 2)
R	https://www.r-project.org/	Calculation of statistical tests and models, creation of graphs (Chapter 3: Articles 1 and 3), for the packages used, see Chapter 3
DeepL	https://www.deepl.com/	Translation from German to English
ScienceDirect	https://www.sciencedirect.com/	Literature search
Springerlink	https://link.springer.com/	Literature search
Scopus	https://www.scopus.com/	Literature search
Google	https://www.google.com/	Literature search
Google Scholar	https://scholar.google.com/	Literature search

Apart from the AI DeepL, no other AI was used for this dissertation. The translation suggestions provided by DeepL have been adopted in an adjusted form.

Affidavit

I hereby declare that I have prepared this dissertation independently and without unauthorized outside assistance. I have not used any sources or aids, including generative models/AI, other than those indicated and have marked as such all text passages taken verbatim or in spirit from published or unpublished writings and all information based on oral information. Likewise, all materials provided or services rendered by others are identified as such.

Bernburg, 6. May 2025



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