Grass growth, matter turnover and herbage production in temperate silvopastoral systems

Dissertation

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Go tell, go tell all the little children.

Tell all the mothers and fathers too.

Now's our last chance to learn to share

What's been given to me and you.

One blue sky above us

One ocean lapping all our shore

One earth so green and round

Who could ask for more.

Pete Seeger

Contents

GENERAL INTRODUCTION

Challenges for agricultural land use systems

Worldwide, the rapid increase of human population results in a raised overexploitation of natural resources and demand for food and energy with critical consequences for agro- and forest-ecosystems (Smith et al. 2013). The intensive and unsustainable management practices in terms of high use of fertilization, pesticides and water as well as increasing mechanization in agriculture and forestry highly impact terrestrial landscapes resulting in environmental degradation putting greater pressure on marginal sites and protected areas (Cumming et al. 2014, Montagnini 2017, Smith et al. 2013). One major threat to ecosystem structure and function is the increasing nitrogen (N) deposition due to raised human activities with several consequences (Freedmann 2013, Galloway et al. 2004). High losses of N through leaching can lead to eutrophication of surface water while the increased N availability can alter biogeochemistry of ecosystems and affect plant growth and nutrient cycling (Farrer et al. 2013). With this, dramatic changes to plant community composition with losses in diversity occur (Bobbink et al. 2010, Field et al. 2014).

Climate change will be a further challenge for agricultural practice. Besides changes in external N inputs mentioned above, climate change will affect also internal N cycling. Rustad et al. (2001) showed in a meta-analysis of experimental warming studies that global warming caused increased N mineralization and thus, N availability, in a wide range of ecosystems. Further, increasing dry summers and more frequent and intense extreme drought events are predicted for central Europe in the next 30 years (IPCC 2013) what will affect ecosystems functions and services as soil fertility, biodiversity and carbon storage (Jentsch et al. 2011).

As in many parts of the globe, also in Europe there is a need for more sustainable agriculture and sustainable intensification, i.e. to raise productivity while at the same time reduce its environmental impacts (Foley et al. 2011). Multifunctional land use forms may be an agricultural management system, integrating the various demands of food and

energy production, biodiversity and environmental conservation, as well as the mitigation of climate change effects (Mosquera-Losada et al 2018).

Grassland growth and nitrogen cycling

Grasslands cover about 25% of all terrestrial ecosystems (Ojima et al. 1993), about 30% of the agricultural area in Europe (Smit et al. 2008) and 28% of the agricultural land in Germany (Statistisches Bundesamt 2019). Managed grasslands are mainly used for livestock either by cutting for silage or hay production and less frequently for grazing (Saha and Butler 2017). Under temperate climate conditions, these grasslands usually consist of a vegetation cover of perennial herbaceous plant species (mainly grasses, legumes and dicotyledonous forbs) with the botanical composition depending on the defoliation intensity and frequency and nutrient input (Boob et al. 2019, Harmens et al. 2004, Pavlů et al. 2011).

Herbage accumulation of grassland is the result of growth and senescence processes (Lemaire & Agnusdei 2000) which are both affected by the availability of resources like light, nutrients and water (Whitehead 1994). The production of photosynthetically active live grassland herbage tissue is primarily dependent on temperature and on a sufficient amount of radiation (Hunt and Thomas 1985). The relation of live and dead herbage accumulation determines the forage quality at harvest as usually morphological parameters change with senescence causing increases in fibre and decreases in nitrogen concentration (Buxton 1996). From an agronomic point of view, grassland production for herbage depends on management intensity, the grassland sward type and their interaction (Belesky et al. 2019, Saha and Butler 2017). Defoliation intensity determines the length of the intervals between defoliation. Intervals between defoliation events longer than the leaf life span of a plant generally increase herbage mass but also the amount of senesced dead herbage (Gastal and Lemaire 2015). An extensive management, i.e. low defoliation frequency, enhance herbage accumulation while under an intensive management digestibility of herbage increase due to the higher amount of live relative to dead herbage (Gastal and Lemaire 2015).

Besides cutting frequency, species diversity, species identity and functional group (grass, legumes, forbs) composition play an important role for grassland production but it is still discussed to which extent they contribute to yielding. Experiments have shown positive effects of species richness on biomass production and more consistent yields (Hector et al. 1999, Nyfeler et a. 2009) due to an improved utilization of resources (niche differentiation), positive interactions and selection effects (Hooper et al. 2005). Küchenmeister et al. (2012) concluded that species identity and functional group composition is more important for yield stability and productivity than species richness. The most common grass species used in cool temperate agricultural grasslands is *Lolium perenne* (L.) due to its high yielding and high feeding quality (Frame 1992, Lamp et al. 1990). Under current climate change scenarios, the more drought resistant grass species *Festuca arundinacea* (Sch.) is of increasing interest (Reheul et al. 2012). In combination with legumes, e.g. *Trifolium repens* (L.), these grass-legume mixtures can lead to overyielding caused by N fixation ability of the legume (Finn et al. 2013, Nyfeler et al. 2009).

Sward nutrient status is, after management and water status, the main factor determining the productivity of grasslands (Duru and Calvière 1996, Klapp 1965, Vitousek and Howarth 1991). Nitrogen, a component of proteins, chlorophyll, and nucleic acids, is essential for plant growth and functions. On an annual basis, sward productivity and forage quality (White et al. 2004) increase with additional N while plant production in temperate ecosystems is often limited by the amount of plant-available N (Chapin 1980, Whitehead 1995).

To be less dependent on current N uptake, nitrogen resorption is a keystone nutrient-conserving mechanism of many perennial plant species in natural and unmanaged ecosystems (Aerts and Chapin 1999, Chapin 1980, Killingbeck 1996). To preserve itself from nutrient loss through senescence and cessation, the plant may retranslocate N from senescing parts to other plant tissues, defined as nitrogen resorption efficiency (percentage of a nitrogen withdrawn from mature leaves before leaf abscission) (NRE) (Killingbeck 1986).

Moreover, the process of nitrogen resorption has profound consequences on nitrogen cycling at community and ecosystem level (Aerts and Chapin 1999, Garnier et al. 2005). Nitrogen that is resorbed during senescence is directly available for plant growth while not resorbed N underlies loss with litterfall and subsequent decomposition pathways (Aerts and Chapin 1999). Plant species compete for mineralized N with micro-organisms (Kaye and Hart 1997) and neighbouring plants or part of the N can become unavailable for plant uptake through incorporation in stable soil organic N (Aerts 1997). Nitrogen resorption efficiency varies with N availability of habitats and of soils (Aerts 1995, Wang et al. 2018, Yuan and Chen 2015), with plant functional group (e.g. legume vs. grasses) (Killingbeck 1996) and several leaf traits (Wright et al. 2005), respectively. In agricultural grassland with no external N input, the presence of legumes in swards will determine the intensity of N resorption (Huang et al. 2008) since legumes are N self-sufficient due to their ability of atmospheric N fixation and, consequently, have a lower NRE than non-legumes (Killingbeck 1996). The NRE is further affected by cutting frequency with longer regrowth intervals leading to higher NRE (Wang et al. 2016).

Although results from a global meta-analysis showed negative effects of N enrichment on plant N resorption (Yuan and Chen 2015), empirical results at the species-level are highly variable, with negative, neutral, and positive effects being reported (van Heerwaarden et al. 2003, Lü et al. 2013). To cope with and to adapt to spatial and/or temporal environmental heterogeneity, species show a phenotypic variability which is determined by genetic variability and by phenotypic plasticity with the latter allowing a genotype to be present in different environments and to respond more flexible and quicker to environmental change (De Witt and Scheiner 2004, Schlichting 1986). To which degree species react to environmental changes, e.g. N fertilization, depends further on their resource-use strategy (Wright et al. 2004). Exploitative species, e.g. *L. perenne*, are found in resource-rich habitats showing a fast tissue turnover, high nutrient capture and a fast growth and are phenotypically highly variable with a high phenotypic plasticity. In contrast, conservative species like *F. arundinacea* are resident in rather N-low habitats, have a slower tissue turnover and growth and show less phenotypic plasticity than exploitative species (Lavoral et al. 2009). The two strategy types can be characterized by

leaf traits involving e.g. leaf N content and leaf dry matter content (Garnier et al. 2001, Wright et al. 2004).

Agroforestry as a sustainable land use system

Agroforestry is beside crop rotation and intercropping one of the appropriate systems for agro-ecological intensification which comprise yield stability and environmental benefits (Tscharntke et al. 2012). Agroforestry systems (AFS) are land use practices which integrate different tree species and agricultural crops on the same unit of land (Jose 2009, Nair 2011). Whereas in the tropics AFS are common land use systems, they have disappeared in temperate regions over the last century because of intensification and mechanization of agricultural production (Nerlich et al. 2013).

AFS provide ecosystem services and environmental benefits, e.g. internal regulations of soil, water and air quality, efficient nutrient cycling, modifying local and global climates, enhanced biodiversity, carbon sinks (Jose et al. 2009, Kay et al. 2019, Smith 2012, Smith 2013, Torralba et al. 2016, Udawatta et al. 2019), and show a raised productivity compared to monocropping systems because of complementary resource capture (Cannell 1996). Nevertheless, AFS still are economically unproven land-use systems partly due to the lack of long-term studies, knowledge transfer and appropriate policies across Europe (Doyle and Waterhouse 2008, Mosquera-Losada et al. 2012).

Silvopastoral systems are AFS combining trees and grassland for herbage production on the same agricultural area (Nair 1993). In Europe and especially Germany, hedgerows designed as windbreaks shape large parts of northern Germany (REF) or orchard grasslands (Paesel et al. 2017) that were used for pasture and fruit production. These long-known less designed and more natural agroforestry systems underline the complex conditions AFS are found in. Until now, designed alley-cropping silvopastoral systems are not very common in today's modern agricultural practice in Europe (Eichhorn 2006, García de Jalón 2018). However, growing research focus is laid on these systems as they provide environmental and ecological benefits of the interaction of trees and grassland/crops (Burgess and Rosati 2018). Especially the integration of short rotation coppice in alley cropping systems, where trees and grassland are arranged in alternating

stripes, can be an adequate method of increasing productivity per unit land area in Europe's limited land resources (Graß et al. 2020, Graves et al 2010, Pent 2020, Quinkenstein et al. 2009). Trees used in these systems are fast-growing species, e.g. willow or poplar, which allow harvest in short rotations of three to six years due to resprout ability after harvest. Diversification of market goods and the superiority on marginal land or land with high environmental risks, e.g. water and/or wind erosion, can further increase attractiveness of alley cropping systems to farmers since opportunity costs are relatively low (Böhm et al. 2014).

In silvopastoral systems, the grassland between the tree lines is exposed to spatially and temporally changing growth conditions due to the competition with trees, i.e. varying distributions of nutrient, light and water (Dodd et al. 2005, Gamble et al. 2016, Gillespie et al. 2000, Jose 2009). Reduced light availability near trees can limit grassland growth by decreasing sward community photosynthesis and herbage accumulation (Devkota et al. 2009, Jose et al. 2009). According to Gastal and Lemaire (2002), leaves receiving less light have a higher N concentration per leaf area and owing to a lower sink demand of growing tissue this may exert towards the N concentration of the senescing tissue (Ono et al. 1996). To optimize the use of spatial, temporal, and physical resources, silvopastoral systems should be intentionally designed by maximizing positive interactions (facilitation) and minimizing negative ones (competition) among the components (Jose et al. 2004). However, as stated by Halvorson et al. (2017) studies on appropriate management in silvopastoral systems are extremely important in order to understand interactions between trees and grassland as these interactions are diverse and largely not understood.

Research objectives

The aim of this dissertation was to understand grassland herbage production and its N cycling in temperate silvopastoral systems as affected by the interaction of trees and grassland and the grassland management.

To investigate the interaction of position to tree lines (i.e., shading) and cutting frequency on the total herbage accumulation, the live and dead herbage tissue and their relation in two in their size and botanical sward composition contrasting silvopastoral systems were

studied (Chapter I). To evaluate the N resorption responses in silvopastoral grassland communities at the small-scale, the N concentration in live and dead herbage as well as the NRE as affected by trees under different cutting frequencies were analyzed at one silvopastoral system (Chapter II). The studied silvopastoral systems of Chapter I and Chapter II were arranged in the form of alley cropping systems comprising willow stripes under short rotation coppice with grassland in the alleyways. Sward botanical composition among sites differed. At one site trees were established into existing permanent grassland and two different original mixtures (grass-clover mixture and 32-species mixture) were sown at the second site, which were termed vegetation compositions in the present thesis assuming that mixtures sown more than five years before study start deviate from the intended sown composition.

The first two studies were carried out within the first phase (2015–2018) of the interdisciplinary project SIGNAL (Sustainable intensification of agriculture through agroforestry), embedded in the BonaRes initiative "Boden als nachhaltige Ressource für die Bioökonomie", funded by the German ministry of education and research (BMBF).

To assess the growth and NRE of agronomic relevant grass species in view of changing climatic conditions, the NRE and growth of two grass species differing in their drought resistance but also in their nutrient-use strategy were investigated under two N supply levels. Therefore, a greenhouse experiment was conducted using *L. perenne* and *F. arundinaceae* grown under a low- and a high-N supply level for 16 weeks (Chapter III).

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CHAPTER I

Impacts of cutting frequency and position to tree line on herbage accumulation in silvopastoral grassland reveal potential for grassland conservation based on land use and cover information

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Abstract

In agricultural grassland, high herbage utilization efficiency (HEFF), which is the proportion of gross live-green herbage production that is utilized before entering senescence, is ensured by frequent defoliation. The decision upon which defoliation frequency to apply depends on the farming intensity. Assuming a reduced total herbage accumulation near trees in silvopastoral systems, frequent defoliations with high HEFF become less worthwhile – at least in specific spatial configurations. This makes an extensive management near trees an interesting option since it promotes other grassland-related ecosystem services such as biodiversity. The present study first analyzed the interaction between defoliation frequency and position to trees on the total, dead and live herbage accumulation and the HEFF at two silvopastoral sites with short-rotation coppices in Germany. In addition, the total grassland-tree interface in Germany was assessed from land use and land cover maps of Germany based on satellite data to approximate the potential of grassland extensification near trees. The total herbage accumulation near trees declined by up to 41% but the HEFF was not affected by the position. Consequently, any intensification is not payed-off by adequate productivity and herbage quality in terms of HEFF and tree-related losses in herbage accumulation are expected up to a distance of 4.5 to 6 m. Applying a 4.5 m border on satellite data, we found that up to 4.4% (~2,200km²) of the total grassland area in Germany is at a tree interface and potentially suitable for extensification. These findings indicate substantial potential for biodiversity conservation in grasslands with low trade-off for high quality yield.

Introduction

Silvopastoralism comprising grassland and trees is gaining importance in Europe (Mosquera-Losada et al., 2018). Alleys of short rotation coppices integrated into agricultural land are the dominating form of agroforestry systems in Germany (e.g. Kahle & Janssen, 2020). Similar to grassland, silvopastoral systems provide a variability of ecosystem services which likely depend on management intensity, the grassland sward type, their interaction (Belesky et al., 2019) and responses on pastures to trees. The latter, for instance, may cause a growth penalty due to litter fall and soil acidification (Muys et al., 1992; Halvorson et al., 2017).

Usually, the herbage accumulation of grassland is the result of the processes of growth and senescence (Bircham & Hodgson, 1983; Lemaire & Agnusdei, 2000). Both processes are affected by the availability of resources, i.e. light, water and nutrients (Whitehead, 1994). In between two tree lines, light becomes a limiting resource at certain spatial positions (Guevara-Escobar et al., 2007) causing losses in grassland herbage accumulation (Ehret et al., 2018; Orefice et al., 2019; Pang et al., 2019). Leaf appearance and growth are functions of light availability and temperature (Hunt & Thomas, 1985; Gastal & Lemaire, 2015). In studies with increasing shading as an experimental factor, net accumulation of herbage in grassland swards consequently declined (Grant et al., 1981; Devkota et al., 2009). Senescence is genetically determined, but the timing and rate of senescence are also controlled by environmental factors (Whitehead, 1994). Shading below the light compensation point causes a negative carbon balance which, in turn, increases the senescence of leaves (Brouwer et al., 2012). Yet, Grant et al. (1981) could not find any change in the senescence rate per tiller due to shading, while, in general, senescence increased linearly with herbage mass. In other words, a constant senescence rate per tiller is an indication of no reaction in the leaf lifespans to shading. All herbage that is not harvested undergoes senescence, and the amount of senesced dead herbage at harvest is therefore influenced by the defoliation intensity and frequency (Parsons & Penning, 1988; Whitehead, 1994). Intervals between defoliation events longer than the leaf lifespan usually increase the herbage mass but also the amount of senesced dead herbage (Gastal & Lemaire, 2015). A modification of the live and dead herbage mass by

defoliation frequency will have an impact on the herbage utilization efficiency (HEFF), which is the proportion of gross live-green herbage production that is utilized before entering senescence (Mazzanti & Lemaire, 1994). Farmers usually adapt their defoliation frequencies in relation to the farming system purposes in order either to increase the herbage accumulation (low HEFF, extensive management) or the amount of digestible herbage (high HEFF, intensive management) (Gastal & Lemaire, 2015).

Several studies on silvopastoral grassland distinguished between the effects on live and dead herbage (e.g. Devkota et al., 2009), but the impact of the defoliation frequency on the HEFF in relation to trees has received little attention under temperate climate in this respect. As stated by Halvorson et al. (2017), studies on appropriate management in silvopastoral systems are extremely important in order to understand interactions between trees and grassland as these interactions are diverse and largely not understood. In silvopastoral grassland with declining growth rates but constant leaf lifespans near trees, no consequences for the HEFF should result from shading. If the HEFF of a specific defoliation system near trees is the same as it is away from trees but herbage accumulation is lower, then intensive use with frequent defoliations is hardly worthwhile near trees. The limitation for the biological process of growth consequently makes infrequent defoliation an interesting option in the area nearer to trees, because it supports other grassland-related ecosystem services such as invertebrate protection (Kruess & Tscharntke, 2002). Presuming the limited growth potential next to trees in silvopastoral systems or, generally speaking, next to trees along roads, hedges or forests, there is a great potential for biodiversity conservation, which depends on extensive management to support e.g. flowering plants (Smart et al., 2002) or birds (Allen et al., 2021). For an assessment aiming at improved biodiversity support in grasslands near trees, land use land cover (LULC) information from remote sensing is a helpful tool to quantify the large-scale grassland-tree interface reliably (Ali et al., 2016).

This study was the first conducted to test the hypothesis that the HEFF is not affected by the defoliation frequency near tree lines. For this, two contrasting silvopastoral sites were studied over two successive years to investigate the interaction of cutting frequency and position to tree lines (i.e. shading) on the total grassland herbage accumulation and the dead and live herbage tissue in order to elucidate any trade-off between management

intensity and herbage production. In addition, we further evaluated to what extent grassland in Germany is potentially affected by tree shading using remote sensing-based LULC information to assess the potential for biodiversity.

Materials and Methods

Field experimental area, setup and climatic conditions

The field study was conducted over two consecutive growing seasons (2016 and 2017) at two silvopastoral sites integrating short rotation coppice and grassland. The site Reiffenhausen (RH), 24 km south of Göttingen (51°23'56.1"N 9°59'13.4"E, 325 m above sea-level), was established in 2011 on former arable land with three tree lines and two grassland sward types on a soil type classified as a Stagnosol (Ehret et al., 2018). After tree planting, two grassland mixtures were established in three replications in a split-plot randomized block design between the tree lines, i.e. either a perennial ryegrass-white clover sward (*Lolium perenne, Trifolium repens*, GC: grass clover) with a sown proportion of 31% legume and 69% grass, or a diverse mixture (DIV) with a proportion of grasses, non-leguminous dicotyledonous herbs and a legume in proportions of 43, 41 and 16%, respectively. A detailed overview is given in Table S2. The different swards at site RH will be termed vegetation compositions hereafter. The vegetation compositions were assigned to the main plot with the cutting system treatments as subplots within main plots. Subplots had a size of 59 m^2 and were sampled at three positions per treatment (see below). The setup of the field study at RH consequently refers to a four-factorial (vegetation composition, cutting system, position and year) field experiment. At the second site, Mariensee (MS) 160 km north of Göttingen (52°33'52''N and 9°27'53''E, 41 m above sea level), three tree lines were established in permanent grassland in 2008. The soil type is a heterogenous mixture with a dominance of Histosol containing a conserved peat layer. As the tree lines at site MS had been established into existing permanent grassland, no factor for vegetation composition was investigated here. At site MS, the field experiment refers to a three-factorial split plot design with the factors cutting system, position and year. The cutting system treatment represented main plots (192 m^2) randomized across the site with the position as subplot and a total of six replicates per treatment (n = 36 plots).

Both sites differed with respect to the initial setup: the distances between two tree lines at site RH were 9 m with a tree line width of 7.5 m. At MS, tree lines were 48 m apart with

a tree line width of 11 m. The tree lines at site RH were planted in a northeast to southwest direction and at site MS from south to north (Figure 1). At RH, the tree lines contained one willow hybrid 'Tordis' ((*Salix schwerinii* × *S. viminalis*) × *S. viminalis*). At MS, a mixture of several willow hybrids, i.e. 'Inger' (*Salix triandra x S. viminalis)*, 'Tora' (*S. schwerinii x S. viminalis*) and 'Tordis' were planted. Trees were harvested for the last time prior to the present study in the beginning of 2015 at RH and 2016 at MS.
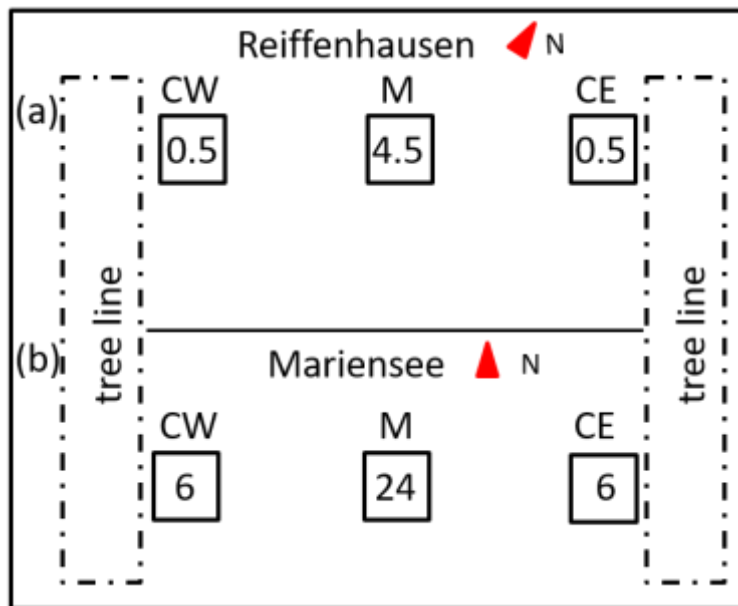
Figure 1 Schematic overview (topview) of the setup of the experimental areas at (a) Reiffenhausen and (b) Mariensee. Every position and cutting system plot is replicated six times and they are denominated uniformly as CW, CE and M, that is, Close West and Close East of tree lines or in the Middle between two tree lines, respectively in this study. Numbers below the position plots show the distances in m to the tree lines

The factor position (three levels) comprised a composition of the compass orientation and the spatial distance to the tree lines located either close to one tree line or in the middle between two tree lines. The position consequently represents a proxy for any potential effects of shading by trees. The actual distances of the positions to the tree line differed between sites in response to the setup with 0.5, 4.5, and 0.5 m at RH and 6, 24 and 6 m at MS (Figure 1). In the following the positions for both sites are named as 'Close West', 'Middle' and 'Close East' (i.e. CW, M, CE, respectively). The factor cutting system (two levels) comprises of two systems with different frequencies of defoliations and length of interval between defoliations, that is, two or four harvests per year. The dates

of harvests are given in Table S1 and only three harvests were realized during 2016 at site RH. During the time of the study, proportions of grass and dicots in the two different vegetation compositions at RH did not differ significantly (Welch two sample t-tests, *p* = 0.335). Legumes were completely absent in the grassland at MS and only scattered shares of herbaceous dicots were present (94% grass on average during 2016 and 2017). No fertilizer was applied.

Both sites are characterized by a long-term (1981 – 2010) temperate climate with an average annual air temperature (± standard deviation) of 9.2°C ± 0.8°C and 9.7°C ± 0.8°C, at RH and MS, respectively. The mean annual precipitation sum (± standard deviation) is 650 ± 131 mm and 665 ± 111 mm at RH and MS, respectively. During the experimental periods, temperatures during the growing season were higher than the long-term values, whereas precipitation sums were lower in 2016 and higher in 2017 than the long-term values, respectively (Table 1).

Table 1 Growing season (April – October) weather data (mean temperature [°C], precipitation sum [mm], mean global radiation [J cm⁻²]) at Reiffenhausen supplied by the German weather service ('Deutscher Wetterdienst' [DWD], station Göttingen) and Mariensee (DWD weather station Hannover airport) during 2016 and 2017 based on daily records compared to the long-term period (1981-2010).

Site		Temperature	Precipitation	Global radiation
Reiffenhausen	2016	14.5 ± 4.4	374.5 ± 30.1	1487.1 ± 517.0
	2017	14.2 ± 3.9	558.7 ± 61.5	1372.6 ± 486.1
	1981-2010	13.7 ± 3.6	379.7 ± 10.2	1407.9 ± 433.5
Mariensee	2016	15.0 ± 4.4	369.7 ± 29.1	1486.0 ± 526.4
	2017	14.6 ± 3.7	541.6 ± 43.9	1334.1 ± 465.0
	1981-2010	14.1 ± 3.7	396.5 ± 9.1	1437.1 ± 454.1

Note: Numbers following ± indicate *SD*

Total, dead and live herbage and herbage utilization efficiency

At harvest, samples of the standing aboveground grassland herbage were taken from areas measuring 9 × 80 cm and 50 × 50 cm at RH and MS, respectively by manual cutting at 3 cm stubble height. Subsequently, a representative subsample of the fresh matter was separated manually into dead (> 80% dead plant area) and live herbage. Separated samples, as well as the remainder were dried in a forced-air oven at 60°C for 48 hours and weighed afterwards to determine the dry-matter (DM) content. The herbage mass at the

second harvest in 2017 at site MS had to be calculated from regular measurements of the compressed sward height (CSH) (Dougherty et al., 2011) based on linear regression between CSH and herbage mass from a common double sampling procedure (t'Mannetje, 2000). In cut grassland swards, the total herbage accumulation may be expressed in a simplified way as the sum of live green and dead herbage mass. In this study, the dead and live herbage masses of each harvest were aggregated to constitute the total herbage accumulation. The herbage utilization efficiency (HEFF) was adapted from Mazzanti and Lemaire (1994) and then calculated from the annual sums as follows:

$$HEFF = \frac{live\ herbage\ mass - dead\ herbage\ mass}{live\ herbage\ mass}$$

Site specific data analysis from field experimental work

Data analyses were performed using R 3.6.1 (R Core Team, 2020). The total herbage accumulation, the live and dead herbage accumulation (g DM m^{-2}) and the HEFF were analyzed using linear mixed-effects models in the *nlme* package (Pinheiro et al., 2018). Model assumptions were tested graphically and data were found generally to follow a Normal distribution. Different variance adjustments were applied in order to meet the criteria of variance homogeneity. Both sites were analyzed separately by estimating global models with vegetation composition, cutting system, position and year as well as all possible interactions as fixed effects for site RH. The random effect constituted of the sampling plot nested in each block within the main plot (vegetation composition), subplot (cutting system) and position. Separate variances per year were allowed in the model for the total herbage accumulation and per position in the model for the live herbage accumulation. For the models of the dead herbage accumulation and the HEFF, separate variances were allowed for each level in the interaction of year cutting system. At site MS, the global models consisted of the cutting system, position and year as well as all interactions as fixed effects and the sampling plot as random effect. The sampling plot resulted from the split-plot design with the main plot (cutting system) and subplot (position). In each model, separate variance was allowed for each level in the interaction of year cutting system. Automated model selection from the global models were

performed using the *MuMIn* package (Barton et al., 2018). The final model was selected based on the lowest Akaike Information Criterion corrected (AICc) for small sample sizes. For significant ($p < 0.05$, *F* test) terms in the final models, treatments were compared post-hoc by least squared means (p < .05) using the package *lsmeans* (Lenth, 2018).

Assessment of the area related to grassland-tree interface based on land use and land cover maps

Remote sensing data from, e.g., Landsat and Sentinel-2, are well suited to map LULC for national scales with high accuracies (e.g. Griffiths et al. 2019; Pflugmacher et al. 2019). The high temporal resolution achieved by combining satellite sensor time series (e.g. Sentinel-1/2, Landsat) allows for creating detailed LULC maps, which provide information on, e.g., grassland and tree cover on a spatial resolution of up to 10m (Chaves et al. 2020. We used two recent LULC maps for 2016 (Griffiths et al. 2019) and 2018 (Blickensdörfer et al. 2021) to obtain tree and grassland cover, respectively. We then derived information on edges between grassland and trees across Germany based on these two LULC classifications by evaluating the 4-pixel neighborhood of each 10x10m grassland pixel with regard to adjacent forest cover or presence of isolated trees or tree rows. In this, permanent and temporary grassland were included. If a grassland pixel was connected to at least one tree pixel, we mapped a grassland-tree boundary of 10m corresponding to the spatial resolution of the map. For each of these pixels, the cardinal direction of the neighboring tree cover was assessed. We calculated the total length of grassland-tree edges for Germany and further evaluated the shares of cardinal directions of those edges. To further characterize the spatial configuration of the grassland-tree interface in Germany, we calculated and compared the grassland area potentially affected by tree shading for federal states corresponding to the Nomenclature of Territorial Units for Statistics (NUTS 2 regions) and administrative districts (corresponding to NUTS 3 regions).

Results

Grassland herbage accumulation at site RH

The importance of each harvest to the herbage accumulation was altered by the cutting system (Figure S1) and the output of the linear mixed effects models is given in Table S3. In RH, years differed significantly ($p < 0.001$, *F* test) with a total herbage accumulation of 711 and 444 g DM m^{-2} in 2016 and 2017, respectively. A significant effect of the vegetation composition ($p < 0.01$, *F* test) revealed that the GC sward produced more total herbage than the DIV sward (652 vs. 503 g DM m^{-2}, $p < 0.01$). The position had a significant influence on the total herbage accumulation ($p < 0.001$, *F* test) and the rank between positions was M > CE > CW (791 > 526 > 417, respectively; Figure 2) with a difference of up to 47%. The same pattern as for the total herbage accumulation followed for the live herbage accumulation except that position had no significant effect (Table S3). The vegetation composition effect ($p < 0.01$, *F* test) revealed that the GC sward produced more live herbage than the DIV sward (531 vs. 434 g DM m^{-2}) and a significant effect of the year resulted in a larger live herbage accumulation in the year 2016 compared with the year 2017 (555 vs. 410 g DM m^{-2}, $p < 0.001$, *F* test). Position tended to have a significant effect ($p < 0.07$, *F* test) on the live herbage with a decline of on average 37% from the middle towards the tree line positions.
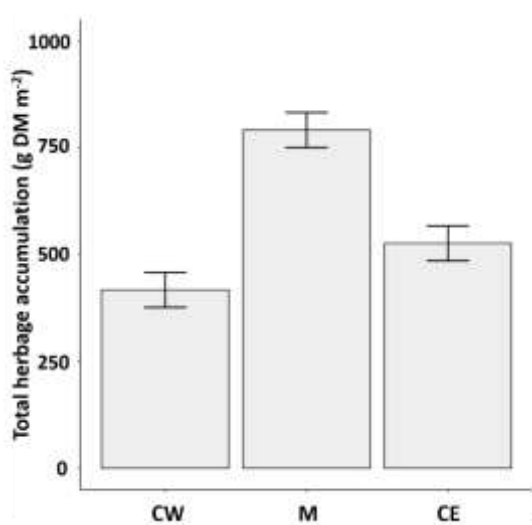

Figure 2 Means (±*SE*) of total herbage accumulation at site RH at each position. CE, Close East; CW, Close West; M, Middle

The interaction of factors vegetation composition and position was significant for dead herbage accumulation ($p < 0.05$, F test). The GC sward accumulated significantly more dead herbage at the M position than the positions adjacent to the tree lines (LSMeans, $p < 0.05$; Table 2(a)). For the DIV sward the dead herbage accumulation at position CE was larger than at position CW (LSMeans, $p < 0.05$) with position M ranging between them (Table 2(a)). Despite the significant interaction between cutting system and vegetation composition ($p < .05$, F test), the dead herbage accumulation was larger in the infrequent compared with the frequent cutting system (on average: 124.8 vs. 65.5 g DM m^{-2}, F test, $p < 0.01$) and the GC sward produced more dead herbage than the DIV sward in both cutting systems (on average: 76 vs. 114.3 g DM m^{-2}, F test, $p < 0.01$). The interaction of vegetation composition and year was also significant for the dead herbage accumulation ($p < 0.001$, F test). In 2017, the swards did not differ in dead herbage accumulation, whereas in 2016 the GC sward produced significantly more dead herbage compared to the DIV sward (191.3 vs. 121.8 g DM m^{-2}, $p < 0.001$, LSMeans). However, a larger dead herbage accumulation in 2016 compared with 2017 was observed in both vegetation compositions (on average: 156.6 vs. 37.4 g DM m^{-2}, $p < .001$, F test). The interaction between cutting system and year was also significant for the dead herbage accumulation ($p < 0.01$, F test). The infrequent cutting system produced more dead herbage than the frequent cutting system in 2016 (203.8 vs. 109.4, $p < 0.001$, LSMeans) and in 2017 (45.8 vs. 21.6, $p < 0.001$, LSMeans); the year 2016 was more productive than 2017 ($p < 0.001$, F test).

The HEFF was affected by the significant interaction between position and year ($p < 0.01$, F test). In both years, the HEFF was higher at position M than at the positions adjacent to the tree lines, although the difference between M and CE was not significant in 2016 (LSMeans, $p = 0.9$, Table 2(b)). The HEFF was also affected by the significant interaction of vegetation composition and cutting system ($p < .01$, F test). The HEFF was significantly larger in the frequent cutting system than in the infrequent one for both vegetation compositions (DIV: 0.87 vs. 0.79, $p < 0.01$, GC: 0.87 vs. 0.72, $p < 0.001$, LSMeans). However, in the infrequent cutting system, the DIV sward had a larger HEFF compared with the GC sward (0.79 vs. 0.72, $p < 0.01$, LSMeans) while in the frequent cutting system the vegetation compositions did not differ (on average: 0.87). A significant interaction

between vegetation composition ans year ($p < 0.001$, F test) resulted from a larger HEFF in the DIV compared with the GC sward in 2016 (0.75 vs. 0.67, $p < 0.01$, LSMeans) while in 2017 no differences were found (on average: 0.9). The interaction between cutting system and year ($p < 0.01$, F test) was also significant but the HEFF was always larger in the frequent cutting system than in the infrequent cutting system (on average: 0.86 vs. 0.75, $p < 0.001$, F test) while in the year 2017 the HEFF was greater than in 2016 in both cutting systems (on average: 0.91 vs. 0.71, $p < 0.001$, F test).

Table 2 Means (±*SE*) of the dead herbage accumulation (g DM m^{-2}) separated for the interaction of vegetation composition and position (a), and of the HEFF separated for the interaction of position and year (b) for site RH

(a) Dead herbage accumulation	Position	DIV	GC
	CW	70.7 ± 8.4	109.2 ± 8.4
	M	73.8 ± 8.4	124.5 ± 8.4
	CE	83.4 ± 8.4	109.2 ± 8.4
(b) HEFF	Position	2016	2017
	CW	0.66 ± 0.03	0.89 ± 0.01
	M	0.74 ± 0.03	0.93 ± 0.01
	CE	0.74 ± 0.03	0.90 ± 0.01

Abbreviations: CE, Close East; CW, Close West; DIV, diverse sward; GC, grass-clover sward; M, Middle; RH, Reiffenhausen.

Grassland herbage accumulation at site MS

At site MS, the total herbage accumulation was affected by the interaction of position and cutting system (p <.01, F test) and by cutting system and year (p <.01, F test). Except at the position CW, the frequent cutting system had significantly larger total herbage accumulation than the infrequent cutting system ($p < 0.05$, LSMeans; Table 3(a)). In the infrequent cutting system, the total herbage accumulation was largest at position CW ($p < 0.01$, LSMeans; Table 3(a)) whereas the positions M and CE did not differ ($p = 0.9$, LSMeans; Table 3(a)). In the frequent cutting system, the total herbage accumulation at position CW was larger only compared to position CE ($p < 0.05$, LSMeans; Table 3(a)). For both cutting systems, the total herbage accumulation in 2016 was larger than in the year 2017 (on average: 908 vs. 443, $p < 0.001$, F test). However, in 2016 the cutting systems did not differ (on average: 908, $p = 0.7$, LSMeans) while in 2017 more total herbage (520

vs. 366, *p* < 0.001, LSMeans) was accumulated in the frequent cutting system. For the live herbage accumulation, a significant effect of the cutting system ($p < 0.001$, *F* test) was observed with a significantly larger amount in the frequent compared with the infrequent cutting system (587 vs. 413, p < 0.001, *F* test). Live herbage differed significantly among years ($p < 0.001$, *F* test) with larger amounts of live herbage in 2016 than in 2017 (653 vs. 347, *p* < 0.001, *F* test). The dead herbage accumulation was significantly affected by the interaction between cutting system and year ($p < 0.001$, *F* test) with a significantly larger dead herbage accumulation in 2016 compared with 2017 in both cutting systems (on average: 272.6 vs. 96.7 g DM m^{-2}, *p* < 0.001, *F* test). However, in 2017 the difference between the cutting systems was not significant (on average: 96.7 g DM m^{-2}) while in 2016 dead herbage accumulation was larger in the infrequent cutting system than in the frequent one (403.8 vs. 141.4 g DM m^{-2}, *p* < 0.001, LSMeans). The dead herbage was also significantly affected by the interaction of position and cutting system ($p < 0.01$, *F* test) and of position and year ($p < 0.01$, *F* test). No clear pattern among positions was observed between years nor in the cutting systems (Table 3(b), (c)). In the infrequent cutting system, the dead herbage was larger at position CW than at position CE (*p* < 0.01, LSMeans; Table 3(b)) while in the frequent cutting system, the dead herbage accumulation was larger at position M than at CW (*p* < 0.05, LSMeans; Table 3(c)).

Table 3 Means (±*SE*) of the total herbage accumulation (g DM m^{-2}) separated for the interaction of cutting system and position (a), and of the dead herbage accumulation (g DM m^{-2}) separated for the interaction of cutting system and position (b) and of position and year (c) for site MS

(a) Total herbage accumulation	Position	Infrequent	Frequent
	CW	706 ± 32.4	736 ± 23.5
	M	608 ± 32.4	717 ± 23.5
	CE	613 ± 32.4	668 ± 23.5
(b) Dead herbage accumulation	**Position**	**Infrequent**	**Frequent**
	CW	276 ± 17.5	103 ± 11.2
	M	257 ± 17.5	136 ± 11.2
	CE	226 ± 17.5	111 ± 11.2
(c) Dead herbage accumulation	**Position**	**2016**	**2017**
	CW	275.2 ± 19.5	103.7 ± 8.0
	M	306.3 ± 19.5	86.1 ± 8.0
	CE	236.3 ± 19.5	100.2 ± 8.0

Abbreviations: CE, Close East; CW, Close West; M, Middle; MS, Mariensee.

The dead herbage accumulation was larger in the infrequent compared with the frequent cutting system across positions (Table 3(b)) and also in 2016 compared with 2017 (Table 3(c)). However, among positions in the year 2017 no differences were observed in the dead herbage accumulation (Table 3(c)), while in 2016 a larger accumulation of dead herbage was found at position M compared with position CE ($p < 0.01$, LSMeans; Table 3(c)). A significant effect of the interaction between cutting system and year ($p < 0.001$, F test) was observed for the HEFF. In both years, the frequent cutting system resulted in a larger HEFF compared with the infrequent one (on average: 0.79 vs. 0.38, $p < 0.001$, F test). The HEFF of the infrequent cutting system in 2016 was significantly lower compared with 2017 (0.14 vs. 0.62, $p < 0.001$, LSMeans) while no difference among years was observed in the frequent cutting system (on average: 0.8).

Assessment of the grassland area potentially interfered by trees in Germany

The length of the estimated grassland-tree boundary amounted to ca. 490,000 km across Germany. Consequently, the estimated grassland area within a distance of 4.5 m to forest or tree cover was 2200 km² corresponding to 4.4% of the total grassland area of Germany (map estimate of 49,700 km^2) (Figure 3). The orientation of the forest-grassland boundary was equally distributed among the cardinal directions. Among the federal states, the share of grassland as interfered by trees ranged between 2.9 (Bremen) and 5.9% (Saarland) (Table 4).

Table 4 Estimates of the grassland-tree interface in Germany based on LULC maps generated from satellite time series.

Region	Federal state	Grassland area	Grassland-tree interface	
		km^2	km^2	%
	Berlin (BE)	10.22	0.58	5.65
	Brandenburg (BB)	3604.72	134.82	3.74
East	Saxony-Anhalt (ST)	1760.95	68.32	3.88
	Saxony (SN)	1823.22	104.02	5.71
	Thuringia (TH)	1633.40	93.57	5.73
	Baden-Württemberg (BW)	5340.96	227.18	4.25
South	Bavaria (BY)	11844.25	448.24	3.78
	Hesse (HE)	3005.15	167.05	5.56
	Saarland (SL)	346.72	20.40	5.88
	Lower Saxony (NI)	7248.93	308.15	4.25
West	North Rhine-Westphalia (NW)	3830.26	207.44	5.42
	Rhineland-Palatinate (RP)	2451.06	127.14	5.19
	Bremen (HB)	77.39	2.25	2.91
	Hamburg (HH)	57.55	2.35	4.08
North	Mecklenburg-Western Pomerania (MV)	2897.78	133.19	4.60
	Schleswig-Holstein (SH)	3755.45	152.82	4.07
Germany		49688.02	2197.53	4.42

Note: Given are the grassland area (km^2) for each federal state in Germany, the grassland tree-interface (km^2) and the share of grassland-tree interface (%) in that state

Analysis on the district level revealed further spatial differences (Figure 3). Districts having both the highest overall grassland proportion and the highest percentage of grassland-tree edges were concentrated in the central regions of Germany. Districts with either a low grassland or edge area percentage predominated in southern regions towards the Alps and northeastern Germany.

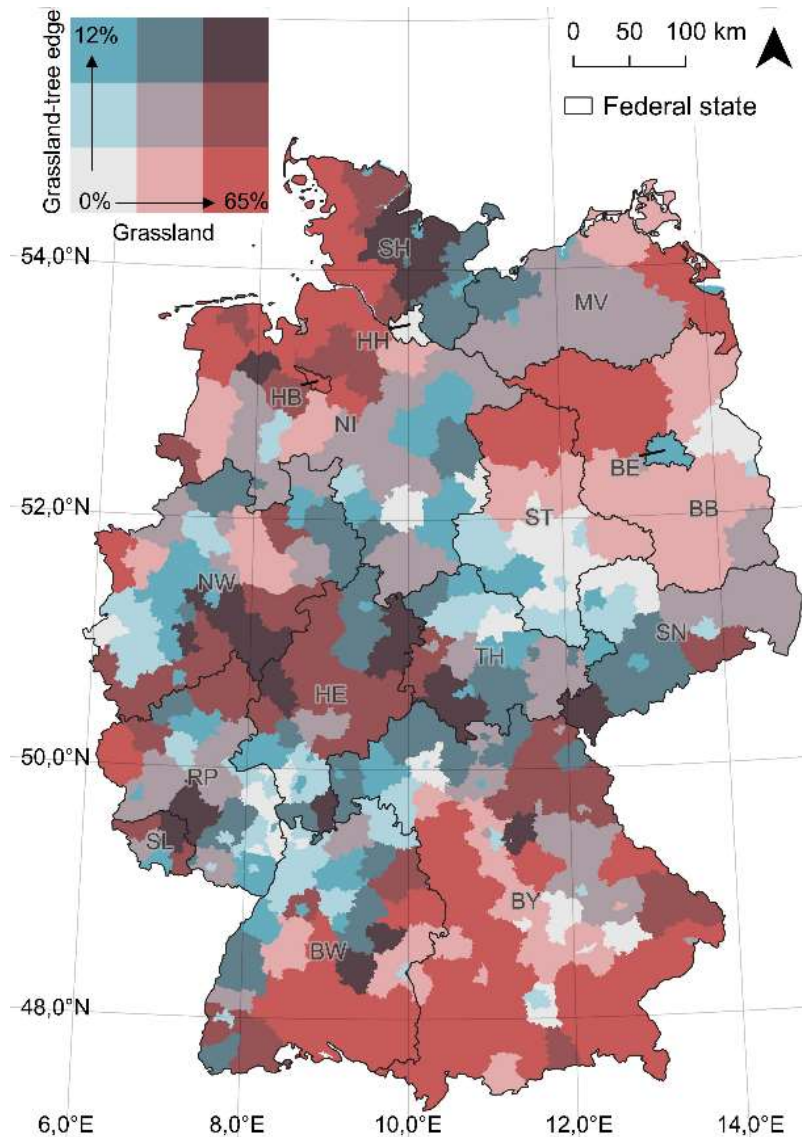**Figure 3 Percentage of grassland and grassland at tree interface on the district level in federal states of Germany (abbreviations explained in Table 4) based on land use and land cover information from satellite time series. Values were grouped into terciles (class intervals of grasslands percentage: 0 – 7.7, 7.8 – 13.7, 13.7 – 65 and class intervals of grassland-tree interface percentage: 0 – 4.5, 4.6 – 5.6, 5.7 – 12**

Discussion

Herbage utilization in silvopastoral grassland

The main results of the field study were that competition by trees played an inconsistent and rather minor role at site MS and that any tree-induced modifications of all investigated herbage accumulation parameters were restricted to site RH. There, the total, live and dead herbage accumulation declined from the middle position towards the tree lines, and the HEFF was related to the cutting system but not strongly to the position or to an interaction between cutting system position.

The average tree height from the beginning until the end of this study increased from 2.4 ± 0.1 m to 4.5 ± 0.9 m at site RH and from 0.5 m to 4.1 ± 0.6 m at site MS, which is a proxy for the strength of shading caused by trees. The photosynthetically active radiation (PAR) was measured above the grass canopy in the respective positions around noon at site MS during a subsequent year in the continuation of the present study (Sutterlütti et al., 2020). The annual average PAR showed no differences between the different positions. It is likely that the absent tree effects observed at site MS were caused by the chosen positioning rather than by the tree harvest prior to the beginning of this study. In a previous study, Ehret et al. (2018), however, reported a decline of the incident light near trees at site RH. Competition for nutrients or water play an important role in agroforestry systems (Guevara-Escobar et al., 2007) and these may also dominate during re-establishment after tree pruning where light is likely less limiting (Jones et al., 1998). In addition, the decomposition of leaf fall is likely to cause growth inhibition of silvopastoral grassland (Halvorson et al., 2017).

We hypothesized no differences of HEFF within one cutting system near or at distance from the tree lines. It was assumed that this mechanism is due to lower growth rates and constant leaf lifespans and specific senescence rates (Grant et al., 1981; Devkota et al., 2009) resulting in a constant proportion of live and dead herbage. We consequently investigated an interaction between position and cutting system for the HEFF in order to assure that the differences between the defoliation systems in the middle position without tree interference are the same as close to trees. This interaction was never

statistically significant which confirms our hypothesis. No interaction with position was observed since the live and also the dead herbage declined consistently while both these variables responded to the defoliation frequency as could be expected (Gastal & Lemaire, 2015). Following Mazzanti and Lemaire (1994), the HEFF is likely to increase with less senescent dead herbage. This is supported by the present study in view of consistently greater HEFF in the frequent compared with the infrequent cutting system.

The absent effect on HEFF in relation to the position may be addressed by questioning of whether growth or ageing is more strongly influenced by trees. An answer to this question requires consideration of the relative changes of live herbage accumulation among positions at site RH. In relative terms, the live herbage mass declined by 37% from the middle towards the tree line while the dead herbage declined by only 7% in the vicinity of the tree lines compared with the middle position (on average across treatments). This stronger effect on the live herbage illustrates that the senescence rate is less likely affected by shading than the growth rate. This is supported by a study reported on *L. perenne*, where shaded tillers continued to produce leaves, albeit, at a reduced rate (Ong & Marshall, 1979). Shaded grasses had lower tiller densities and, thus, lower herbage accumulation (Thomas & Davies, 1978) which can be attributed to carbohydrate-limited leaf expansion rates (Fulkerson & Donaghy, 2001). An unchanged senescence rate is supported by Grant et al. (1981) who have also found reduced growth rates under shading. The 2-year average senescence rates (± average standard errors of means) resulting from the minimum and maximum dead herbage accumulation for the period from the beginning of April until the last harvest of a year in the present study ranged from 0.5 ± 0.04 to 2.1 ± 0.14 g m^{-2} day^{-1} at site MS and from 0.1 ± 0.01 to 1.10 ± 0.1 g m^{-2} day^{-1} at site RH. These values are lower than those reported by Bircham and Hodgson (1983) who have found average daily senescence rates of between 1 and 4.4 g m^{-2} day^{-1} in fertilized swards. Larger values can be attributed to different climatic conditions and fertilization or are due to different sward types (Binnie and Chestnut, 1994). Especially at site RH greater proportions of dicot species were present which can have effects on the amount of produced dead herbage. For instance, Calvière and Duru (1995) reported that the species-specific growing degree days until 25% of all leaves per shoot show signs of senescence differ between 800 (monocots) and 2000 (dicots).

Changes of species-specific leaf-life spans between monocot and dicot species could also explain differences between the two sites observed in the present study since legumes were present at site RH. Legumes are important components for maintaining the nitrogen supply within pasture systems (Andrews et al., 2007) and they obviously contributed positively to the herbage quality at site RH in terms of HEFF which was generally high.

Potential implications of grassland extensification near trees

The constantly low HEFF in the infrequent cutting system provides an opportunity to promote other grassland-related ecosystem services near tree lines such as invertebrate protection which benefit from extensive management (Kruess & Tscharntke, 2002): any intensification of defoliation in order to increase the HEFF is not worth the effort in view of the low productivity and the trade-off with high-quality herbage accumulation is consequently low. This is in accordance with Smart et al. (2002) who attributed a high potential of field edges to promote biodiversity of British grasslands under appropriate management. The frequency of defoliation is critical to attain a high productivity, quality or floristic performance (Belesky et al., 2019) or to support birds (Allen et al., 2021). Cong et al. (2020) showed that designed diverse grassland mixtures harvested twice a year, increased the provision of flower resources in three landscapes of Denmark. A higher value for insect conservation of extensified grassland is also in line with Ekroos et al. (2020), who sampled nearly 300 grasslands across Europe and found a significant decline in bee species richness among other flower visiting invertebrates with intensification in fertilizer use.

The comparison of the two sites in our study allows for an estimation at which distance effects of trees on grassland herbage are likely to decline under the present climate. The middle position differed compared with the close positions at site RH. At MS, no differences between 6 m and 24 m distances were observed. Consequently, effects of tree shading by short-rotation coppices reaching a height of up to five meters will likely decline at distances of between 4.5 (or less) to 6 m. Transferring these results to grasslands across Germany, we estimated that 4.4% of the grassland area is affected by shading of trees and is, therefore, potentially suitable for decreasing management

intensity without substantial declines of high-quality herbage production. The LULC classifications were validated with high accuracies (overall accuracy ≥80%). Class area estimates were well in line with official statistics (Griffiths et al., 2019; Statistisches Bundesamt, 2019), confirming the reliability of the estimated area of grassland-tree interface. However, we likely overestimated the grassland area near trees that is suitable for extensification as we could not identify different management intensities and thereby were not able to limit the investigation to intensively used grasslands only. Based on our district-level estimates (Figure 3), a high share of grassland-tree edges was found in the middle of Germany which is typically hilly and less intensively utilized than the coastal areas and grasslands in southern Germany. So far, national-scale information on different management systems and their management intensity on grassland has not been derived reliably from remote sensing time series. It is therefore not yet possible to further specify the suitability for extensification of grasslands from remote sensing data alone without consideration of the actual management on site. Most intensive grassland is found in dairy enterprises. In 2018 approximately 61,000 dairy farmers were registered with an average herd size of 64 cows/farm (Tergast et al., 2019). Given that a farm has an average stocking rate of 1.7 livestock units/ha, roughly 38 ha of agricultural land are required per average farm. Assuming dairy livestock is fed on grassland and arable land each with a share of 0.5, then 19 ha of mainly intensive grassland per farm is utilized. The total grassland area under intensive dairy farming would then roughly be 1.16 million ha in Germany. Applying a constant value of 4.4% of grassland at the tree interface would give a rough estimate of 51,040 ha of extensification potential on intensively managed grassland due to trees. However, one has to take into account differences in shade intensity and shadow length between e.g. forests and short rotation coppices as the canopy affects irradiance that is reached by understorey vegetation (Valladares et al., 2016). Additional remote sensing-based information from satellite sensing regarding tree species, tree height and density of tree cover would further enable the assessment of the shading intensity on grassland and enable a better estimation. By including these factors, most suitable grasslands for the proposed conservation measure of small scale extensification near trees in intensive grassland could be identified to promote the conservation value of grassland around trees in general. However, the vegetation

(whether natural or sown) should be adapted to the low-light environment near trees. When Pang et al. (2019) studied herb production and survival of 22 forage species under artificial shade, they concluded that most species are adapted to less bright environments, although grasses tended to be more suitable than flowering dicot species such as *Trifolium pratense*. On the other hand, coniferous trees in particular may exert strong adverse effects on the understorey vegetation since leaf litter fall decreases the soil pH (Muys et al., 1992; Halvorson et al., 2017). Studies of the tree-grassland interface can contribute to insights in finding appropriate management schemes and vegetation for particular regions and purposes.

Implications

Shading by trees reduces grassland growth more severely than it increases senescence in silvopastoral grassland and this effect is not modified by the defoliation frequency. Although high herbage utilization efficiency could be maintained by increased defoliation frequencies near tree lines, the total herbage production is generally low. Consequently, shorter harvesting intervals are less desirable near the tree lines. This reveals a potential for providing other grassland-related ecosystem services which benefit from infrequent defoliation since any intensification is not payed-off by adequate productivity and herbage quality. This serves as a basis for setting up an agri-enviromental scheme focusing on management extensification at field edges near trees in any grassland. We estimated that approximately 4.4% of the German grassland is at a tree interface and potentially available for extensification, which goes far beyond silvopastoral alley-cropping systems.

Acknowledgements

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Supporting information

Table S1 Harvest dates at sites Mariensee (MS) and Reiffenhausen (RH) during the study. * harvests of extensive cutting systems. Because of technical issues, the harvest in July 2016 at site RH had to be split between the intensive and extensive cutting systems. Consequently, 19.07.2016 represents the second harvest of the intensive cutting system and 06.07.2016 the first harvest of the extensive one.

2016		2017	
MS	RH	MS	RH
24.05.	06.06.	17.05.	19.05.
20.06.*	19.07./06.07*	15.06.*	29.06.*
08.08.	-	17.08.	19.08.
05.10.*	27.09.*	17.10.*	13.10.*

Table S2 Seed mixture composition (%) of the diverse mixture sward which was established at Reiffenhausen initially in 2011.

Grasses	%	Legume	%	Non-leguminous Herbs	%
Agrostis capillaris	5.0	Lotus corniculatus	0.3	Achillea millefolium	0.8
Anthoxanthum odoratum	5.0			Agrimonia eupatoria	1.0
Bromus hordeaceus	5.0			Agrostemma githago	2.0
Cynosurus cristatus	5.0			Ajuga reptans	0.2
Festuca brevipila	20.0			Campanula patula	0.2
Festuca nigrescens	15.0			Centaurea cyanus	2.0
Festuca rubra subsp. Rubra	10.0			Centaurea jacea	1.5
Poa nemoralis	3.0			Crepis biennis	1.0
Poa trivialis	2.0			Galium mollugo	1.5
				Galium verum	0.5
				Hypericum perforatum	1.5
				Knautia arvensis	1.0
				Leucanthemum ircutianum	1.5
				Lychnis flos-cuculi	0.5
				Malva moschata	1.5
				Papaver rhoeas	1.5
				Pimpinella saxifraga	1.5
				Prunella vulgaris	1.5
				Salvia pratensis	2.0
				Sanguisorba minor	2.5
				Silene dioica	1.0
				Silene vulgaris	1.0
				Tragopogon pratensis	2.0

Table S3 Output of linear mixed effects models on the total herbage, dead and live herbage accumulation (g DM m^{-2}) and the herbage utilization efficiency (HEFF). Presented are F-values, corresponding degrees of freedom and p-values. Dashes /- indicate that an effect was not retained in the final model. Vegetation composition was a factor at site Reiffenhausen, not at Mariensee. *Abbreviations: RH: Reiffenhausen, MS: Mariensee. N/A: not available, * p< .05; ** p< .01; *** p< .001, +p<0.1.*

Parameter	Fixed and interactions effects	RH	MS
Total herbage (g m^{-2})	Vegetation composition	$F_{1,32}=12.5$**	N/A
	position	$F_{1,32}=27.7$***	$F_{2,20}=6.9$**
	cutting system	-/	$F_{1,10}=0.3$
	year	$F_{1,35}=53.7$***	$F_{1,34}=101.6$***
	cutting system year	-/	$F_{1,34}=8.3$**
	position x cutting system	-/	$F_{2,20}=8.3$**
Live herbage (g m^{-2})	Vegetation composition	$F_{1,32}=8.8$**	N/A
	position	$F_{2,32}=3.1$+	-/
	cutting system	-/	$F_{1,10}=102.1$***
	year	$F_{1,33}=10.5$**	$F_{1,35}=214.5$***
	position x year	$F_{2,33}=2.8$+	-/
Dead herbage (g m^{-2})	Vegetation composition	$F_{1,28}=12.1$**	N/A
	position	$F_{2,28}=2.6$+	$F_{2,20}=4.6$*
	cutting system	$F_{1,28}=11.5$**	$F_{1,10}=60.6$***
	year	$F_{1,33}=28.6$***	$F_{1,32}=74.7$***
	Vegetation composition position	$F_{2,28}=4.7$*	N/A
	Vegetation composition cutting system	$F_{1,28}=6.9$*	N/A
	Vegetation composition year	$F_{1,33}=11.9$**	N/A
	cutting system year	$F_{1,33}=8.6$**	$F_{1,32}=76.4$***
	position cutting system	-/	$F_{2,20}=5.9$**
	position year	-/	$F_{2,32}=5.8$**
HEFF	Vegetation composition	$F_{1,30}=16.6$***	N/A
	position	$F_{2,30}=6.8$**	-/
	cutting system	$F_{1,30}=10.8$**	$F_{1,10}=52.2$***
	year	$F_{1,31}=15.0$***	$F_{1,34}=24.6$***
	Vegetation composition cutting system	$F_{1,30}=8.2$**	N/A
	Vegetation composition year	$F_{1,31}=21.9$***	N/A
	position year	$F_{2,31}=5.9$**	-/
	cutting system year	$F_{1,31}=8.4$**	$F_{1,34}=26.8$***
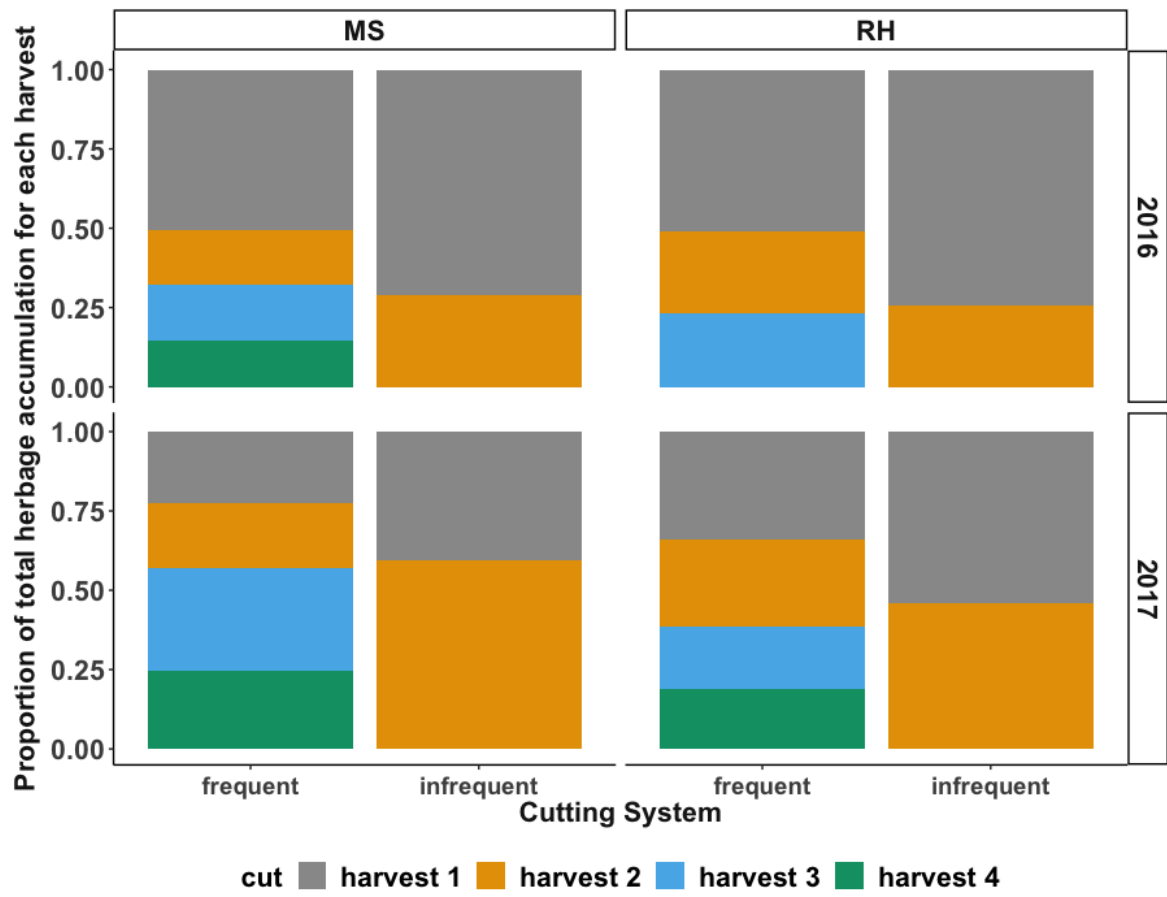
Figure S1. Mean proportion of each harvest to the annual herbage accumulation within cutting system, year and site. *Abbreviations: MS: Mariensee, RH: Reiffenhausen.*

CHAPTER II

Trees in silvopastoral systems reduce legume proportion with no consequence for internal N resorption efficiency

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Abstract

In legume-based grassland, legumes supply the sward with nitrogen (N) from biological N_2 fixation. Previous studies in silvopastoral systems have emphasized that legume proportions decline near trees which would cause spatial variation of the N supply and the concentration of N in the harvested herbage of grass swards between trees. In natural ecosystems, it was shown that the N resorption efficiency (NRE) increases with nutrient limitation, hence, near trees where legumes are scarce. We, therefore, tested the hypothesis that the NRE increases near trees and compensates for the loss of legumes with respect to N concentration in live herbage. For this, two vegetation compositions consisting of legume-based grass swards were analyzed across positions between tree lines in an alley cropping system established five years prior to this study. Legume proportion declined up to 45% towards the tree line and thus, also N concentration in live and dead herbage but on average only by 15%. As a consequence of a reduced N concentration and also herbage mass close to trees, the N yield decreased by up to 50%. Despite the loss of legumes near trees, the NRE was unaffected by the tree line irrespective of vegetation composition. Further, the results indicate that internal N cycling of managed grass swards in silvopastoral grassland with short defoliation intervals between harvests is of lesser relevance than in unmanaged ecosystems. Legume proportions control the N concentrations of live and dead herbage irrespective of tree shading in silvopastoral systems.

Introduction

Alley cropping systems can be combined with grassland to form a silvopastoral system (Ehret et al. 2018). Such systems can be sustainable and productive especially under conditions of a changing climate (Tsonkova et al. 2012; Smith et al. 2012, 2013; Torralba et al. 2016; Kay et al. 2019). The grassland between tree lines underlies spatially and temporally varying growth conditions because the availability of light, nutrients, and water for the grass sward is affected by competition with the trees (Jose 2009; Gamble et al. 2016). Herbage accumulation of grassland is the result of the processes of growth and senescence (Bircham & Hodgson 1983; Lemaire & Agnusdei 2000) and both processes are affected by the availability of light, water and nutrients (Whitehead 1994). In the grass sward, under sufficient water availability, the production of photosynthetically active live herbage tissue is primarily dependent on temperature and the sufficient amount of radiation (Hunt and Thomas 1985). Reduced radiation near trees in silvopastoral systems will limit grassland growth as sward community photosynthesis is reduced (Devkota et al. 2009; Jose et al. 2009). Schmiedgen et al. (2021) in their experiments on two silvopastoral systems in Central Europe found that accumulation of live herbage was stronger reduced than senescence under conditions of shade near the tree lines.

The internal process of resorption of nitrogen (N) from senescing tissues into still live and photosynthetically active tissues is an adaptive strategy of plants to conserve N. This internal N translocation from dead to live herbage tissue is defined as N resorption efficiency (NRE) and describes a mechanism of nutrient conservation particularly under N limited conditions (Kobe et al. 2005). The extent of N resorption within plants thus plays an important role in ecosystem N cycling (Whitehead 2000). In agronomic terms, a high NRE would make plants less dependent on external N input. The NRE is therefore positively related to the nutrient use efficiency of dry matter production (Güsewell 2005; Hayes et al. 2014). Grass sward leaves that receive less light usually have a higher N concentration (Barro et al. 2012; Wang et al. 2016). When, however, growth is reduced also the function of growing live tissue as a sink for N is lowered. Further, when growth is reduced, N concentrations in plants are likely to increase and this may also result in a higher N concentration of the dead herbage tissue (Ono et al. 1996). As growth was

particularly limited close to tree lines, we can see an effect of the position on the N concentration in live and dead herbage of the grass sward within silvopastoral systems.

Generally, studies analyzing the importance of NRE in temperate grassland are scarce. Reported values for NRE range between 29 and 74% (Mao et al. 2011) and seem to a large degree determined by species-specific functional group characteristics and by the occurrence and proportion of legumes in the sward (Huang et al. 2008). Most information on NRE in grassland biomes is available from cold-arid steppes along with shrub-infested arid rangelands (e.g. Van Heerwaarden et al. 2003; Zhang et al. 2015; Li et al. 2018). So far, to our knowledge no study has investigated the N resorption in temperate managed legume-based silvopastoral grassland.

Legumes provide N in legume-based grassland from the biological N_2 fixation (Nyfeler et al. 2011). As nitrogen resorption is dependent on the level of nutrient input (Yuan and Chen 2015; Huang et al. 2008), legumes are therefore anticipated to have a lower NRE than non-legumes (Killingbeck 1996). In swards with no external N input, the proportion of legumes will thus determine the intensity of N resorption at the sward scale (Huang et al. 2008). Reduced light availability may modify sward botanical composition; especially legumes are sensitive to shade (Frame 2019). In previous studies it was shown that trees in silvopastoral systems reduce the proportion of legumes in shaded areas (Ehret et al. 2018; Graß et al. 2020). Whether the tree effect on live and dead herbage N concentration varies between grass swards with different legume proportion in the vegetation has not been tested. This effect can best be studied in silvopastoral systems, where varying legume proportions occur naturally due to light-induced selection.

In grassland, the resorption of N from dead leaves depends on the time for regrowth between two defoliation events (Wang et al. 2016). The amount of dead herbage will increase significantly with a longer duration of the regrowth cycle (Parsons and Penning 1988; Gastal and Lemaire 2015). For grassland managed for provision of forage, the implications for NRE are therefore complicated by defoliation frequency: on the one hand, longer regrowth intervals allow higher nutrient resorption (Wang et al. 2016) and on the other hand, litter accumulation at longer regrowth intervals reduces the volume of growing tissue as a sink for N (Gastal and Lemaire 2015). An interaction of the defoliation management with the grass sward composition on live and dead herbage N concentration

and NRE were, however, not analyzed despite the importance of N concentration for livestock production. It follows that legume-based grass swards should have little variation in N concentration of the live herbage if NRE is an important mechanism that compensates for the loss of legumes near trees.

In the present study we set out to test whether NRE in legume-based silvopastoral grassland is affected by an interaction of the vegetation composition with the position (distance) with respect to tree lines and the defoliation frequency. The legume-based grass swards tested in the present study should have little variation in N concentration of the live herbage if NRE compensates for the loss of legumes near trees. Thus, we understand the effect of position as an effect of legume proportion and hypothesized that NRE increases towards tree lines

Materials and methods

Experimental area, setup and climatic conditions

The experimental field study was conducted over a period of two consecutive growing seasons (2016 and 2017, April-October) at an AFS site located in Reiffenhausen (RH) 24 km south of Göttingen (51°39'83''N and 9°98'75''E, 325 m above sea level), which integrated a short rotation coppice and grassland. The predominant soil type is a Haplic Stagnosol with deposits of loamy sand and silty clay (Hartmann et al. 2015). The AFS had been established on former arable land in 2011. The experimental outline consisted of a factorial design with four tree lines and alternating grassland stripes between the tree lines (Ehret et al. 2018). The tree lines were planted on an area of 0.7 ha in the orientation from south-east to north-west. The distance between tree lines was 9 m with a tree row width of 7.5 m and a length of 80 m. The cultivated tree species was the willow hybrid 'Tordis' ((*Salix schwerinii* × *S. viminalis*) × *S. viminalis*). The experimental setup of the present study, which was conducted on the grassland areas between the tree lines, refers to a four-factorial field experiment with the factors vegetation composition (diverse / grass-clover), cutting system (frequent / infrequent), position to tree line (middle, close east, close west) and harvest date (up to four dates per year).

Between the tree lines, two different grassland seed mixtures of varying diversity were sown in a randomized block design in 2011. The sown mixtures were i) a perennial ryegrass-white clover sward (GC) comprising of *Lolium perenne* L. and *Trifolium repens* L. (proportion of 31% in the seed mixture) representing the standard of grassland for dairy cows in organic agriculture and ii) a diverse vegetation composition (DIV) with 32 species containing grasses, legumes and dicotyledonous non-leguminous forbs (see Table S1 for details) as reference for extensive grassland swards. According to a first assessment of the botanical composition of the DIV grass sward in 2013 (as part of the study by Graß et al. 2020) showed proportions of 43%, 16% and 41% of grasses, legumes and non-leguminous dicotyledonous species, respectively. Instead of white clover, birdsfoot trefoil (*Lotus corniculatus* L.) was chosen as a legume in that mixture (a list of sown species is

available in the supplementary Table S1). These sown mixtures are denominated as 'vegetation composition' in the present study as swards develop over time and the sowing mixture is not appropriate to describe the vegetation five years after sowing. Within this randomized block design of vegetation compositions, the factor 'cutting frequency' was implemented randomly resulting in a split-plot randomized block design of three replicates (vegetation composition main plot, cutting frequency subplot). The factor cutting frequency describes either a two-cut system (infrequent) common for hay meadows in the region or an intensive cutting frequency (frequent) with three to four cuts annually. The harvests of the infrequent cutting frequency coincided with the second and the last harvest of the frequent cutting frequency and refer to the factor harvest date.

The tree lines were established from south-east to north-west and the experimental plots in the grassland were laid out in transects from tree line to the next tree line. The positions close to the tree lines differed with respect to the cardinal point and were located either south-west (Close West = CW) or north-east (Close East = CE) of a tree line each 0.5 m apart. A third position was located in the middle (Middle = M) between two tree lines at a distance of 4.5 m (for details see e.g. Schmiedgen et al. 2021). The factor 'position' thus allowed the quantification of distance and orientation to the tree line on biomass production of the grassland. Following the study of Graß et al. (2020) trees have caused a spatially distinct variation in legume proportion which declined from the middle between tree lines towards areas close to trees irrespective of legume identity in the originally sown sward. The grassland had not been fertilized since 2012. Trees were harvested for the last time prior to the present study in early 2015. Consequently, the experimental years refer to the second and third years following tree harvest. The height of tree regrowth was on average 2.4 ± 0.1 m to 4.5 ± 0.9 m from 2016 to 2017.

The site is characterized by a temperate climate with an average annual air temperature of 9.2°C and a mean annual precipitation sum of 651 mm (German Meteorological Service (DWD); 1981–2010). The average temperatures during 2016 and 2017 were 9.8 and 9.9°C, respectively with precipitation sums of 544 mm and 775 mm, respectively. During the experimental period, the temperature during the growing season was higher than the

long-term average, whereas precipitation was lower in 2016 and higher during 2017 (Fig1).
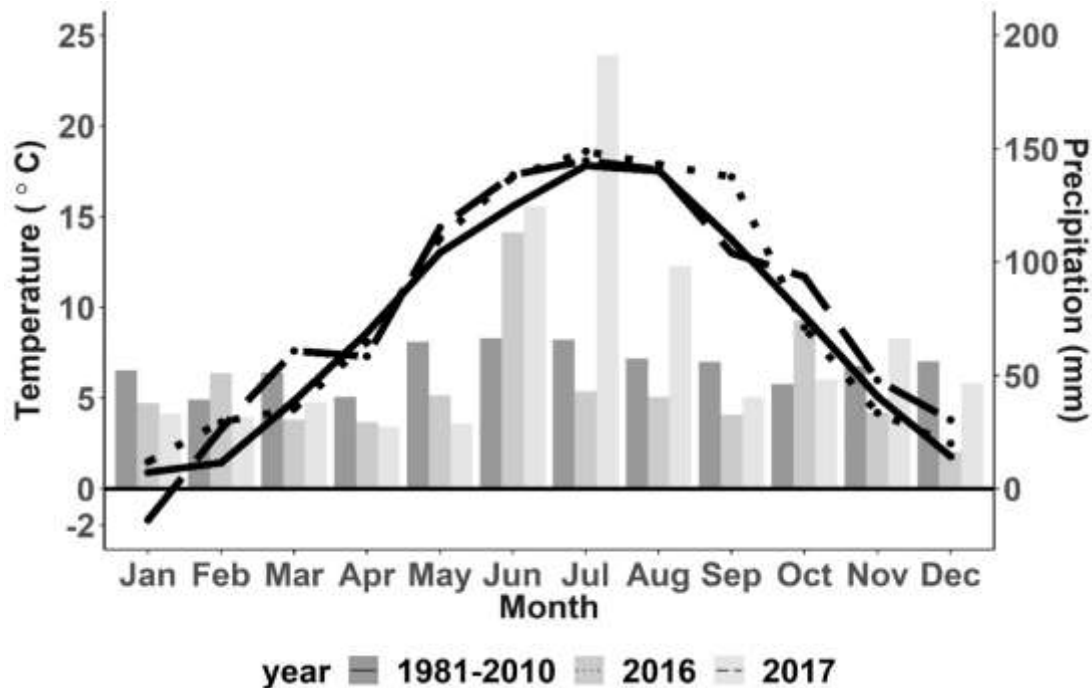
Figure 1 Monthly temperature and precipitation (average daily temperatures, sum of precipitation per month) for 2016, 2017 and long-term average (1981-2010) at Reiffenhausen (DWD weather station "Göttingen").

Measurement of herbage production

The herbage production of the grassland was determined by two approaches that aimed at i) capturing the temporal dynamics during the growing season and at ii) determining the herbage accumulation at harvest within each cutting frequency. With the first approach, we assessed the seasonal dynamics of the standing aboveground grassland herbage biomass with a double sampling method ('t Mannetje, 2000). Approximately every two weeks the compressed sward height (CSH) was measured using a rising-plate meter of 30 cm diameter and 200 g plate weight (Castle, 1976). This was done in every plot at the three positions in a square of 0.25 m2. In total, nine and 13 dates of CSH measurements were obtained in 2016 and 2017, respectively, including the dates around cutting frequency-specific harvests. To calibrate the relationship between CSH (sward height) and grassland herbage mass based on linear regression models as described by

Şahin Demirbağ et al. (2009) four 50 cm × 50 cm quadrats per treatment (cutting frequency × vegetation composition) laying outside of the experimental plots, were cut to near ground surface level. This was done every four weeks in addition to CSH measurements. Hereby, the full gradient of sward heights and different distances to the tree line were considered. Sampled quadrats close to tree line were not included due to limitations in the spatial arrangement of the study site. The biomass samples were oven-dried at 105°C for at least 24 hours to obtain the dry matter content (DM). Based on the relationship between CSH and herbage dry-matter of the respective quadrats, the herbage mass was modeled for the dates without calibration sampling. The R^2 of modelled vs. determined herbage mass was 0.95 with an RSME and RSE of 8.04 and 8.7, respectively.

The second approach was based on manual herbage sampling at the harvest dates of each cutting frequency and aimed at determining the amount of live and dead herbage accumulation at harvest of each treatment (Table 1).

Table 1. Dates of harvest of the frequent and infrequent cutting frequency (CS) in 2016 and 2017. The infrequent cutting frequency was harvested at every second date of the frequent cutting frequency with one exception. *for data analysis, harvests were used as factors irrespective of cutting frequency-specific dates.

	Harvest date 1	Harvest date 2	Harvest date 3	Harvest date 4
2016				
Frequent CF	06.06.	19.07.*	21.09.	
Infrequent CF		06.07.	21.09.	
2017				
Frequent CF	19.05.	29.06.	14.08.	13.10.
Infrequent CF		29.06.		13.10.

Within the second approach, all treatments were sampled together at every harvest date in order to determine the potential performance of the infrequent cutting frequency at the harvest dates of the frequent cutting frequency. Consequently, up to four annual harvests are obtained irrespective of cutting frequency treatment. However, only two harvests per year are actually valid for calculating herbage accumulation of the infrequent cutting frequency. The first harvest date of the infrequent cutting frequency in the year 2016 was not identical with the second harvest of the frequent cutting frequency due to technical reasons (Table 1). The samples taken at harvest in the second approach were

obtained by cutting to a residual stubble height of three centimeters in quadrats measuring 0.04 m^2 in size. The harvested fresh matter was immediately taken to the laboratory and separated manually into dead (> 80% chlorotic plant material) and live green herbage mass. Within the live green herbage, the botanical composition was determined by means of separating between grasses, legumes and dicotyledonous non-leguminous herbs. The separated samples were dried in a forced-air oven at 60°C for 48 hours and weighed afterwards to determine the DM content of live and dead herbage in the different functional groups. To combine and compare the two approaches for the determination of herbage production in this study, the proportions of live and dead herbage as determined in the second approach were scaled to the herbage mass as determined by the first approach based on the CSH method. The amount of live and dead herbage consequently refers to the proportion expressed on the herbage production from the calibration model of CSH measurements.

For each sample of the actual harvest date, the carbon (C) and N concentration of the dead and the live green herbage were determined by DUMAS combustion using a CN elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany). For this, the manually separated subsamples of live herbage (legume, forb, grasses) were pooled before analysis and each sample was milled to pass a sieve size of 0.2 mm using a Retsch mill after drying.

The herbage parameters considered in the present study were i) the live herbage mass at harvest (g DM m^{-2}) (as based on proportions obtained from sampling at harvest and translated to the values determined by the first approach using double sampling), ii) the dead herbage at harvest (g DM m^{-2}), and iii) the proportion of legumes in the live herbage at each harvest.

Assessments of N resorption were based on the NRE and N resorption proficiency (NRP), which represents N concentration in senesced dead herbage and is considered N remaining in dead herbage at harvest before being resorbed into green leaves (Killingbeck 1996). Thus, N resorption was calculated based on the N concentration in live and dead herbage. The live herbage DM and its N concentration thus characterizes the relevant quantity and quality of harvestable herbage regardless of whether it is potentially taken up by a grazer or harvested by cutting.

The NRE represents the percent reduction of nitrogen (NREperc) between green and senesced leaves and was calculated by the following equation (Killingbeck 1996):

$$NREperc = \frac{(N\ concentration\ live - N\ concentration\ dead)}{N\ concentration\ live}$$

Herbage mass-related NRE (NREmass) describes the proportion of N mass in the live-green herbage as a proxy for the N in the green herbage which can be utilized efficiently by grazing herbivores or by harvesting. NREmass was determined as follows:

$$NREmass = \frac{((N\ yield\ live\ herbage) - (N\ yield\ dead\ herbage))}{(N\ yield\ live\ herbage)}$$

The N yield of the live and dead herbage was calculated using the formula:

$$N\ yield\ herbage\ \left[\frac{g}{m2}\right] = \frac{DM\ herbage\ [g/m2]}{100} * Nconcentration\ [\%]$$

In interpreting NRP, we consider low values of N content in dead herbage per unit mass as high NRP.

Data analysis

All data analyses were performed in R 3.6.2 (R Core Team, 2019). A global generalized least squares model, including the single and interactive effects of CSH, vegetation composition, cutting frequency and date (of CSH measurement), was fitted to predict standing biomass based on the calibration cuttings. The final model for biomass from CSH measurements included the interaction between CSH and date as well as CSH and vegetation composition. All total herbage data presented in this study refer to modelled values obtained from the regular CSH measurements.

Linear Mixed Effects Models (lme) were applied to analyze the effects of the fixed factors harvest date (including year), position, cutting frequency and vegetation composition and their interactions, as well as block. This model was applied to the dead and live herbage production, the proportion of legumes, the N concentration of the live and dead herbage, the CN ratio of the live and dead herbage, the NREpercent and NREmass, the live and

dead herbage yield and the live and dead herbage N yields by using the nlme package (Pinheiro, 2019). Owing to the varying sampling dates within both years, the factor date corresponds to a combination of year × harvest date to analyze all data within one model. The random factor plot accounted for repeated measurements on the same experimental unit throughout the study years. Automated model selection was assessed using the "dredge" function of the MuMIn package (Barton, 2018). Subsequent analyses of variance (ANOVA) were followed by multiple contrasts performed using the emmeans package (version 1.3.2, Lenth, 2020).

Model assumptions were tested graphically for the criteria of normal distribution and variance homogeneity. Significance level for analysis was set at $P < 0.05$. To improve normal distribution, the proportion of legumes was logit transformed. Further, data was log or logit transformed and variance heterogeneity was accounted for by using "weights" function of the stats package (version 3.6.2, R core Team, 2019) where required.

Results

Legume proportion

The legume proportions differed among position × vegetation composition (F=3.0**) and date × vegetation composition (F=3.4**). In both vegetation compositions, the legume proportion was significantly greater at position M (21.7% ± 3.7) than at both positions close to tree lines (CE, CW, 12.1% ± 2.1; Table 2). The GC sward had a legume proportion that was two times greater than those of DIV at positions CW and M (23.9% ± 3.8 vs. 11.2% ± 2.1; Table 2).

Table 2. Estimated means ± SE (standard error of the mean) of legume proportion (%) averaged over the harvest dates as affected by the interaction of vegetation composition × position. Lowercase letters show significant differences between the positions within vegetation composition and uppercase letter show differences between the vegetation compositions within positions.

Factor	CW	M	CE
Diverse (DIV)	7.3 ± 1.3 aA	15.0 ± 2.8 bA	9.2 ± 1.8 aA
Grass-Clover (GC)	19.5 ± 3.0 bB	28.3 ± 4.5 cB	12.4 ± 2.3 aA

N concentration and internal N cycling

The interaction of cutting frequency and position had no significant effect on any of the measured or calculated N-related parameters. A position × vegetation composition interaction effect was found for the N concentration in live and dead herbage and the CN ratio of live herbage (Table 3).

Table 3. Results of the linear mixed effects models for the N concentration in dead (NRP, respectively) and live herbage (mg g-1 DM), CN live and dead, the NREperc and the NREmass with F- and P-values. Significance level was set at P < 0.05 with * P < 0.05, ** P < 0.01, *** P < 0.0001. If no effect on respective variable was found it is marked with "–".

Effect	N concentration live [mg g^{-1}]	N concentration dead [mg g^{-1}]	CN live	CN dead	NREperc [%]	NREmass [%]
	F	F	F	F	F	
Position (P)	21.5***	10.0***	25.1***	5.4**	-	8.3**
Date	61.3***	21.9***	36.3***	22.4***	21.6***	26.5***
Vegetation composition (VC)	11.2*	5.3	10.7*	4.9	0.2	-
Cutting frequency (CF)	2.3	-	2.7	-	2.6	6.4*
Date × P	4.7***	-	2.9**	-	-	-
Date × VC	2.9**	-	3.3**	-	3.7**	-
Date × CF	15.6***	-	10.4***	-	7.9***	4.3**
P × VC	7.3**	3.0*	4.5*	-	-	-

Regarding differences in the N concentration of live herbage between the swards, GC had with 24.2 mg g^{-1} ± 0.7 a 1.2 times higher N concentration at the M and CW positions than the DIV sward (Table 4A). Nitrogen concentration in live herbage of GC at position M was 1.2 times higher than at the positions close to the tree lines (Table 4A). In DIV, the N concentration in live herbage at position M was significantly higher than at position CE (Table 4A). The CN ratio in live herbage was significantly wider in DIV than in GC, but not affected by position. In the GC sward, CN ratio was wider at positions closer to the trees (Table 4B) explaining the interaction. Dead herbage in the GC swards had 1.3 times higher N concentration in the middle than in the positions close to tree lines. At the M position N concentration in dead herbage was significantly higher in GC than in DIV (Table 4C). Variation as caused by the dynamics of the growing season was captured by the effects of date with all other factors (Table 3) (see Text and Tables S2 − S7 in supplement for details).

Position had a significant effect on NREmass (Table 3) which was significantly higher at the M position (74.7% ± 1.8) compared to the positions close to tree lines (CW, CE, 70.3% ± 2.1).

Table 4. Estimated means ± SE (standard error of the mean) of the N concentration (mg g^{-1} DM) A) in and the CN ratio of live herbage B) and the N concentration (mg g^{-1} DM) in the dead herbage C) as affected by position × vegetation composition across harvest dates. Lowercase letters show significant differences between the positions within vegetation composition (rows). Uppercase letters show significant differences between vegetation compositions within positions (columns).

Factor	CW	M	CE
A) N concentration live			
Diverse (DIV)	19.5 ± 0.6 abA	21.0 ± 0.8 bA	19.2 ± 0.6 aA
Grass-Clover (GC)	22.1 ± 0.6 bB	26.2 ± 0.8 cB	20.5 ± 0.6 aA
B) CN ratio live			
Diverse (DIV)	22.4 ± 0.7 aB	22.0 ± 0.7 aB	23.2 ± 0.7 aA
Grass-Clover (GC)	19.4 ± 0.7 bA	17.8 ± 0.7 aA	21.4 ± 0.7 cA
C) N concentration dead			
Diverse (DIV)	11.8 ± 0.6 aA	12.7 ± 0.8 aA	11.1 ± 0.5 aA
Grass-Clover (GC)	12.4 ± 0.6 aA	16.1 ± 0.8 bB	12.8 ± 0.5 aA

Responses of herbage production and N yield

Here we present data obtained at harvest dates (second approach); the growth dynamics determined by the first approach can be found in the supplement – Table S2).

Table 5. Results of the linear mixed effects models for the live and dead herbage accumulation in the harvested herbage and for the live and dead herbage N yield with F- and P-values. Significant level was set at P < 0.05 with * P < 0.05, ** P < 0.01, *** P < 0.0001. If no effect on respective variable was found it is marked with "-".

Effect	Live herbage yield[a] [g DM m^{-2}]	Dead herbage yield[a] [g DM m^{-2}]	Live herbage N yield[+] [g N m^{-2}]	Dead herbage N yield[+] [g N m^{-2}]
	F	*F*		
Position (P)	212.2***	2.1	6.9**	0.1
Date	102.2***	71.9***	8.0***	15.1***
Vegetation composition (VC)	5.1	3.2	0.4	1.1
Cutting frequency (CF)	0.03	57.2**	6.9*	0.2
Date × P	13.6***	-	7.0***	-
Date × VC	4.6**	-	-	-
Date × CF	24.1***	15.1***	-	5.9***
P × VC	4.8**	2.9	15.8***	3.4*
P × CF	2.4	4.7*	-	0.6
VC × CF	0.3	0.7	-	0.9
P × VC × CF	11.9***	4.7*	-	3.3

[a] Tests are performed on the log scale.
[+] The variance structure varIdent allowed for separate variances per date and cutting frequency.

There was a significant interaction between position × vegetation composition × cutting frequency for the dead and live herbage at harvest (Table 5).

Generally, GC was more productive and consequently had 1.4 and 1.3 times more live herbage yield than the DIV sward at two positions under infrequent defoliation (M and CE) and at one position under frequent defoliation (position CW; Fig. 2a). At other combinations of the factors cutting frequency × position, differences between GC and DIV in live herbage yield were not significant. The live herbage yield was generally greater (1.6 times) in the infrequent than in the frequent defoliation frequency (Fig. 2a), however, this was not the case for the GC sward at the CW position. At position M live herbage yield was two times greater than at the positions close to the tree line (Fig. 2a). Accumulation of dead herbage was 1.7 and 3.3 times greater for positions M and CE of the GC swards, respectively, in the infrequent than in the frequent defoliation frequency (Fig. 2b). Dead herbage yield in position M under infrequent defoliation was almost two times higher in GC than in DIV while no differences were found for the other positions (Fig. 2B).
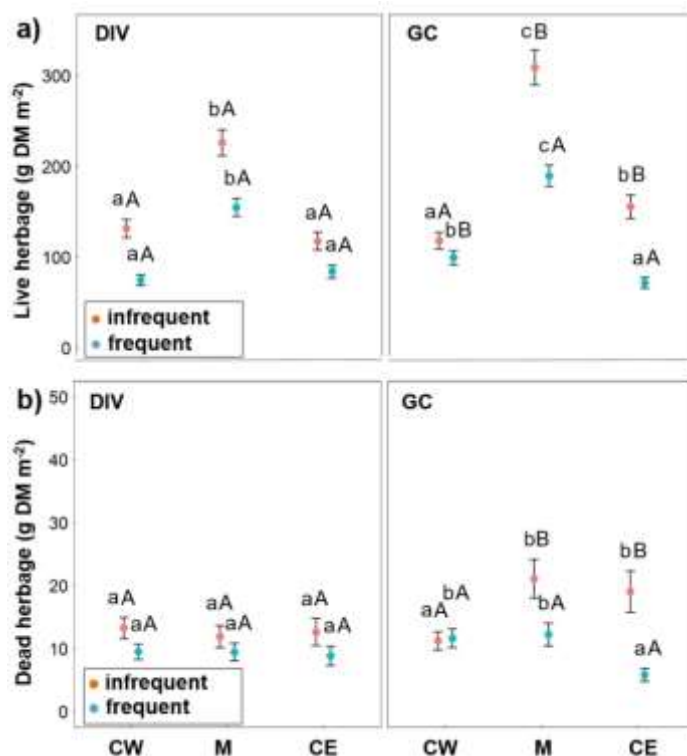
Figure 2. Estimated means of live herbage accumulation (± standard error of the mean) a) and dead herbage accumulation at harvest b) averaged over up to four harvest dates per year and given for each position × cutting frequency of the two vegetation compositions (DIV, GC). Lowercase letters show significant differences between positions within cutting frequency and vegetation composition. Uppercase letters show differences between vegetation compositions within cutting frequency and position (P<0.05). CW: close west; M: middle; CE: close east; DIV: diverse; GC: grass clover.

A significant cutting frequency effect was found on the live N yield (Table 5) which was caused by significantly greater N yield in the infrequent compared to the frequent defoliation frequency (3.7 ± 0.2 vs. 3.2 ± 0.1 g N m^{-2}). The significant interaction of position × vegetation composition (Table 5) was related to a significant difference between DIV and GC at the M position (4.2 ± 0.2 vs. 6.3 ± 0.2 g N m^{-2} for DIV and GC, respectively) whereas the two vegetation compositions did not differ in N yield close to the trees. For both vegetation compositions N yield of live herbage was greater at the M position compared with the positions close to trees (Table 6A). The N yield in dead herbage of GC was greater than of DIV at the M position (Table 6B). At position M dead herbage N yield was increased in GC but not in DIV explaining the significant interaction of position × vegetation composition (Table 3).

Table 6. Estimated means ± SE (standard error of the mean) of the N yield in the live A) and dead herbage (g N m^{-2}) B) as affected by position × vegetation composition across harvest dates. Lowercase letters show significant differences between the positions within vegetation composition (rows). Uppercase letters show significant differences between vegetation compositions within positions (columns).

Factor	CW	M	CE
A) N yield live			
Diverse (DIV)	2.5 ± 0.6 aA	4.2 ± 0.2 bA	2.5 ± 0.3 aA
Grass-Clover (GC)	2.8 ± 0.2 aA	6.3 ± 0.2 bB	2.4 ± 0.3 aA
B) N yield dead			
Diverse (DIV)	0.3 ± 0.03 aA	0.3 ± 0.03 aA	0.3 ± 0.03 aA
Grass-Clover (GC)	0.3 ± 0.03 aA	0.4 ± 0.03 bB	0.3 ± 0.03 aA

Discussion

Internal nitrogen cycling in silvopastoral grassland and the role of legumes

It is known from more natural ecosystems that internal nutrient recycling contributes to the effective use of limited resources and thus to the conservation and productivity of these natural areas (Eckstein et al. 1999; Freschet et al. 2010). In contrast, little attention has been paid to the internal N recycling in an agronomic context. In forage production plant communities with a diverse species composition may contain a variety of traits and survival strategies which also affects the internal N resorption on both, plant and community level (e.g. Ladouceur et al. 2019; Lachaise et al. 2020). In order to take a first step towards elucidating the importance of internal N recycling in legume-based grassland, we have examined two differently composed vegetation types in a silvopastoral system. Apart from intra- and interspecific competition among the grassland species there is also competition with trees, which exert a strong effect on several functions such as biomass production (e.g. Schmiedgen et al. 2021).

The hypothesis of the present study was that the N resorption efficiency (NRE) of legume-based silvopastoral grassland is affected by the interaction of distance to tree line and cutting frequency. In addition, NRE was assumed to compensate for the loss of legumes and thus the loss of N concentration in the sward near trees. In contrast, the reaction of N resorption in the different positions to tree line and to the two cutting frequencies was very limited. It is suggested that NRE increases with increasing N concentration of the live leaf (Kobe et al. 2005). However, N concentration along with herbage yield and N yield in dead and live herbage followed the same pattern: they all declined from the mid position in the grassland alley to the positions close to trees. The decline of N concentration in live and dead herbage with proximity to trees was of similar magnitude, which resulted in similar NRE. In contrast, a decline of N concentration of live herbage was observed towards the tree lines often irrespective of vegetation composition and in the grass clover this also occurred in the dead herbage N concentration. Generally, and in contrast to our findings, we would have expected an increase of N concentrations in live and dead

herbage under shade and therefore closer to the trees (Awada et al. 2003; Buergler et al. 2005; Pang et al. 2019). Both in temperate climate (Graß et al. 2020) and in the tropics (e.g. Gomes et al. 2020), shade competition leads to reductions in biomass production. In a previous study, it was shown that herbage accumulation was lower near trees (Schmiedgen et al. 2021). It follows that N dilution effects in the biomass, which usually occur during growth under common environmental conditions (Duru et al. 1997), played no role in the present study. This is particularly evident in the fact that GC usually resulted in more biomass production than the DIV sward, but the N concentration of dead and live herbage was still mostly above that of DIV (see Table 4, Figure 2). In addition, we expected increasing N concentrations in dead herbage tissue under shade than in the middle of the field away from the tree lines due to the lower N demand of slower growing leaves under shaded conditions. Against this expectation of patterns, N concentration in the dead herbage (along with live herbage N concentration) declined towards trees. It is therefore more likely that the lower N concentration in the positions close to trees in our experiment was directly related to much smaller legume proportions at these positions (Table 2). In both vegetation compositions, the proportion of legumes declined strongly from the middle between tree lines towards the trees, by 45% on average. This finding is in line with other investigations at the study site (Ehret et al. 2018; Graß et al. 2020) who explained this decrease by shading. Dodd et al. (2005) described a significant decrease in the proportion of *Trifolium repens* and *Lotus spp.* with increasing shade level (0–90% shade intensity) and shade duration (3–12 months). Reduced light incidence beneath trees in silvopastoral systems was often used as explanatory variable for reactions in herbage accumulation (e.g. Gomes et al. 2020; Mercier et al. 2020) in studies across the globe. In our experiment, shading appeared to have a strong influence on botanical composition and internal N partitioning between dead and live herbage. This has, to our knowledge, not been shown previously. Frame (2019) explained smaller proportions of *Trifolium repens* and *Lotus corniculatus* under shade with their sensitivity to reduced light interception, which can be transferred directly to the situation of the present experiment where both legumes were part of the vegetation. The different direction of the decrease in legume proportions between GC and DIV is worth noting (Table 2). While in GC the greater decrease of approximately 56% took place towards CE (viewed from M), the

decrease of approximately 50% in DIV was irrespective of position (viewed from M) (Table 2). The stronger decline in legume proportion within GC towards position CE was likely not the result of light availability because CW had lower light than CE in a previous study (Ehret et al., 2018). Differences between the two legume species that is *Trifolium repens* in GC and *Lotus corniculatus* in DIV, might have played a role, too. The possibly better adapted legume *L. corniculatus* was rather subdominant in DIV and the legume proportion was lower than the legume proportion in GC (see Table 2). The lack of effect in response to position in DIV may therefore be due to legume identity and proportion in the vegetation. Mechanisms of recruitment and survival of individual legume identities in shaded areas under varying light orientations require further investigation.

Potential role of non-leguminous companion species may help in explaining the lack of effect on internal nitrogen cycling

Legumes as N fixing species contain usually a higher N concentration than the herbage of unfertilized non-legumes, especially in live green herbage (Killingbeck 1996; Whitehead 2000). Nutrient resorption can also be quantified by resorption proficiency (NRP), a parameter describing the level to which senescing tissue is depleted by N translocation to new growing tissue. Higher NRP corresponds to lower final N concentration in dead herbage and likely lower litter quality, as indicated by a wider ratio of carbon (C) to N. Dead herbage of legumes also has higher N concentrations than non-legumes which would correspond to a lower NRP, smaller CN ratio and higher litter quality (Oikawa et al. 2020; Martin et al. 2017). Generally, the positive response in N concentration in legumes to a reduced light availability is less strong than in grasses. In legumes, less light availability reduces the leaf:stem ratio by elongation of stems leading to reduced N concentrations in the whole plant biomass (Buxton and Mertens 1995; Lin et al. 2001). A lower N concentration of a legume under shade than under full sunlight was also found in a study of Barro et al. (2012) in the tropical regions of southern Brazil where they tested responses in yield and N dynamics of warm-season native forage grasses and one legume under two shading levels compared with full sun. The response of the legume was contrasting to that of the grasses, which showed higher N concentrations under shade

than under full sun. In the latter study, the negative response of the N concentration of the legume under restricted light incidence was explained by a negative effect of shading on the biological N-fixation because of lower nodulation, which also decreased forage yield. In contrast to that, shaded grasses in temperate climate reacted much stronger with a 50% higher N concentration compared to unshaded controls (Koukoura 2009; Wang et al. 2016). With respect to N cycling in the present study it is noteworthy that despite a reduction in legume proportion of on average 45% from the middle towards the tree positions, the reduction in N concentration was less pronounced with an average of 11 and 18% for the DIV and GC vegetation, respectively, when averaged across harvests and cutting frequencies. Consequently, the non-legume vegetation might have had reacted to tree shade with much higher N concentrations as expected. Support for this assumption can be derived from the fact that, despite the different cutting systems, no clear significant effect on the internal N concentration could be found in the present study. In comparison to infrequent disturbance by defoliation, frequent disturbance of the plant biomass by defoliation generally results in higher N concentration in the harvested herbage of plant communities (Sheldrick et al. 1986). Species-specific reactions of the internal N-cycles of grasses, legumes or herbs in mixed grass swards were not the subject of the current study, which deals with the plant community-weighted reactions, because it is usually the standing biomass as a whole that is harvested (Mercier et al. 2020) or ingested by herbivores during grazing (Rosenthal et al. 2012). It remains open whether the functional groups (i.e. grasses, forbs and legumes) in the live herbage within the mixed legume-grass swards reacted differently in their N concentration. In a study in South America, Distel et al. (2003) showed that grasses adapted to nutrient-poor conditions generally recycle nutrients more efficiently. We believe that our results reinforce the need for future research on the topic.

Evaluation of nitrogen resorption efficiency by NREperc and NREmass

The NREmass largely followed the patterns of herbage mass and N concentration and was greater in the middle of the grassland alley. This may be due to differences between the positions in 1) N concentration and 2) total herbage mass. Both were greater in the live

and dead herbage in the mid position than in the positions close to tree lines irrespective of cutting system and vegetation composition. The NREperc as such describes the process of internal N translocation from dead to live herbage tissue (Killingbeck 1986) and increases with increasing live leaf N concentration (Kobe et al. 2005; Vergutz et al. 2012). In both vegetation compositions, GC and DIV, NREperc did not vary among the positions. This seems surprising, since the N concentration of the live herbage was higher in the middle of the alley, which could be explained by higher legume proportions. The NREperc also increases with lower dead herbage N concentration (higher NRP). However, this effect may have been counteracted by the negative effects of shading on NREperc close to the tree line. It is likely that shading reduces growth and lowers the demand of the sink organs accordingly (Hikosaka et al. 2005, Yasumura 2007). Also, NREperc did not differ among the vegetation compositions. While the GC sward had higher legume proportions, the DIV had a 1.7 times higher proportion of dicotyledonous non-leguminous forbs than GC; the proportion of grasses was similar in both swards. As monocotyledons have a higher NRE than dicotyledons (Wang et al. 2018; Vergutz et al. 2012) the counteracting effects of proportion of legumes and forbs would then lead to similar NREperc for both vegetation compositions. The ability of certain grass species to recycle nutrients is also determined by their natural origin (Distel et al. 2003).

We also expected an effect of cutting frequency on NRE, as longer growing intervals allow for greater nutrient resorption (Wang et al. 2016). However, even in our defoliation management with rather long intervals of up to 12 weeks between defoliations (infrequent cutting system), we did not find a clear response in internal N resorption between the cutting frequencies. It therefore seems likely that in managed grassland swards, defoliation intervals between harvests are often too short for a relevant contribution of internal N resorption. Studies in more natural ecosystems such as fens and bogs (Eckstein et al. 1999), where hardly any biomass removal occurs within one year, have shown that the internal resorption is particularly relevant. Under such conditions, however, there is no disturbance due to defoliation. In managed grassland, defoliation affects the resorption process, because both the sink (green leaves) and the source (old senescing leaves) organs are directly removed at harvest.

We conclude that the effects on the internal N cycling of swards in silvopastoral grassland managed for forage production with short defoliation intervals are far less relevant than in natural and unmanaged ecosystems. Short intervals between defoliation events also result in a shorter time for N resorption from dead senescent tissue back to live tissue.

Legume-based plant communities for silvopastoral systems require shade-tolerant legumes

The change in N yields caused by variation in defoliation frequency and the reduced productivity in this respect with more frequent disturbance compared to less frequent ones (Sheldrick et al. 1986) can also be confirmed in the present study. In the present case, however, this was due to reductions in both dead and live herbage production in the infrequent cutting system at unchanged N concentration.

In view of the lower legume proportions near the tree lines, the combination of trees with legume-based grassland is consequently challenging – this would particularly affect organic farming where legumes play a great role. In legume-based grassland, legumes fulfil several functions all of which rely on the provision of N from biological N2 fixation (Suter et al. 2015). Especially organic agriculture relies on this N input and, therefore, a consistent and homogenous distribution of N provision through legumes is relevant for nutrient cycling in these agricultural systems. In general, a reduction of legumes near trees in silvopastoral systems is relevant to management if no additional N is applied (Fox et al. 2020; Komainda et al., 2021). The overruling importance of legume presence is therefore significant for future sustainable agricultural production. Another important agronomic variable that could be affected by changes of the botanical composition and especially by the legume proportion in silvopastoral systems is the soil organic carbon (SOC) stock as a relevant advantage of silvopastoral systems (Cardinael et al. 2017). Legumes alter SOC through their positive biomass production and the related N-input into the soil (De Deyn et al. 2011). In our study we could show strong spatial differences in legume proportion depending on distance to the tree line – the long-term consequences of this effect need to be addressed in future research.

However, the use of sward botanical compositions that are designed for the special situation in silvopastoral systems can help to improve the grass production. For instance, Mercier et al. (2020) in a multi-year study under temperate climate showed that the more shade tolerant grass species Dactylis glomerata (Belesky et al. 2005) as valuable grass species from an agronomic point of view is well suited for mixed cultivation with legumes (Jones and Tracy 2015), especially with Lotus corniculatus (Farnham and George 1994).

No assured influence of vegetation composition of mixed plant communities, no effect of varying defoliation frequency, and no influence of tree shading suggest that little control of varying N availability from modified legume proportions via internal N recycling occurs in managed silvopastoral grasslands. Defoliation intervals are likely too short and therefore N resorption far less relevant than in natural and unmanaged ecosystems. It remains to be seen whether a plasticity of functional groups with regard to N recycling comes into play in mixed swards under shade. For this, precise investigations of the individual species or functional groups and their N concentration are first required.

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Supporting information

Table S1. Sown species and proportions (% weight basis) of the diverse vegetation composition at RH at sowing in 2011. The mixture was established at a seed rate of 5 g m^{-2}.

Species	%	Species	%
grasses			
Agrostis capillaris	5.0	*Galium mollugo*	1.5
Anthoxanthum odoratum	5.0	*Galium verum*	0.5
Bromus hordeaceus	5.0	*Hypericum perforatum*	1.5
Cynosurus cristatus	5.0	*Knautia arvensis*	1.0
Festuca brevipila	20.0	*Leucanthemum ircutianum*	1.5
Festuca nigrescens	15.0	*Lychnis flos-cuculi*	0.5
Festuca rubra subsp. Rubra	10.0	*Malva moschata*	1.5
Poa nemoralis	3.0	*Papaver rhoeas*	1.5
Poa trivialis	2.0	*Pimpinella saxifraga*	1.5
		Prunella vulgaris	1.5
legumes		*Salvia pratensis*	2.0
Lotus corniculatus	0.3	*Sanguisorba minor*	2.5
		Silene dioica	1.0
dicotyledonous forbs		*Silene vulgaris*	1.0
Achillea millefolium	0.8	*Tragopogon pratensis*	2.0
Agrimonia eupatoria	1.0		
Agrostemma githago	2.0		
Ajuga reptans	0.2		
Campanula patula	0.2		
Centaurea cyanus	2.0		
Centaurea jacea	1.5		
Crepis biennis	1.0		

Table S2. Estimated means ± SE of the mean for the total herbage production as affected by the interaction of A) date × position, B) date × vegetation composition and C) date × cutting frequency. Letters show significant differences between the positions within date and year A), between the vegetation compositions within date and year B) and between cutting frequencies within date and year C). Date 3, 5, 8 of 2016 and 4, 6, 9, 13 of 2017 refer to the harvest dates of the frequent cutting frequency and dates 3 and 8 of 2016 and 4 and 13 of 2017 of the infrequent one. VS: vegetation composition, CF: cutting frequency. Shaded areas represent harvest dates within year according to Table 1.

Year	Factor	Date 1	Date 2	Date 3	Date 4	Date 5	Date 6	Date 7	Date 8	Date 9	Date 10	Date 11	Date 12	Date 13
A) position														
2016	CW	111.2 ± 8.1 a	207.0 ± 14.3 a	262.2 ± 20.4 a	149.3 ± 10.6 a	210.2 ± 11.9 a	52.5 ± 5.6 a	88.1 ± 6.3 a	90.3 ± 5.4 a					
	M	130.0 ± 6.6 ab	241.9 ± 11.6 b	337.8 ± 18.1 b	194.6 ± 9.6 b	271.8 ± 10.9 b	93.1 ± 7.2 b	151.4 ± 7.5 b	213.2 ± 9.0 b					
	CE	134.5 ± 9.3 b	249.8 ± 16.2 b	346.3 ± 25.3 b	166.0 ± 11.1 a	200.7 ± 10.7 a	52.2 ± 5.6 a	79.0 ± 5.3 a	83.9 ± 4.7 a					
2017	CW	55.4 ± 3.7 a	60.8 ± 3.6 a	89.5 ± 5.2 a	135.4 ± 11.0 a	76.2 ± 17.6 a	115.8 ± 13.8 a	40.8 ± 2.9 a	43.3 ± 4.4 a	79.6 ± 4.6 a	47.2 ± 5.4 a	59.2 ± 5.9 a	86.5 ± 10.6 a	67.7 ± 9.9 b
	M	58.1 ± 2.7 a	75.7 ± 3.2 b	126.5 ± 5.3 c	216.2 ± 12.0 b	234.8 ± 35.8 b	331.5 ± 26.5 b	47.8 ± 2.4 b	86.4 ± 5.9 b	169.6 ± 7.0 b	72.6 ± 5.6 b	110.1 ± 7.4 b	195.2 ± 16.0 b	173.1 ± 17.8 c
	CE	72.8 ± 4.6 b	74.4 ± 4.2 b	105.0 ± 5.8 b	155.6 ± 11.9 a	92.4 ± 20.0 a	117.7 ± 13.1 a	44.8 ± 3.0 ab	55.0 ± 5.2 a	82.3 ± 4.5 a	49.4 ± 5.3 a	59.7 ± 5.5 a	68.0 ± 7.8 a	44.6 ± 6.1 a
B) VC														
2016	DIV	131.1 ± 6.6 a	215.3 ± 10.4 a	335.3 ± 176 a	175.5 ± 8.7 a	216.4 ± 9.5 a	70.1 ± 5.1 a	106.3 ± 5.3 a	116.1 ± 5.1 a					
	GC	118.9 ± 7.9 a	250.3 ± 15.9 a	292.2 ± 20.6 a	162.6 ± 10.6 a	235.0 ± 12.2 a	57.4 ± 5.8 a	97.5 ± 6.4 a	118.6 ± 6.7 a					
2017	DIV	79.3 ± 3.8 a	80.9 ± 3.5 a	102.8 ± 4.5	148.5 ± 8.1 b	96.5 ± 13.4 a	138.4 ± 10.2 a	53.5 ± 2.6 b	62.7 ± 4.0 a	100.3 ± 4.3 a	63.1 ± 4.5 a	72.0 ± 4.6 a	89.7 ± 6.8 a	65.8 ± 5.8 a
	GC	47.9 ± 3.0 b	60.5 ± 3.4 b	109.2 ± 6.0 a	185.0 ± 13.5 a	144.9 ± 28.7 a	197.5 ± 20.6 b	36.7 ± 2.4 a	55.5 ± 5.0 a	107.0 ± 5.9 a	48.5 ± 4.9 a	74.1 ± 6.5 a	122.1 ± 13.1 a	98.7 ± 12.5 b
C) CS														
2016	Frequent	124.2 ± 7.2 a	226.1 ± 12.4 a	305.1 ± 18.5 a	62.5 ± 3.5 a	137.1 ± 6.7 a	45.1 ± 3.8 a	91.6 ± 5.2 a	105.5 ± 5.2 a					
	Infrequent	125.5 ± 7.2 a	238.4 ± 13.1 a	321.2 ± 19.4 a	456.7 ± 25.8 b	370.8 ± 17.2 b	89.2 ± 7.6 b	113.0 ± 6.4 b	130.6 ± 6.5 b					
2017	Frequent	57.6 ± 3.1 a	67.0 ± 3.3 a	98.7 ± 4.8 a	160.9 ± 10.1 a	47.1 ± 7.2 a	90.3 ± 7.9 a	45.7 ± 2.6 a	59.1 ± 4.5 a	99.4 ± 4.8 a	33.2 ± 2.8 a	50.0 ± 3.7 a	69.6 ± 6.2 a	57.1 ± 6.0 a
	Infrequent	66.0 ± 3.6 a	73.0 ± 3.6 a	113.7 ± 5.5 a	170.7 ± 10.7 a	297.1 ± 48.7 b	302.6 ± 26.5 b	43.0 ± 2.4 a	59.0 ± 4.5 a	108.0 ± 5.2 a	92.1 ± 7.8 b	106.5 ± 7.9 b	157.5 ± 14.1 b	113.8 ± 12.0 b

Text S1. The CN ratio and N concentration of the live herbage, the NREperc and the NREmass as affected by date x position, date × vegetation and date × cutting frequency

The CN ratio and N concentration of the live herbage, the NREperc and the NREmass as affected by date × position, date × vegetation and date × cutting frequency

The interaction of date × position was significant for the N concentration in and CN ratio of the live herbage (Table 2, Table S3). The date × vegetation composition interaction was found to be significant for the N concentration in and CN ratio of the live herbage and the NREperc (Table 2, Table S4) while the interaction of date × cutting frequency was also significant for NREmass (Table 2, Table S5).

The NREperc did mostly not differ between vegetation compositions except at harvest date 1 of 2016, where GC showed a 30.8% greater value than DIV (43.5 ± 2.0 vs. 29.6 ± 2.3, Table S3). Regarding the cutting frequencies, the NREperc of the infrequent cutting frequency was 22.1% higher than that of the frequent cutting frequency at the first harvest date of 2016, whereas at the second harvest date the frequent cutting frequency revealed 66.6% higher NREpercent than the infrequent cutting frequency (Table S5). For the NREmass no differences between the cutting frequencies at the harvest dates were found except for the first harvest in 2017, where the frequent cutting frequency showed significantly higher NREmass than the infrequent cutting frequency (Table S5).

Table S3. Estimated means ± SE of the N concentration live (mg g^{-1} DM) A) and the CN ratio live B) as affected by date × position across vegetation compositions and cutting frequencies. Lowercase letters show significant differences between the positions within harvest date (rows).

Year	Factor	Harvest 1	Harvest 2	Harvest 3	Harvest 4
A) N concentration live					
2016	CW	15.7 ± 0.7 b	19.9 ± 1.0 b	20.2 ± 0.7 a	
	M	12.9 ± 1.0 a	22.7 ± 1.5 b	25.4 ± 1.1 c	
	CE	14.2 ± 0.7 ab	17.3 ± 0.9 a	22.4 ± 0.7 b	
2017	CW	25.7 ± 0.8 b	20.1 1.1 ab	21.6 ± 0.7 a	22.4 ± 0.8 a
	M	27.1 ± 1.2 b	22.2 ± 1.7 b	28.3 ± 1.1 b	26.5 ± 1.2 b
	CE	22.2 ± 0.8 a	18.2 ± 1.0 a	22.2 ± 0.7 a	22.4 ± 0.8 a
B) CN ratio live					
2016	CW	26.4 ± 1.6 a	22.5 1.8 a	21.1 0.7 b	
	M	32.3 ± 1.6 b	21.9 ± 1.8 a	17.4 ± 0.7 a	
	CE	29.1 ± 1.6 ab	26.8 ± 1.8 a	19.1 ± 0.7 a	
2017	CW	16.7 ± 0.6 a	21.8 ± 1.6 ab	19.3 ± 0.6 b	18.7 ± 0.7 b
	M	15.8 ± 0.6 a	20.1 ± 1.6 a	15.4 ± 0.6 a	16.5 ± 0.7 a
	CE	19.0 ± 0.6 b	24.6 ± 1.6 b	18.5 ± 0.6 b	19.0 ± 0.7 b

Table S4. Estimated means ± SE of the N concentration of live herbage (mg g^{-1} DM) A), the CN ratio of live herbage B) and the NREperc C) as affected by date × vegetation composition across cutting frequencies and positions. Lowercase letters show significant differences between the vegetation compositions within harvest date (rows).

Year	Factor	Harvest 1	Harvest 2	Harvest 3	Harvest 4
A) N concentration live					
2016	**Diverse (DIV)**	13.1 ± 0.7 a	18.2 ± 1.0 a	20.8 ± 0.8 a	
	Grass-Clover (GC)	15.5 ± 0.7 b	21.7 ± 1.0 b	24.5 ± 0.8 b	
2017	**Diverse (DIV)**	22.5 ± 0.8 a	18.1 ± 1.1 a	22.8 ± 0.7 a	23.9 ± 0.8 a
	Grass-Clover (GC)	27.5 ± 0.8 b	22.2 ± 1.1 b	25.3 ± 0.7 b	23.7 ± 0.8 a
B) CN ratio live					
2016	**Diverse (DIV)**	31.1 ± 1.3 a	26.4 ± 1.5 b	20.8 ± 0.7 b	
	Grass-Clover (GC)	27.1 ± 1.3 a	21.1 ± 1.5 a	17.6 ± 0.7 a	
2017	**Diverse (DIV)**	18.6 ± 0.6 b	24.0 ± 1.3 b	18.7 ± 0.6 a	17.8 ± 0.7 a
	Grass-Clover (GC)	15.7 ± 0.6 a	20.3 ± 1.3 a	16.8 ± 0.6 a	18.3 ± 0.7 a
C) NREperc					
2016	**Diverse (DIV)**	29.6 ± 2.3 a	43.1 ± 3.5 a	27.4 ± 2.8 a	
	Grass-Clover (GC)	43.5 ± 2.9 b	36.6 ± 4.4 a	26.2 ± 3.5 a	
2017	**Diverse (DIV)**	38.7 ± 2.9 a	25.1 ± 4.1 a	52.7 ± 2.3 a	50.7 ± 2.3 a
	Grass-Clover (GC)	42.1 ± 3.6 a	32.1 ± 5.0 a	47.8 ± 2.8 a	44.7 ± 2.8 a

Table S5. Estimated means ± SE of the N concentration of live herbage (mg g^{-1} DM) A), the CN ratio of live herbage B), the NREperc C), the NREmass D) and the HEFF E) as affected by date × cutting frequency across vegetation compositions and positions. Lowercase letters show significant differences between the cutting systems within harvest date (rows).

Year	Factor	Harvest 1	Harvest 2	Harvest 3	Harvest 4
A) N concentration live					
2016	**Frequent**	12.9 ± 0.7 a	23.8 ± 1.0 b	23.3 ± 0.8 a	
	Infrequent	15.6 ± 0.7 b	16.1 ± 1.0 a	22.0 ± 0.8 a	
2017	**Frequent**	25.1 ± 0.8 a	21.5 ± 1.1 a	23.6 ± 0.7 a	26.1 ± 0.8 b
	Infrequent	24.9 ± 0.8 a	18.8 ± 1.1 a	24.5 ± 0.7 a	21.4 ± 0.8 a
B) CN ratio live					
2016	**Frequent**	31.2 ± 1.3 a	18.0 ± 1.5 a	18.8 ± 0.7 a	
	Infrequent	27.4 ± 1.3 a	29.5 ± 1.5 b	19.6 ± 0.7 a	
2017	**Frequent**	17.0 ± 0.6 a	20.1 ± 1.3 a	18.2 ± 0.6 a	16.0 ± 0.7 a
	Infrequent	17.3 ± 0.6 a	24.2 ± 1.3 a	17.3 ± 0.6 a	20.1 ± 0.6 b
C) NREperc					
2016	**Frequent**	32.0 ± 2.5 a	57.6 ± 3.6 b	26.6 ± 2.8 a	
	Infrequent	41.1 ± 2.9 b	22.1 ± 4.3 a	27.0 ± 3.5 a	
2017	**Frequent**	39.6 ± 2.9 a	29.5 ± 4.1 a	52.1 ± 2.3 a	49.7 ± 2.3 a
	Infrequent	41.2 ± 3.5 a	27.8 ± 4.9 a	48.4 ± 2.8 a	45.7 ± 2.8 a
D) NREmass					
2016	**Frequent**	84.5 ± 1.2 a	30.2 ± 10.4 a	44.3 ± 8.6 a	
	Infrequent	84.5 ± 1.5 a	38.4 ± 13.4 a	29.8 ± 11.1 a	
2017	**Frequent**	89.4 ± 1.8 b	93.5 ± 1.3 a	88.a ± 2.6 a	89.0 ± 4.2 a
	Infrequent	73.8 ± 2.4 a	94.2 ± 1.6 a	88.2 ± 3.4 a	77.8 ± 5.4 a
D) HEFF					
2016	**Frequent**	0.90 ± 0.01 a	0.78 ± 0.05 b	0.59 ± 0.08 a	
	Infrequent	0.92 ± 0.01 a	0.61 ± 0.05 a	0.60 ± 0.08 a	
2017	**Frequent**	0.95 ± 0.01 a	0.90 ± 0.03 a	0.93 ± 0.01 a	0.93 ± 0.01 b
	Infrequent	0.97 ± 0.01 a	0.91 ± 0.03 a	0.95 ± 0.01 a	0.86 ± 0.01 a

Text S2. The live herbage production as affected by the interaction of date × position, date × cutting frequency and date x vegetation composition

At each harvest, the live herbage accumulation was significantly greater in the M position compared to the positions close to tree line with one exception, where the CE position was similar to the M position (harvest date 1, 2016) (Table S6A). If there was a harvest of the frequent cutting frequency, the live and the dead herbage responded mostly with significantly greater values in the infrequent than in the frequent cutting frequency (Table S6B, Table S7). During 2016, the amount of live herbage at harvest did not differ between vegetation compositions, while during 2017 GC mostly produced higher amounts than DIV (Table S6C) explaining the interaction of harvest × vegetation composition.

Table S 6. Estimated means ± SE (standard error of the mean) of the live herbage production as affected by the interaction of date × position A), date × cutting frequency B) and date × vegetation composition C). Lowercase letters show significant differences between the positions A), cutting frequencies B) and vegetation composition C) within harvest date and year.

Year	Factor	Harvest 1	Harvest 2	Harvest 3	Harvest 4
A) Position					
2016	**CW**	250.0 ± 19.0 a	161.1 ± 12.3 a	62.3 ± 6.0 a	
	M	304.3 ± 16.4 b	210.1 ± 11.4 b	162.7 ± 11.0 b	
	CE	323.0 ± 26.6 b	156.5 ± 12.9 a	54.2 ± 5.7 a	
2017	**CW**	125.5 ± 9.7 a	99.9 ± 12.5 a	72.4 ± 4.2 a	56.5 ± 8.4 a
	M	175.0 ± 11.7 b	315.1 ± 27.07 b	166.8 ± 7.0 b	168.8 ± 17.2 b
	CE	147.4 ± 12.3 a	106.9 ± 14.5 b	73.4 ± 4.5 a	37.6 ± 6.1 a
B) CF					
2016	**Frequent**	279.5 ± 17.4 a	111.9 ± 7.0 a	73.0 ± 5.6 a	
	Infrequent	302.4 ± 18.8 a	271.6 ± 16.9 b	92.0 ± 7.0 a	
2017	**Frequent**	150.6 ± 9.5 a	84.9 ± 8.1 a	90.5 ± 5.9 a	52.2 ± 5.9 a
	Infrequent	166.4 ± 10.5 a	264.5 ± 25.3 b	102.0 ± 5.1 a	96.7 ± 10.9 b
C) VC					
2016	**DIV**	311.8 ± 19.3 a	173.1 ± 10.8 a	84.4 ± 6.4 a	
	GC	271.1 ± 16.8 a	175.6 ± 10.9 a	79.5 ± 6.1 a	
2017	**DIV**	141.3 ± 8.9 a	124.0 ± 11.9 a	91.5 ± 4.6 a	57.4 ± 6.5 a
	GC	177.3 ± 11.2 b	181.1 ± 17.3 b	100.9 ± 5.1 a	87.9 ± 9.9 b

Table S7. Estimated means ± SE (standard error of the mean) of the dead herbage accumulation as affected by the interaction of harvest date × cutting frequency. Letters show significant differences between cutting frequencies within date and year.

Year	Cutting frequency	Harvest 1	Harvest 2	Harvest 3	Harvest 4
2016	Frequent	25.2 ± 2.7 a	23.0 ± 2.4 a	27.7 ± 4.6 a	
	Infrequent	23.0 ± 2.5 a	91.7 ± 9.0 b	31.0 ± 5.2 a	
2017	Frequent	5.0 ± 1.3 a	5.7 ± 1.0 a	4.1 ± 1.3 a	3.2 ± 0.6 a
	Infrequent	2.4 ± 0.6 a	19.1 ± 3.4 b	3.2 ± 1.0 a	13.0 ± 2.3 b

CHAPTER III

Effects of nitrogen supply on internal nitrogen resorption of
Lolium perenne* and *Festuca arundinaceae

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Abstract

Adapting grass swards to future climate change conditions is becoming increasingly important. In grassland production in temperate climates, the most commonly used grass species, *Lolium perenne*, is limited in its productivity and persistence under drought events and other forage grass species are needed. The effects of nitrogen (N) availability (low and high N supply) on the N resorption of L. perenne and of the more drought-tolerant grass species *Festuca arundinacea* were investigated in a pot experiment under standardized environmental conditions. Leaf N concentrations of both grass species were greater under high than under low N supply and higher in *F. arundinacea* than in *L. perenne*. At pot level, *L. perenne* showed greater N concentrations than *F. arundinacea* under high N supply what might be caused by dilution effects since *F. arundinacea* revealed higher total DM herbage. The N resorption efficiency (NRE) and proficiency (NRP) of the two grass species showed distinct responses to high N supply while NRP was more responsive than NRE. Under both N supply levels, *F. arundinacea* had the highest NRE and NRP. Concluding, *F. arundinacea* might be a suitable grass species in future grassland production due to its ability to recycle N under varying N availability while maintaining high total DM production.

Introduction

In temperate agricultural grassland of North-West Europe, the most important and most widely used grass species is *Lolium perenne* L. (Lop) due to high yields and nutritional composition (Wilkins 2000, Hopkins and Wilkins 2006). *Lolium perenne* is adapted to the mild winter and cool moist summer conditions prevailing in western and north-western Europe (Sampoux et al. 2013) and is known to have little drought tolerance (Thomas and Evans 1990). Under predicted climate change scenarios, mean summer temperatures are expected to increase while precipitation rate is expected to decline in Central Europe resulting in more extreme weather situations (IPCC 2013). This drought type, called Mediterranean drought type, is characterized by rain-fed winters and irregular precipitation and periods of drought in summer (Lobell and Gourdji 2012). These climatic conditions will have a great impact on Lop with reduced persistence and performance of the grass sward resulting in increasing interest in grass species that are more tolerant to drought stress (Nijs et al. 1996, Westermeier et al. 2016). A more drought-tolerant grass species which also performs well under cutting management is the highly competitive *Festuca arundinacea* Schreb. (Fea; Reheul et al. 2013, Cougnon 2013). It also has a higher yield potential than Lop (da Silveira Pontes et al. 2007, van Eekeren et al. 2010). These features of Fea are gaining increasing attention as they represent an adaption to moisture stress (Reheul et al. 2013, Turner et al. 2012). One constraint is the lower digestibility of Fea compared to Lop due to the chemical composition and the proportion of lignin in leaves and stems. Research already focuses on breeding varieties with higher feeding quality (Suter et al. 2009, Baert et al. 2014).

Different morphological and physiological traits are found for Fea and Lop describing their nutrient use strategy ranging in a spectrum from fast acquisition to efficient conservation of nutrients (Wright et al. 2004). Exploitative species are common in N-rich habitats, with a rapid turnover of N in leaves which is related to fast growth, high rate of resource acquisition, high N concentrations and uptake per unit leaf mass (Lambers & Poorter 1992, Ryser 1996, Wright et al. 2004). These traits can be attributed to the grass species Lop (Grime and Hunt 1975). In contrast, Fea as conservative species (Maire et al. 2009,

Duchini et al. 2018), resident in rather N-poor habitats, is characterized by the conservation of N resulting in slower growth, higher values for dry matter content, high concentrations of cell walls and secondary compounds and a greater leaf and root longevity (Aerts and Chapin 1999, Chapin 1980, Ryser 1996, Reich 1998, Wright et al. 2004).

The nitrogen (N) status of the grass sward is of high importance for forage production since N is one main nutrient most frequently limiting plant growth and production (Chapin 1980, Wedin 1995). To be less dependent on current nutrient uptake the recycling of N is a keystone nutrient-conserving mechanism of many perennial plant species (Chapin 1980, Killingbeck 1996, Aerts and Chapin 1999). To preserve itself from nutrient loss through senescence and cessation, the plant may mobilize N from senescing parts and transport them to other plant tissues, also defined as N resorption efficiency, the percentage of N withdrawn from mature leaves before leaf abscission (NRE; Killingbeck 1986). The process of NRE has profound consequences on N cycling at community and ecosystem level (Aerts and Chapin 1999). Another quantification of N resorption is the resorption proficiency, the concentration of N in senesced leaves (NRP), which is not subject to temporal variation in live leaves and timing of sampling (Killingbeck 1996).

A higher NRE is an adaption advantage for plants in low soil N habitats (Eckstein et al. 1999, Aerts and Chapin 1999). To which extent N availability affects NRE is still much debated. The NRE is predicted to be controlled by soil N availability (Aerts and Chapin 1999, Yuan and Chen 2009, Zhao 2017) or N concentration in plant tissues (Kobe et al. 2005, Vergutz et al. 2012). In a recent meta-analysis with a global data set of 9703 observations at 306 sites from 508 published articles examining the effects of N fertilization on plant leaf N concentrations and resorption efficiency, Yuan and Chen (2015) found decreasing NRE and NRP (increasing N concentrations in senesced leaves) as a response to N fertilization. Within species, these relationships are highly variable with positive, neutral or negative effects of increased fertility on NRE (Aerts 1996, Eckstein et al. 1999, van Heerwaarden et al. 2003b, Lü et al. 2013). According to Vitousek (1998), NRP seems to be more responsive to N availability than NRE potentially detecting varying

responses within species to increased N accessibility. Further, lower N in the dead leaf is associated with a longer leaf lifespan (Wright and Westoby 2003) both strategies to minimize nutrient loss.

Until now, studies examining the growth and N cycling of grasses focused rather on fast-growing exploitative species due to their agronomic relevance than on conservative slower-growing ones (Chapin 1980, Schulte auf'm Erley 2001). There are studies investigating growth and yielding of Lop and Fea (e.g. Becker et al. 2020) but information about the leaf N resorption of the two contrasting grass species is scarce. Therefore, a greenhouse experiment was conducted with Lop and Fea to investigate the effects of two N supply levels on N resorption, i.e. NRE and NRP, and growth of the grass species. The legume *Trifolium repens* L. (Trr) was used as reference species since it is not N limited due to its N fixing ability (Ledgard and Steele 1992). It is hypothesized that i) under high N supply both grass species have a similar NRE while at low N availability the NRE of Fea is greater than of Lop, and ii) that the conservative grass species Fea shows a higher NRP than the exploitative grass species Lop under low and high N supply.

Materials and methods

Experimental set up

The experiment was carried out in a temperature-controlled greenhouse from December 2015 to June 2016 with measurements starting on 4 March 2016 for the duration of 16 weeks. To understand the responses of investment in the production of plant organs and internal N cycling in response to the grass strategy and nutrient supply, a one-factorial experiment was established as a randomized block design with twelve replications. Of this, six replications each were used for destructive and non-destructive measurements, respectively. The experimental factor was 'treatment', defined by the combination of species and N supply. The chosen grass species differed in their nutrient-use strategy: *Lolium perenne* L. (Lop; variety 'Barylou') as an exploitative grass species and *Festuca arundinacea* Schreb. (Fea; variety 'Lipalma') as a conservative grass species. *Trifolium repens* L. (Trr; variety 'Rivendel') as N-fixing species served as a reference. Nitrogen supply started with measurements and comprised two levels of calcium nitrate fertilizer application (N concentration of 15.5%; YaraTera® CALCINIT): either a weekly N supply to ensure unlimited conditions ("high" and "+", respectively; 3.9 g N pot^{-1}) or a two-weekly N supply for N limited conditions ("low" and "-", respectively; 1.2 g N pot^{-1}). Fertilizer was applied as suspension solubilized in 250 ml of water with a concentration of 600 mg l^{-1} and 300 mg l^{-1}, respectively. Water was unlimited for plants throughout the experimental period. Species were sown in Petri dishes in December of 2015 and plants were potted in soil as seedlings in January of 2016. A number of 16 plants per species were arranged equally spaced in 60 square pots in total (two grass species x two N supply rates + Trr reference x twelve replicates) with a width of 18 cm x 18 cm and 15 cm height each. Pots per treatment were later split equally for non-destructive or destructive measurements resulting in six replications per treatment finally. Pots were two-thirds filled with greenhouse compost soil and one-third (on top) with a mixture of 1:10 of sand (sieved with a sieve grain size of 0.4 mm) and greenhouse compost soil. Plant available nutrients and pH value were determined by calcium-acetate-lactate extraction (CAL) and calcium chloride extraction method, respectively (Verband deutscher landwirtschaftlicher

Untersuchungs- und Forschungsanstalten – VDLUFA 1991, chapter A 5.1.1: pH, chapter A 6.2.4.1: magnesium (Mg), chapter A 6.2.1.1: phosphor (P), potassium (K)). The organic matter content of the mixed soil was 9.7%, soil P, K and Mg contents were 103.1 mg 100 g^{-1}, 136.4 mg 100 g^{-1} and 30.8 mg 100 g^{-1}, respectively, and the pH 7.7. The organic matter content of the compost soil was 9.7%, soil P, K and Mg contents were 103.0 mg 100 g^{-1}, 136.0 mg 100 g^{-1} and 30.2 mg 100 g^{-1}, respectively, and the pH 6.8. Plants were cut three times to a residual height of 3 cm (28 January, 9 and 22 February 2016) before measurements started. The day and night temperature (mean ± standard deviation) during the measurement period was 22.2 ± 4.4°C and 14.4 ± 1.9°C, respectively.

Measurements

On plants growing in pots designated for destructive measurements, we randomly selected the youngest fully developed leaf on ten tillers to form five pairs of tillers for studying live and dead leaves of the same cohort and developmental stage. In sum, this makes 30 repetitions per treatment (species and N supply level) for live and dead leaves. At the beginning of April, five weeks after the first N supply, the laminae of five leaves of these youngest leaves were clipped right at the leaf base on the border to the sheath (grasses) or petiole (white clover) and collected. These collected laminae will be termed live leaves in the following. Leaf area of the live leaf (Larea) was measured by scanning the leaf (Epson Perfection V700 Photo) and using the software WinFolia (Régent Instruments, Quebec, Kanada). The remaining five youngest fully developed leaves that were not harvested were marked with a metal wire and the tip of the previous leaf was marked white in case the metal wire was lost. The laminae of the marked leaves were harvested by removing them from the plant when fully senesced at the border to the sheath and categorized into dead leaves. The experiment of the destructive pots was finished by harvesting the last fully senesced marked leaf 16 weeks after the experiment had started (28 June 2016). Harvested live and dead leaves were oven-dried at 60 °C for 48 h to determine the dry matter (DM) weight. Analyses of N and carbon (C) concentration of the leaves were conducted through DUMAS combustion using a CN elemental analyzer (vario EL III, elementar, Langenseibold, DE). Based on these analyses,

the N mass in live and dead leaves was calculated as a product of dry weight and N concentration divided by 100.

The leaf appearance rate (LAR) is an important parameter in the production efficiency of agricultural grassland and describes the number of days required for the emergence of one leaf (Lemaire and Agnusdei 2000). For obtaining the LAR, ten tillers per pot of the non-destructive pots were identified with the first fully developed (from previous cutting undamaged) leaf marked with a metal wire for identifying investigated tillers. A new leaf (leaf tip) and its length were recorded every three days until the end of the experiment. Leaf appearance rate (day^{-1}) was calculated by the mean leaf appearance from the third to fourth leaf and from the fourth to fifth leaf.

Plants of the non-destructive pots were clipped at 3 cm stubble height after 13 weeks and oven-dried at 60 °C for 48 h to determine DM weight. The C and N concentrations were determined by DUMAS combustion using a CN elemental analyzer (vario EL cube, elementar, Langenseibold, DE). The tiller number per pot (tiller density) was determined by counting the tiller of each pot 14 days after clipping plants of the non-destructive pots. The number of tiller per unit area is a common indicator of agronomic status and gives information about the productivity of a sward (Matthew et al. 1996).

Nutrient resorption efficiency variables

The ability of a plant to resorb N from senescing to live leaves plays a major role in nutrient conservation and to be less dependent on external growth conditions, especially on N-poor soils (Pugnaire and Chapin 1992, Lin and Wang 2001). The NRE represents the percent reduction of N (NREperc) of the senesced leaves relative to the live green leaves and was calculated by the following equation (Killingbeck 1996):

$$NREperc = \left(\frac{N\ concentration\ live\ leaf\ (\%) - N\ concentration\ dead\ leaf\ (\%)}{N\ concentration\ live\ leaf\ (\%)} \right) * 100$$

The N mass which is resorbed from the senescing to the live leaf relative to the N mass of the live leaf (NREmass), describes the extent of resorbed N mass in relation to the tissue

DM. To determine the N mass resorption from the dead to the live leaf, NREmass was considered as follows:

$$NREmass = \frac{(N\ yield\ live\ leaf - N\ yield\ dead\ leaf)}{(N\ yield\ live\ leaf)}$$

The N yield of the live and the dead leaf was calculated using the formula:

$$Leaf\ N\ yield\ [mg] = \frac{DM\ leaf\ [mg]}{100 * N\ concentration\ leaf\ [\%]}$$

Nitrogen resorption proficiency (NRP) was quantified as the absolute level of remaining N in the dead leaf which is not resorbed (Killingbeck 1996). Therefore, low N concentrations in the dead leaf signify a high value of NRP.

Data analysis

Since white clover was not tested under the two N rates, the fixed factor treatment, which represents a combination of species and N supply, was used as predictor of the response variables at leaf level (Larea, DM, N concentration and CN ratio of the live and the dead leaf, NREperc, NREmass), at pot level (tiller density, LAR, DM, N concentration and N mass, CN ratio) using linear mixed effects models (lme). To reveal any interacting effects of grass species and N supply level on NREperc, NREmass and the NRP, lme with grass species and N supply as predictor variables were applied. Block was treated as a random factor. Model assumptions were tested graphically. To meet the criteria of variance homogeneity, data transformations and weighting were applied if necessary. Automated model selection from the global models was performed to obtain minimum adequate models based on Akaike's Information Criterion for small sample sizes (AICc). In case of significant effects, Tukey tests were used *post-hoc* to compare the least squared means.

Analysis was performed with the statistical software R 3.6.1 (R Core Team, 2020) and the packages 'nlme' (Pinheiro et al., 2019), 'MuMIn' (Barton et al., 2019) and 'lsmeans' (Lenth, 2018).

Results

Pot level

Treatment had a significant effect on all investigated variables at pot level (Table 1). The leaf appearance rate (LAR) was significantly greater under high than under low N supply with 1.4 times in Fea and 1.1 times in Lop (Table 1). Under the low N supply, Lop had a 1.2 times higher LAR than Fea.

Tiller density was 1.8 times higher in the highly fertilized plants (average of 495.5 tillers pot^{-1} ± 16.2) compared to the low fertilized plants (average of 268 tillers pot^{-1} ± 16.2) with Fea showing 1.3 times greater values than Lop (Table 1).

At final harvest, both grasses under high N supply showed a 2.7 times increased total DM herbage compared to under low N supply. Under high N supply, total DM of Fea was 1.2 times greater than that of Lop whereas under low N supply Lop produced 1.1 times more total DM than Fea (Table 1). Significantly highest total DM herbage per pot was achieved by Trr with 98.8 ± 8.0 g (Table 1). Further, also the N concentration in total DM herbage per pot was highest in Trr (Table 1). The two grass species showed a 1.3 times increased N concentration in total DM herbage under high N supply compared to low N supply. In accordance with the biomass production, under high N supply Lop showed a 1.1 times greater N concentration than Fea (Table 1). As a result of the total DM and N concentration, N mass per pot revealed significantly higher values under high N supply than under low N supply. The legume Trr showed significantly greater values than Fea and Lop (Table 1).

Leaf level

At leaf level, treatment significantly affected all investigated variables (Table 2). The Larea was similar for the grass species within N supply levels and increased 1.3 times under high N supply compared to low N supply (Table 2). The DM of the live leaf was two times greater under high compared to low N supply. Moreover, under low N supply, the values

of Fea were significantly lower than those of Lop and Trr (Table 2). The DM of the dead leaf of Fea was 1.5 times greater under high N supply (Table 2).

The N concentration of the live leaf was affected by treatment with significantly greater values in the grass species under high N supply than under low N supply and in Fea than in Lop (Table 2). Both grasses under high N supply showed a 1.5 times greater N concentration in the live leaf than under low N supply whereas Fea had significantly higher N concentration than Lop: under low N supply 1.3 times and under high N supply 1.1 times, respectively. The legume Trr showed the highest N concentration in the live leaf with 2.8 times greater values than the average of the two grass species (Table 1).

Further, the N concentration of the dead leaf was significantly highest in Trr and lowest in Fea under low N supply (Table 2). Both grasses had a significantly higher N concentration in the dead leaf under high N supply but Lop showed 1.5 times greater values than Fea (Table 2). In turn this means that NRP was greater in the grass species under low N supply compared to under high N supply with Fea showing the highest values under each N supply level. The lme with the variables grass species and N supply as separated predictors revealed significant main effects of grass species (F = 35.6, P < 0.0001) and N supply (F = 60.8, P < 0.0001) on the NRP, i.e. on the N concentration in dead leaves. Significantly higher NRP was found in Fea compared to Lop and under low N supply compared to under high N supply.

As a consequence of the N concentration, the CN ratio in the live leaf was lowest under low N supply with Lop having 1.4 times higher values than Fea and 1.5 greater values than Lop under high N supply (Table 2). Under low N supply Fea showed 1.3 times greater CN ratio in the live leaf than Fea under high N supply. The legume Trr had the lowest CN ratio in the live leaf (Table 2).

The CN ratio of the dead leaf followed the opposite pattern of the N concentration in the dead leaf. Both grass species showed greater CN ratios under low N supply with 1.3 times in Fea and 1.5 times in Lop (Table 2). The grass species Fea had 1.3 times and 1.5 times greater CN ratio than Lop under the low and the high N supply, respectively (Table 2). The lowest CN ratio in dead leaf was found for Trr (Table 2).

The NREperc was with 1.4 times significantly higher in Fea than in Lop (Table 2). The legume Trr showed similar values to Fea (Table 2). In contrast, the NREmass of Fea under high N supply was 1.1 times greater than under low N supply and 1.2 times higher than of Lop under high N supply (Table 2). The analysis of effects of grass species and N supply as separated variables in the lme revealed significant main effects of species on NREperc ($F_{grass\ species}$ = 82.2, P < 0.0001) and species and N supply on NREmass ($F_{grass\ species}$ = 34.0, P < 0.0001; $F_{N\ supply}$ = 12.0, P = 0.0032).

Table 1 Estimated means ± SE (standard error of the mean) of leaf appearance rate (LAR), tiller number (tiller density), leaf area (Larea), total dry matter herbage (DM pot) and N concentration (N pot), N mass and CN ratio (CN pot) at pot level of the pot experiment. Wald Test shows F and p values of the effect of treatment (combination of species and fertilization: Fea+, Lop+, Fea-, Lop+, Trr; Fea = *Festuca arundinacea*, Lop = *Lolium perenne*, Trr = *Trifolium repens*, + = high N supply, - = low N supply) on the response variables. Lowercase letters show significant differences between the treatments with P < 0.05.

	Treatment					Wald Test	
	Fea+	Fea-	Lop+	Lop-	Trr	F	p
LAR [leaves d^{-1}]	0.1 ± 0.00 bc	0.07 ± 0.00 a	0.1 ± 0.00 c	0.09 ± 0.00 b	-	29.0	< 0.001
Tiller density [n pot^{-1}]	567 ± 16.2 c	292 ± 16.2 a	424 ± 16.2 b	244 ± 16.2 a	-	79.9	< 0.001
DM [g pot^{-1}][+]	79.4 ± 3.1 d	26.8 ± 1.6 a	70.3 ± 2.7 c	29.5 ± 1.9 b	98.8 ± 8.0 e	220.6	< 0.001
N pot [%]	1.8 ± 0.07 b	1.6 ± 0.07 a	2.0 ± 0.07 c	1.4 ± 0.07 a	3.8 ± 0.07 d	381.6	< 0.001
N mass [g pot^{-1}][+]	1.4 ± 0.1 b	0.4 ± 0.0 a	1.4 ± 0.1 b	0.4 ± 0.0 a	3.7 ± 0.3 c	705.2	< 0.001
CN pot[+]	23.7 ± 1.1 b	27.0 ± 1.1 c	21.7 ± 1.1 b	29.9 ± 1.1 d	11.1 ± 1.1 a	68.0	< 0.001

[+] The variance structure varIdent allowed for separate variances per treatment

Table 2 Estimated means ± SE (standard error of the mean) of leaf area (Larea), dry matter (DM) of live and dead leaf, N concentration and CN ratio of the live and dead leaf and N (percent, mass) at leaf level of the pot experiment. Wald Test shows F and p values of the effect of treatment (combination of species and fertilization: Fea+, Lop+, Fea-, Lop-, Trr; Fea = *Festuca arundinacea*, Lop = *Lolium perenne*, Trr = *Trifolium repens*, + = high N supply, - = low N supply) on the response variables. Lowercase letters show significant differences between the treatments with P < 0.05.

	Treatment					Wald Test	
	Fea+	Fea-	Lop+	Lop-	Trr	F	p
Larea [cm²]	5.8 ± 0.3 b	3.5 ± 0.1 a	5.6 ± 0.3 b	3.7 ± 2.0 a	5.2 ± 0.2 b	122.8	< 0.001
DM live [mg]⁺	52.1 ± 2.2 c	19.9 ± 1.3 a	47.8 ± 4.6 c	30.6 ± 3.8 b	25.9 ± 1.5 b	45.7	< 0.001
DM dead [mg]	23.7 ± 2.1 b	15.5 ± 2.1 a	24.8 ± 2.1 b	19.2 ± 2.1 ab	18.1 ± 2.6 ab	3.4	< 0.05
N live [%]	2.2 ± 0.08 d	1.7 ± 0.08 b	2.0 ± 0.08 c	1.3 ± 0.08 a	5.0 ± 0.08 e	324.7	< 0.001
N dead [%]⁺	0.6 ± 0.03 b	0.4 ± 0.02 a	1.0 ± 0.06 c	0.6 ± 0.05 b	1.4 ± 0.15 d	42.0	< 0.001
CN live[a]	17.7 ± 0.9 b	22.4 ± 1.1 c	20.1 ± 1.0 bc	30.3 ± 1.5 d	8.3 ± 0.4 a	126.6	< 0.001
CN dead	57.3 ± 3.2 c	77.3 ± 3.2 d	39.4 ± 3.2 b	59.5 ± 3.2 c	25.4 ± 3.9 a	44.5	< 0.001
NREperc	70.8 ± 2.7 b	72.6 ± 2.7 b	51.3 ± 2.7 a	51.1 ± 2.7 a	71.8 ± 3.3 b	17.7	< 0.001
NREmass	87.0 ± 2.0 c	78.9 ± 2.0 b	74.4 ± 2.0 ab	69.3 ± 2.0 a	79.0 ± 2.4 b	10.7	< 0.001

[a] Log transformation was used

⁺ The variance structure varIdent allowed for separate variances per treatment

Discussion

The present study investigated the effects of N availability on the growth and N cycling processes of *Festuca arundinacea* (Fea) and *Lolium perenne* (Lop), two in their resource-use strategy contrasting grass species. It was first hypothesized that both grass species show similar NRE under high N supply while at low N supply NRE of Fea is greater than of Lop, and second, that Fea has a higher NRP than Lop under low and high N supply. Fea showed the highest N concentration in live leaves under both N supply levels but lowest in dead leaves which is in line with the greater NREperc than that of Lop. While NREperc was unaffected by high N supply within each grass species NREmass of Fea increased. The NRP decreased in both grass species under high N supply whereas the conservative grass species Fea showed greater NRP than the exploitative Lop.

Responses of N concentration, the NRE and the NRP to different N supply levels

Generally, the N concentration of the live and dead leaves of Trr was greater than of Lop and Fea what can be attributed to the N fixing ability of legumes causing unlimited N availability (Killingbeck 1996). The N concentration of the live leaves of the two grass species under both N supply levels showed with an average of 1.8% ± 0.6 similar values than those presented in a global meta-analysis by Vergutz et al. (2012) examining 86 studies with the most data points coming from Europe and North America (N concentration of 1.9% ± 0.2).

As expected, N concentration of live leaves of both grass species increased under high N supply and differences disappeared under low N supply what is in line with the literature (van Arendonk et al. 1997, Wright et al. 2004). Contradicting our expectations, the N concentration of the live leaf of Fea was greater compared to Lop under both N supply levels. However, N concentration of the total DM herbage of the high N supply plants at final harvest was greater in the exploitative Lop than in the conservative Fea while differences were not found under low N supply confirming findings of literature from above. The varying N concentrations of the harvested leaves and the total DM herbage at the end of the measurements may emerge from the different developmental stages at

which the leaves and total herbage, respectively, were harvested. With increasing plant maturity the N concentration decreases (Gordon et al. 1962). Further, an increasing plant mass consequently results in reduced N concentration due to dilution effects (Wilman 1975, Peyraud & Astigarraga 1998).

The resorption of N from senescent to live leaves can increase nutrient use efficiency (Yuan et al. 2006) and reduce plants' dependence on external nutrient uptake (Killingbeck 1996). In the present study, NREperc ranged from 51% to 73% with 1.4 times higher values of Fea than of Lop under high N supply what is in line with Aerts (1996) reporting of an NRE of graminoids of 60% while Eckstein et al. (1999) described values of 20% to 60% but of plants in temperate-arctic regions. In general, conservative grass species are likely to have a higher NRE than exploitative grass species as an adaption to N-poor soil conditions (Aerts and Chapin 1999).

In this study, NREperc of the exploitative and conservative grass species was not affected by N availability. Literature is not clear on the response of NRE to availability and shows contrasting findings. It is described that NRE declines with increasing leaf N status (Kobe et al. 2005) caused by a higher N availability. Schulte auf'm Erley (2001) found an increasing NRE of the conservative grass species *Festuca rubra* L. under low N supply while the exploitative *L. perenne* showed no response. In contrast, in the study of van Heerwarden (2003b), only two of six sub-arctic bog species responded to higher N supply with a decreasing NRE. However, it is also reported, that NRE is rather little responsive to N availability (van Heerwarden 2003b).

In contrast to the NREperc, NREmass, the N mass which is resorbed from senescent leaf relative to live leaf, increased in Fea under high N supply. Since the NREmass is calculated of DM live leaf and its N concentration, this result can be attributed to the relatively higher N concentration in the live leaf of Fea compared to that of Lop. Given the results of NREperc and NREmass, the first hypothesis has to be rejected.

Varying definitions and estimations of NRE may be responsible for the inconsistency in the literature concerning its responses to different N levels and among species (van Heerwaarden 2003a, Kobe et al. 2005). This inconsistency could arise from biological and methodological sources such as low light, water availability and the variation between

individuals, sites and years as well as the thresholds of complete and incomplete resorption on the one hand (Chapin and Moilanen 1991, Escudero et al. 1992, Eckstein et al. 1999). On the other hand, different methodologies could cause inconsistent results. Mass-based N concentrations do not take translocations of carbon and other nutrients into account which may increase or decrease during senescence (van Heerwarden et al. 2003a). Thus, determined on a mass basis, NRE may be underestimated up to 10% because of mass loss during senescence (van Heerwaarden 2003a). However, we did not consider this aspect when calculating NRE. By comparing the NREperc of this study with NRE calculated on the mass-basis of other investigations, which form the majority when studying NRE (Kobe et al. 2005), similar values could be observed.

The N concentration in the dead leaf had a mean of 0.8% and ranged from 0.4% in Fea under low N supply to 1.4% in Trr (Table 2). The high variation of NRP, i.e. N concentration in the dead leaf, was also reported by Killingbeck (1996) investigating leaf nutrients of 77 woody perennial plants. He also described a two times greater N concentration of potential N-fixers than of nonfixers confirming an evolutionary trade-off between N fixation and effective N resorption (Killingbeck 1993).

As expected, the NRP of both grass species decreased under high N supply, i.e. the dead leaf N increased with increasing N availability (van Heerwarden 2003b, Kobe et al. 2005, Yuan and Chen 2015). The higher NRP of both grasses under the low N supply demonstrated the limited N availability in this study since plants under N limitation are more likely to resorb N to complete levels compared with unlimited N conditions (Aerts and Chapin 1999). However, the within-species variability of NRP was higher than that of NREperc (Table 2) confirming its greater sensitivity to N availability relative to NRE (Vitousek 1998). This was found for plant species of different habitats. Lü et al. (2021) examined the effects of six different N fertilization rates on perennial plants in a temperate steppe and found in all seven species a reduced NRP with increasing N availability while for NRE only three out of seven correlated negatively with N addition. Also, sub-arctic bog species showed greater responses of NRP than of NRE to N fertilization (van Heerwarden 2003b).

The NRP of Fea was generally higher than that of Lop underlining its conservative character (Maire et al. 2009). Even under high N supply, Fea showed significantly greater NRP than Lop confirming the second hypothesis of this study. This might be related to the slow growth and consequently longer leaf lifespans (Eckstein et al. 1999, Lemaire et al. 2009) which are attributes of *F. arundinacea*. Wright and Westoby (2003) found a negative correlation between N in senesced leaves with leaf lifespan in evergreen sclerophyllous species in their native habitats leading to the assumption that nutrient loss is rather minimized through long leaf lifespans and low concentration of nutrients in senesced leaves than maximizing NRE.

Besides nutrient resorption leaching processes determine the N concentration in senescent leaves (Reich et al. 1992) with potentially higher leaf leaching in species of N-rich environments due to lower leaching resistance (Aerts 1999). This would rather affect species under high N supply. But since leaching occurs by the action of aqueous solutions like rain, fog or surface water (Tukey et al 1966, 1970) leaching of N in the present study under greenhouse conditions and specific watering might have been little.

Responses of growth to N supply

The total DM herbage was four times higher in the legume Trr than in the grass species (averaged over N supply levels). This suggests that the "high" N supply of this study was rather of intermediate level than of high and unlimited N availability, respectively. However, our results showed positive effects of high N supply on growth characteristics of both grasses what is in line with literature studying N availability on growth and aboveground productivity (Ryle 1963, Elberse and Berendse 1993, da Silveira Pontes et al 2010). Thereby, the high N supply caused greater tiller density and total DM herbage in Fea than in Lop. Further, Fea responded to a greater magnitude to high N supply with 1.9 times and 3 times greater tiller density per pot and total DM herbage while Lop with 1.7 and 2.4. This is contrasting to literature which describes a general greater response magnitude, i.e. a higher level of phenotypic plasticity, of exploitative species to N fertilization than conservative species (Grassein 2010, Van Arendonk 1997). Regarding the species-specific responses of Fea and Lop, a study of Elberse and Berendse (1993)

investigated eight perennial grass species from habitats differing in soil fertility grown under nutrient-poor and nutrient-rich conditions. They found increasing plant DM with increasing Ellenberg nitrogen indicator value (Ellenberg 1988), from which *F. arundinaceae* and *L. perenne* are defined with 4 and 7, respectively. They also showed that *L. perenne* weakened this correlation because of its shorter growth form in relation to the other high N rank grass species. In line with our results, Schulte auf'm Erley (2001) described a higher tiller density for the conservative grass species *F. rubra* than of *L. perenne* under high N supply whereas under low N supply values were similar. The conservative grass species *F. arundinaceae* might maintain and increase its tiller density by low tiller mortality while exploitative grass species have high tiller mortality and turnover due to their high tissue turnover in general (Duchini et al. 2018). Although the results of the present study are in line with other investigations, it has to bear in mind, that the tiller density of the present study was measured 14 days after harvesting total herbage of the non-destructive pots resulting in greater tiller density than at harvest date (Grant et al. 1981). Reduced light competition and cutting may have enhanced tillering of the grass species (Gautier et al. 1999). The weight per tiller and the number of tillers per unit area determine yield (Kays and Harper 1974, Matthew et al. 1996). Greater yields under N fertilization of *F. arundinaceae* compared to *L. perenne* were found in several studies. Collins et al. (1991) studied DM yielding on three cultivars of *F. arundinaceae* and two of *L. perenne* under three levels of N fertilization (0, 75, 150 kg ha^{-1} N annually) and showed generally higher yields in *F. arundinaceae* than in *L. perenne*. Also, Becker et al. (2020) recently reported in a study about the performance of *F. arundinaceae* and *L. perenne* as main species in mixtures on three soil types and three management regimes the superiority of *F. arundinaceae* against *L. perenne* regarding yielding and persistence.

Conclusion

In the face of climate change, modifications of the grass sward by, inter alia, using alternative forage species adapted to drought are of great importance. In this study, the conservative grass species *Festuca arundinaceae* revealed a high ability to recycle internal N showing superiority to the exploitative grass species *Lolium perenne*. The high resorption efficiency and proficiency of *F. arundinaceae* have profound consequences for the conservation of N in the plant. In turn, this means that N losses of *F. arundinaceae* are lower than of *L. perenne* making it less dependent on external N supply. The high productivity and herbage quality in terms of N concentration may make *F. arundinaceae* a suitable grass species in agricultural grasslands on both N-rich and N-poor soils and under high and low N fertilization, respectively. Internal N cycling and growth responses of *F. arundinacea* to N fertilization and also to management like defoliation might be altered in a sward community with other grasses and legumes and competition effects may occur. Further research on internal N cycling processes as well as on persistence and performance of *F. arundinacea* at species- and community-level grown in mixtures under varying management would give more information on the suitability of *F. arundinacea* for future grassland production.

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GENERAL DISCUSSION

Grassland in silvopastoral systems is exposed to complex spatially and temporally environmental conditions due to the competition with trees for resources. The overarching aim of the present dissertation was to understand effects of tree-grass interactions and grassland management on the productivity and internal N cycling of grassland herbage in silvopastoral systems. Further, this study aimed to evaluate N cycling processes of agronomically important grass species.

In temperate silvopastoral systems, light is a major constraint of grassland growth because of increased shading by trees of the understorey plant species (Dufour et al. 2013, Gillespie et al. 2000). Therefore, in the first study, two silvopastoral sites were investigated with regard to interactions of cutting frequency and position to tree lines (i.e., shading) on the total grassland herbage accumulation and the dead and live herbage tissue in order to reveal any trade-off between management intensity and herbage production (Chapter I). In a concomitant study, data on responses of internal N cycling and growth in grassland communities as affected by trees under different cutting frequencies at one silvopastoral site was collected (Chapter II). To gain a better understanding of the internal N cycling of *Lolium perenne* and *Festuca arundinacea* (two agronomically important grass species) the responses of nitrogen resorption to different N supply levels were investigated in a greenhouse experiment (Chapter III).

Grassland herbage production in silvopastoral systems

The analysis of the field study revealed diverging results regarding effects of trees on total herbage DM production, live and dead herbage accumulation at both experimental silvopastoral sites (Chapter I). The tree-induced effects on all herbage accumulation parameters were mainly found at the narrow alley cropping site in Reiffenhausen (RH). There, analysis of the herbage accumulation showed declining total, live and dead herbage accumulation by up to 36%, 37% and 7%, respectively, from the centre between

two tree lines towards the tree line. The main reason for reduced yield is the competition for light, water and nutrients (Jose et al. 2009). The unequal decline of live and dead herbage led to a lower HEFF (Mazzanti and Lemaire 1994) in the vicinity of the trees which was not modified by defoliation intensity although it was anticipated (Chapter I and II). It is common sense that grassland swards with longer regrowth intervals (two-cut defoliation system vs. 4-cut defoliation system) accumulate much more dead herbage. The process of growth (i.e. live herbage production) with its sigmoidal pattern is reduced under limited light conditions while the senescence rate is unchanged (Grant et al. 1981). The first study has shown that senescence was much less reduced than growth and consequently a lower HEFF resulted. Although higher defoliation intensity maintained a high HEFF, i.e. a high proportion of live herbage in the total herbage accumulated at harvest, total herbage accumulation was rather low. Low herbage accumulation makes intensive defoliation near trees muss less interesting and opens pathways for other ecosystem services. Several studies have reported a compensation of reduced crop yields close to trees by greater yields in the centre of the crop alley (Grünewald et al. 2007, Winterling et al. 2017). However, grassland production throughout the whole alleyway at RH might have been negatively affected by tree lines. This assumption is supported, for instance, by Graß et al. (2020) who reported that the silvopastoral grassland yield was significantly lower compared to an adjacent open grassland site.

The tree-grass sward interaction played a rather minor and inconsistent role at the wider silvopastoral site in Mariensee (MS). This may be explained by some experimental considerations:

First, the studied positions 'close to tree line' at RH site were closer to trees than those at the wide site (0.5 m vs. 6 m) so that incident light reaching the sward was less at RH first as shown in studies of Ehret et al. (2018) and Sutterlütti et al. (2020), respectively. in addition to shading effects, increasing below-ground competition in grassland-tree interface zones s may also contribute to declining herbage accumulation (Gamble et al. 2020). This is in accordance with several studies observing more severe yield reductions the closer the distance to trees is (Graß et al. 2020, Swieter et al. 2019, Yang et al. 2019). At which distance tree effects on grassland herbage are likely to decline may be drawn by

the comparison of the two study sites with the result that tree effects by short-rotation coppices reaching a height of up to 4.5 m (as was the case in our studies) will decline at distance between 4.5 (or less) to 6 m. Several studies reported yield reductions up to a distance from the trees of two times their height (Nuberg 1998, Lamerre 2016). Second, the missing effects at MS might also emerge from the north-south orientation of the tree lines since this design seems to be optimal to minimize competition for light at both sides of the tree lines lowering yield and quality reductions (Chalmin 2009, Dupraz et al. 2005). The tree lines at RH were oriented from south-east to north-west causing greater shadows at the zenith and longer diurnal shading periods. Third, the tree harvest at MS prior to the beginning of this study might have had attenuated the tree effects on the grassland since shading was lowered. The distance to which light availability is limited by trees and thus, also herbage yield, is largely affected by the maturity of the AFS and age of the trees, respectively, which determine tree height and canopy size (Nerlich et al. 2013, Pardon 2018). Competition for water might also be reduced due to a lowered demand of the cut trees (Kang et al. 2009). Fourth, the botanical composition in silvopastoral grassland might be of great importance since grassland species have a wide range of shade tolerance (Devkota et al. 1997). The permanent grassland at MS was dominated by Yorkshire fog (*Holcus lanatus* L.), a species tolerant to moderate to heavy shade resulting in a better performance, i.e. absolute and relative number of tillers per plant, close to trees (Devkota et al. 1997). In contrast, at RH two temporary grass swards were sown in 2011 comprising of significant shares of legumes which are sensitive to shade (Frame 2019). Particularly white clover and ryegrass, forming a legume-grass mixture of high productivity (Nyfeler et al. 2011), are less shade tolerant than Yorkshire fog and show low production and persistence under heavy shade (Devkota et al. 1997).

With regard to the total herbage accumulation of the grassland within the tree lines MS site was superior to RH site with 1.3 times greater herbage production in 2016. Total herbage production in 2017 was generally low and thus, also differences between the two sites. Considering the potential tree effect at a distance from trees up to 4.5 m, tree lines at RH might have affected the herbage accumulation of the whole 9 m wide grassland alley resulting in lower total herbage production. In contrast, given a potential tree affected area up to 4.5 m distance from the tree line at MS this would comprise 18% of

the total grassland alley area. The percentage of the zone which is negatively affected by trees is greater in alley cropping systems comprising a narrow grassland alley than a wide alley and consequently, also the impact on the total herbage accumulation (Swieter et al. 2019).

The rather great distance of the positions 'close to tree line' at MS site arose out of experimental issues. At this site the original intended experimental setup included sampling plots at 1.75 m distance to tree lines. Technically, unintended harvests were conducted by the practical site manager who has cut a 4 m wide stripe along each tree line in June 2016 and 2017 and in September 2017. Therefore, no sampling was possible close to tree lines because the actual growth was interrupted and, consequently, no true herbage growth assessment was realizable. Hence, the sampling plots within this zone could not be investigated. Therefore, the data analysed in Chapter I excluded any existing data points obtained from the plots at 1.75 m in both years. However, three harvest dates in 2016 and two in 2017 (2016: 24 Mai, 8 August, 5 October; 2017: 15 Mai, 16 August) were unaffected by these wrong harvests (in the frequent cutting system only). This data was analysed by applying linear effects model (lme) with the experimental factors position to tree line (5 levels with two times 1.75 m and 6 m, and 25 m denominated as W, MW, M, ME, E) and harvest date (5 levels) and the response variable total herbage accumulation. The analysis showed significant interacting effects of position and date ($p <$ 0.0001, $F_{date \times position}$ = 8.9) with no clear and rather inconsistent effects of distance to tree line on total herbage accumulation (Figure 1). Although only five harvest dates were analyzed, these results support the importance of inter alia the orientation of tree line and botanical composition for grassland production in silvopastoral systems.
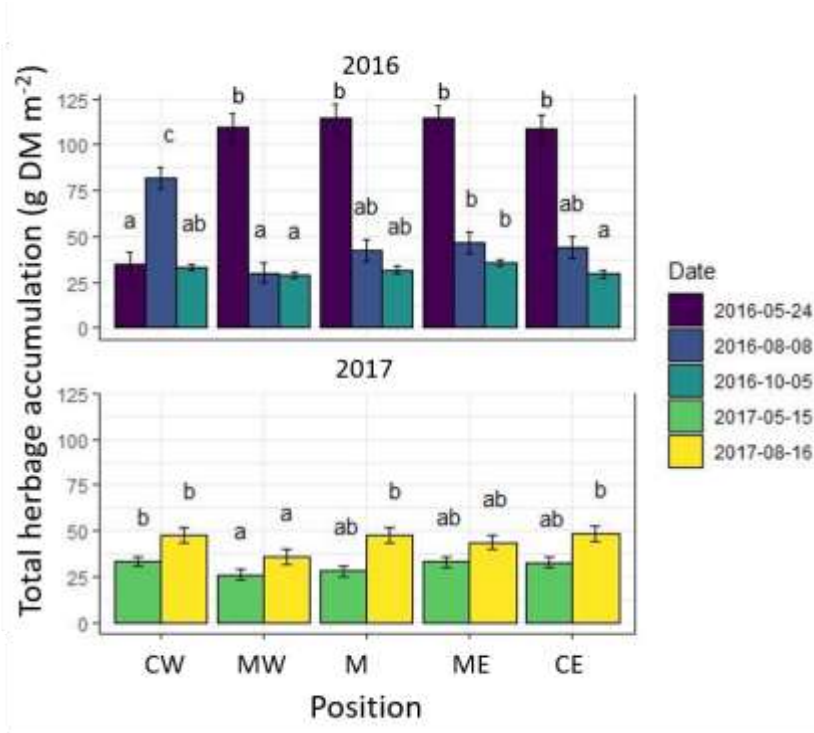
Figure 1 Means (±SE) of total herbage accumulation at site MS at each position. CE, Close East; CW, Close West; M, Middle; ME, Middle East; MW, Middle West

The data on grassland yields from this silvopastoral systems can improve the knowledge on developing ideal alley cropping designs for temperate regions. An implementation of grassland reference plots into the experimental design would further support information on grassland production in alley cropping systems. This poses challenges since those experiments require a large scale at limited space and resources (Stamps and Linit 1998, Dupraz et al. 1999). In addition, provided ceteris paribus conditions, e.g. homogeneity of soil, for different treatments has to be ensured as well (Seserman et al. 2019).

The role of legumes in silvopastoral grassland systems

The botanical composition not only has considerable impact on grassland productivity but also on internal N cycling processes (Nyfeler et al. 2011). Legume based swards are among the most productive grassland mixtures under temperate climate conditions (Peyraud et al. 2009). The N-fixing ability of legumes enhances nitrogen accumulation and biomass production (Spehn et al. 2002). Legume-grass swards can achieve similar yields

than pure grass swards albeit a reduced N fertilization of up to 88% if legume proportion of the sward is 50 to 70% (Nyfeler et al. 2009).

In the present study legume proportion decreased up to 45% towards the tree line in both grass swards at RH site which can be attributed to the shading of trees (Chapter II). The lower proportions of white clover and birdsfoot trefoil under shade are due to their sensitivity to reduced light interception (Frame 2019). Since the level of N concentration of a legume-grass sward is the result of the N fixing ability of legumes and the positive grass-legume interactions (Ledgard and Steele 1992, Nyfeler et al. 2009), the decline of legumes causes a decreased nitrogen concentration of the grassland biomass near trees. In our study (Chapter II), the N concentrations of live and dead herbage declined by up to 8% and 9%, respectively, for the diverse mixture and up to 20% and 22%, respectively, for the grass-clover mixture. Whole sward crude protein content for grass-clover in the middle of the alley and close to trees were consequently 15.9% and 12.8% and 12.8% and 12.0% for the diverse mixture. Thus, crude protein content close to trees might be insufficient to meet the crude protein requirements of higher performing dairy cows of 14 to 18% (Botts et al. 1979).

The severe reductions of clover proportion in the grass-clover sward will negatively affect N fixation. Legume-dominated swards with a legume proportion of over 80% transferred 11 g of N kg^{-1} dry weight more to grasses than legume-poor swards with less than 20% legume proportion (Nyfeler et al. 2011). In our study, legume proportion close to tree line was about 8% and 15% in the diverse and grass-clover sward, respectively. Given the reduced total herbage accumulation in the grassland-tree interface (Chapter I) and its declined N concentrations N yields are drastically reduced. Thus, benefits of white clover-ryegrass mixtures are strongly limited in silvopastoral systems close to trees. However, one has to bear in mind that the swards at site RH had been established in 2011 (i.e. five years prior to the beginning of our experiments). That means that the legume proportions were higher in the initial years. Comparing proportions of white clover of the present study with those of two to three years after establishment of the silvopastoral site RH (Ehret et al. 2018) legumes were 1.3 times higher in the middle of the alley and 1.8 times close to trees indicating decreasing legume proportion and thereof more severely close to

tree line. Given that grass-legume swards are mainly part of temporary ley grassland systems (Peeters et al. 2014), planting trees will not improve grass-clover production when it is the aim to utilize the multi-functions of grass-clover swards (Suter et al. 2021). For instance, grass clover is an essential part of the crop rotation in organically managed fields. Hence, reduced clover proportions will likely reduce the value of grass-clover in terms of nitrogen provision to subsequent crops (Fox et al. 2020).

Conclusion and implications for silvopastoral systems

The findings of this study show diverging effects of trees on herbage production of two silvopastoral systems. However, negative effects of trees on the herbage production, the HEFF and the N budget of silvopastoral grassland are detected while grass-clover swards are more severely affected. Since the defoliation frequency did not modify these effects and frequent harvesting is less worthwhile an extensive defoliation at the tree-affected grassland zone could be applied. Thus, there is the chance to include a broader range of ecological and environmental objectives since an extensive management, with respect to defoliation frequency and fertilizer input, may promote other grassland-related ecosystem services like botanical and faunistic diversity (Allen et al. 2020, Di Giulio et al. 2001, Marini et al. 2008, Weiss et al. 2013).

Considering the increased demand on food and feed for livestock, the use of legume based swards in silvopastoral systems might be critical since herbage production and feeding quality is drastically reduced due to tree shading. However, there are management options mitigating the negative effects of tree shade on these swards. A regular renovation of the grass sward by reseeding clover could maintain legume proportion but reseeding costs might further reduce the economic advantages of the reseeded swards (Hopkins 2006). Moreover, tree management such as tree pruning or thinning (Devkota et al. 2009, Garrett 2009) and a lowered tree density (Ivezic et al. 2021) would increase transmitted light and may maintain herbage yield and quality since white clover is able to deal with light to moderate shade (Ehret et al. 2015). Under those conditions, white clover-ryegrass swards might be suitable for grassland production in silvopastoral systems under temperate climate. However, trade-offs between the

production of woody crops and grassland has to be evaluated. Moreover, research has to focus on grassland species, in particular legumes, or varieties with broader amplitudes of shade tolerance persisting under limited light conditions close to trees while also promising high N yields. Additionally, investigations of effects of leaf litter, tree root exudates and decomposition as well as the function of tree roots as nutrient pump (Mosquera-Losada et al. 2011) may give more information on N mineralization providing soil N for understorey vegetation.

The comparison of the site MS, the wide alley cropping system with permanent grassland, and site RH, the narrow one with temporary grassland, led to the conclusion that the design, i.e. the orientation of the tree lines, of the alley cropping system can significantly reduce tree impacts on grassland production (Chalmin 2009, Dupraz et al. 2005). Further, while designing alley cropping systems the width of the grassland alley between tree lines has to be considered since i) tree effects occur up to a distance to trees twice of its height (Nuberg 1998) and ii) the proportion of the tree affected zone increases the less wide the grassland alley is.

In the view of above discussed effects of trees and potential management options to reduce negative tree effects on herbage yield and quality, silvopastoral grassland production might be an alternative to intensively managed agricultural grassland providing a range of ecological benefits like reduction of nutrient leaching (Böhm et al. 2013), soil enrichment and carbon sequestration (Jose 2009, Mayer et al. 2022). Additional, they can serve as windbreaks reducing wind erosion, evaporation and mitigating microclimatic extremes (Brandle et al. 2004, Tsonkova et al. 2012). Silvopastoral systems also have the potential to promise productivity, yield stability while designed as alley cropping systems management can be facilitated by machinery (Jose et al. 2009, Orefice et al. 2016, Quinkenstein et al. 2009, Tscharntke et al. 2012). Thus, especially in marginal and / or intensively managed unfertile areas with high environmental risks, e.g. summer droughts and wind erosion, agroforestry systems could be developed (Quinkenstein et al. 2009). In Germany, a focus is given inter alia to the dry-sandy areas of Brandburg (Kanzler et al. 2021, Mirck et al. 2016, Seserman et al. 2018).

In recent times, agricultural practices are changing towards a more sustainable land use to preserve natural resources as basis for future food and fuel security and to mitigate impacts of climate change (FAO 2014). There is an international consensus about an agriculture that can "multi-functionally" increase food production while simultaneously enhancing social and environmental goals, as committed to in the sustainable development goals (SDGs) by the United Nations (UN General Assembly 2015). Agroforestry can contribute to the implementation of nine out of the 17 SDGs, with the strongest impact potential for poverty reduction (SDG 1) and hunger alleviation (SDG 2), as well as for climate action (SDG 13) and life on land (SDG 15; Agroforestry Network 2018, Goparaju et al. 2020). At the European level the 'Farm to Fork Strategy' (European Union [EU] 2021) calls for a fair, healthy and environmentally-friendly food system in the context of the European Green Deal (European Commission 2020) while civil society organisations set out 10 key priorities (European Environmental Bureau 2021) for the European Parliament to enable the transition to sustainable food systems in its report. One is to promote agroecological and organic farming practices such as agroforestry. Also, at national level the potential of agroforestry in Germany is considered to be very high, especially for climate protection, climate adaptation and biodiversity and soil conservation (WBGU 2020).

However, despite its ecological and economically advantages the establishment of agroforestry systems in the European agricultural landscape has been rather low, with a current area of approximately 358.000 ha (Herder et al. 2015). Reasons might be, amongst others, the high establishment costs (Nerlich et al. 2013), lack of information (Graves et al. 2009) and financial incentives (Smith et al. 2012) as well as management challenges (Jose et al. 2019). Until now, Europe's and thus also Germany's legislative constraints limit the implementation of AFS as agricultural practice (Tsonkova et al. 2018). In the recent final report of the Zukunftskommission Landwirtschaft (2021), a commission of the Federal Government, farming and animal husbandry are described to be more sustainable and socially acceptable but the implementation of agroforestry as a potential land use practice was not considered. In contrast to other agricultural practices farmers do not receive financial support for the establishment of agroforestry systems in Germany since they are not registered with a code in the first pillar of the Common Agricultural

Policy (CAP) of the EU which is essential for administration by the Integrated Administration and Control System (IACS; Tsonkova et al. 2018). Currently, the CAP is reformed since the EU aims to adapt agriculture to changing social and ecological requirements without losing its competitiveness. All EU member states must develop National Strategic Plans for the 1st and 2nd pillars of the CAP for the first time for the new CAP funding period running from 2023 to 2027. In the package of laws Germany has passed to implement the reform of the CAP agroforestry is mentioned as part of the 'Öko-Regelung'. Thus, agroforestry on arable land and grassland is part of the support of the CAP for which 25% of direct payments are reserved. In addition, some states are preparing support programs for agroforestry in the 2nd pillar. These include Brandenburg, Mecklenburg-Western Pomerania, Thuringia, Saxony-Anhalt and Rhineland-Palatinate. However, the actual draft for the regulation on the implementation of CAP direct payments of the Federal Ministry of Food and Agriculture (2021) describes limiting specifications of agroforestry implementation (e. g. use of only energy and value wood) with low financial funding (60 Euro ha^{-1} woody area year^{-1}). Agroforestry societies are calling for extensive need for improvement of the position of agroforestry in agriculture at European and national level (e. g. European Agroforestry Federation, Deutscher Fachverband für Agroforstwirtschaft e.V.). The reform of the EU's CAP is now a chance to foster agroforestry implementation in Germany and Europe.

Also grassland management has to be adapted to the challenges facing agricultural land use mentioned above (Hopkins et al. 2006). A greater use of forage legumes instead of N-fertilizers and alternative forage species and mixtures adapted to drought would be some of potential management strategies (Hopkins and Del Prado 2007, Reheul et al. 2013). The more drought tolerant grass tall fescue (*Festuca arundinacea* Schreb.) may be a suitable grass species in agricultural grasslands due to its superiority to perennial ryegrass when drought is an issue. The present study (Chapter III) has shown that tall fescue is superior under varying N availability regarding the resorption and thus, conservation, of N in the plant making it less depend on external N supply (Aerts and Chapin 1999). Growth and internal N cycling responses of *F. arundinacea* to N fertilization and also to management like defoliation may change in a sward community with legumes or other grasses and competition effects may occur (Surault et al. 2006, Cougnon et al. 2014).

Therefore, further research on internal N cycling processes as well as performance and persistence of *F. arundinacea* at species- and community-level grown in mixtures under different management would give more information on the suitability of *F. arundinacea* for future grassland production.

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SUMMARY

In recent times, silvopastoral systems gain increasing attention due to their various ecological and economic benefits. They have the potential to be a more sustainable alternative to common intensive land-use practices and monocultures and to mitigate climate change effects in agriculture. Interactions between woody and non-woody components in agroforestry can enhance e.g., nutrient and water cycling, microclimatic conditions and raise productivity compared to non-agroforestry systems because of complementary resource capture. Still, silvopastoral systems are economically unproven land-use systems due to, inter alia, limited understanding of tree-grass sward interactions and their development over time. Further, agricultural grassland systems face changing growth conditions like increasing dry summers due to climate change. Hence, alternative grasses need testing for future climate change conditions.

The present dissertation comprises three studies of which the first two aimed to reveal interacting effects of trees, cutting frequency and sward botanical composition on herbage production and nitrogen (N) resorption processes of the grass sward in between tree lines in silvopastoral systems. The aim of the third study was to investigate the effects of N availability on growth and N resorption of tall fescue (*Festuca arundinacea*), a grass known for its drought tolerance, compared to perennial ryegrass (*Lolium perenne*) as the most widely used grass.

The first two studies were carried out as part of the SIGNAL collaborative project as part of the BonaRes (Soil as a Sustainable Resource for the Bioeconomy) funding program at the University of Göttingen, which investigated the sustainable intensification of agriculture through agroforestry. Therefore, silvopastoral systems were studied at two sites in Central Germany in the years 2016 and 2017. Both silvopastoral systems were arranged in the form of alley cropping systems comprising willow stripes under short rotation coppice with grassland in the alleyway. At one site, the grassland sward can be

characterized as long-term permanent grassland with the other site consisting of two levels of temporary grassland sown five years prior to the present studies (a standard white clover perennial ryegrass and a diverse sward). Two defoliation frequencies were implemented with either two cuts or three to four cuts per year. Measurements were conducted along a transect between the tree lines at various distances (close to trees and farther away from the trees). The main experimental factors (distance to tree line, cutting frequency and for one site sward botanical composition) led to four and six treatments, respectively, with six and twelve replicates. The plots were laid out in a split-plot randomized block design and arranged in that way in order to evaluate any shade effects of trees on the grassland sward. The experiment of the third study was conducted as a pot experiment in a temperature-controlled greenhouse from December 2015 to June 2016. The investigated species were the conservative and more drought-tolerant grass species *Festuca arundinacea* (Schreb.), the exploitative less drought-tolerant grass species *Lolium perenne* (L.) and the legume *Trifolium repens* (L.) as reference species due to its N-fixing ability. Two N levels were applied with either a low or a non-limiting N supply level. The two experimental factors (species, N supply) were adjusted to a randomized block design with twelve replications.

The first study indicates that the distance to which tree effects may occur ranges from at least 4.5 to 6 m. Shading by trees reduced grassland growth more severely than it increased senescence and this effect was not modified by defoliation frequency. Shorter defoliation intervals maintained a higher proportion of live relative to dead herbage accumulation close to trees but total herbage accumulation was generally low. In consequence, shorter harvesting intervals close to trees are not worthwhile. Alternatively, longer defoliation intervals at the grassland-tree interface may promote other grassland-related ecosystem services that benefit from extensive management like biodiversity. Additionally, 4.4% of the German grassland is at a tree interface and potentially suitable for such extensification.

Nitrogen resorption processes in the silvopastoral grassland were affected by tree shading. Nitrogen concentration in the grassland alley of both sward mixtures was clearly determined by the proportion of legumes. Non-legumes dominated the area close to

trees while legumes were remarkably more present apart from trees. Consequently, N concentrations in the live and dead herbage close to trees decreased. The N resorption efficiency (NRE), i.e., the proportion of N that is resorbed from the senescing herbage, showed no differences along the grassland transect due to an overruling effect of trees on the functional composition of the grass sward irrespective of initial grass sward composition.

According to the third study, leaf N concentrations of both grass species were greater under high than under low N supply and higher in *F. arundinacea* than in *L. perenne*. The grass species *L. perenne* showed greater N concentrations in total DM herbage than *F. arundinacea* under high N supply what might be caused by dilution effects since *F. arundinacea* revealed a higher total DM herbage. The NRE of both grass species varied but it was not affected by N supply level. Generally, *F. arundinceae* showed a greater NRE and a lower N concentration in the dead leaves than *L. perenne* under low and high N supply.

Trees reduce herbage production and change the botanical composition with legumes suffering most from the competition. A simultaneous provision of high yields of digestible herbage close to trees will likely be challenging. Applying extensive management to the border zones near trees may be a chance to support biodiversity. Nevertheless, studies are required to evaluate potential long-term effects of the tree-induced changes in botanical composition with respect to soil nutrient cycling. Furthermore, the results suggest that *F. arundinacea* may be a suitable grass species for temperate agricultural grasslands under future climate changes due to its drought tolerance and ability to recycle nitrogen efficiently.

ZUSAMMENFASSUNG

In jüngster Zeit gewinnen silvopastorale Systeme aufgrund ihrer vielfältigen ökologischen und wirtschaftlichen Vorteile zunehmend an Aufmerksamkeit. Sie haben das Potenzial, eine nachhaltigere Alternative zu herkömmlichen intensiven Landnutzungspraktiken und Monokulturen darzustellen und die Auswirkungen des Klimawandels in der Landwirtschaft abzuschwächen. Wechselwirkungen zwischen holzigen und nicht holzigen Komponenten in der Agroforstwirtschaft können z. B. den Nährstoff- und Wasserkreislauf sowie die mikroklimatischen Bedingungen verbessern und die Produktivität im Vergleich zu nicht agroforstwirtschaftlichen Systemen steigern, da Ressourcen komplementär genutzt werden. Dennoch sind silvopastorale Systeme nach wie vor wirtschaftlich eher unerprobte Landnutzungssysteme, was unter anderem auf das begrenzte Wissen um die Wechselwirkungen zwischen Baum und Grasnarbe und ihrer Entwicklung im Laufe der Zeit zurückzuführen ist. Darüber hinaus sind landwirtschaftliche Grünlandsysteme mit veränderten Wachstumsbedingungen konfrontiert, z. B. mit zunehmend trockenen Sommern aufgrund des Klimawandels. Daher müssen alternative Gräser für die zukünftigen Bedingungen des Klimawandels getestet werden.

Die vorliegende Dissertation umfasst drei Untersuchungen, von denen die ersten beiden darauf abzielten, die Wechselwirkungen zwischen Bäumen, Schnitthäufigkeit und botanischer Zusammensetzung der Grasnarbe auf die Grünlandproduktion und den internen Stickstoffkreislauf des Grünlands zwischen den Baumreihen zu untersuchen. Ziel der dritten Studie war es, die Auswirkungen der Stickstoffverfügbarkeit auf das Wachstum und den internen Stickstoffkreislauf von Rohrschwingel (*Festuca arundinacea*), einem Gras, das für seine Trockentoleranz bekannt ist, im Vergleich zum Deutschen Weidelgras (*Lolium perenne*), dem in der Grünlandwirtschaft am häufigsten verwendeten Gras, zu prüfen.

Die ersten beiden Untersuchungen wurden im Rahmen des Verbundprojektes SIGNAL als Bestandteil des Förderprogrammes BonaRes (Boden als nachhaltige Ressource für die Bioökonomie) an der Universität Göttingen durchgeführt, das die nachhaltige Intensivierung der Landwirtschaft durch Agroforstwirtschaft untersuchte. Dazu wurden in den Jahren 2016 und 2017 silvopastorale Systeme an zwei Standorten in Mitteldeutschland untersucht. Beide silvopastoralen Systeme waren als Alley-Cropping Systeme angelegt, bestehend aus abwechselnden Reihen von Grünland und Weiden. Das Grasland bestand an einem Standort aus langjährigem Dauergrünland und am anderen Standort aus zwei temporären Grünlandmischungen (Gras-Klee und diverse Mischung), welche fünf Jahre vor der Untersuchung eingesät wurden. Die Schnitthäufigkeit betrug entweder zwei Schnitte oder drei bis vier Schnitte. Die Messungen in den silvopastoralen Systemen wurden entlang eines Grünlandtransekts zwischen den Baumreihen in verschiedenen Abständen (nahe der Bäume und weiter entfernt von den Bäumen) durchgeführt. Die Hauptversuchsfaktoren (Abstand zur Baumgrenze, Schnitthäufigkeit und für einen Standort die botanische Zusammensetzung der Grasnarbe) führten zu vier bzw. sechs Behandlungen mit sechs bzw. zwölf Wiederholungen. Die Untersuchungsparzellen wurden in einem randomisierten Split-Plot-Blockdesign angelegt und so angeordnet, um etwaige Beschattungseffekte von Bäumen auf die Grasnarbe zu feststellen. Der Versuch der dritten Studie wurde als Topfexperiment in einem temperaturgeregelten Gewächshaus von Dezember 2015 bis Juni 2016 durchgeführt. Untersucht wurden die nährstoff-konservative und trockenheitstolerantere Grasart *Festuca arundinacea* (Schreb.) und die nährstoff-ausbeutende, weniger trockenheitstolerante Grasart *Lolium perenne* (L.) sowie die Leguminose *Trifolium repens* (L.) als Referenzart. Beide Grasarten wurden ab Messbeginn mit zwei Stickstoffniveaus behandelt, einer nicht-limitierenden und einer reduzierten Stickstoff-Versorgungsstufe. Die beiden Versuchsfaktoren (Art, Stickstoff-Versorgung) wurden in ein randomisiertes Blockdesign mit zwölf Wiederholungen angepasst.

Die erste Studie deutet darauf hin, dass der Abstand, bis zu dem Baumeffekte auftreten können, mindestens 4,5 bis 6 m beträgt. Die Beschattung durch Bäume verringerte das Grünlandwachstum stärker als sie die Seneszenz erhöhte, und diese Wirkung wurde durch die Schnitthäufigkeit nicht verändert. Durch kürzere Schnittintervalle konnte ein höherer

Anteil an lebender relativ zur toten Biomasse nahe der Bäume erhalten werden, aber die gesamte Grünlandbiomasse war im Allgemeinen gering. Folglich lohnen sich kürzere Ernteintervalle in der Nähe von Bäumen nicht. Alternativ könnten längere Schnittintervalle an der Grünland-Baum-Grenze andere Ökosystemleistungen des Grünlands fördern, die von einer extensiven Bewirtschaftung profitieren, wie z. B. die Artenvielfalt. Es befinden sich 4,4 % des deutschen Grünlands an einer Baumgrenze und sind potenziell für eine Extensivierung geeignet.

Die Stickstoffresorptionsprozesse im silvopastoralen Grünland wurden durch die Beschattung durch Bäume beeinflusst. Die Stickstoffkonzentration beider Grünlandmischungen wurde eindeutig durch den Anteil der Leguminosen bestimmt. In der Nähe von Bäumen dominierten Nicht-Leguminosen, während Leguminosen weiter von Bäumen entfernt deutlich stärker vertreten waren. Folglich nahm die Stickstoffkonzentration in der lebenden und toten Grünlandbiomasse in der Nähe von Bäumen ab. Die Stickstoffresorptionseffizienz (NRE), d. h. der Anteil des Stickstoffs, der aus dem absterbenden Pflanzengewebe resorbiert wird, zeigte keine Unterschiede entlang des Grünlandtransekts, was auf einen übergeordneten Effekt der Bäume auf die funktionelle Zusammensetzung der Grasnarbe unabhängig von deren ursprünglichen Zusammensetzung

In der dritten Studie waren die Blatt-Stickstoffkonzentrationen beider Grasarten höher unter hoher als unter niedriger Stickstoffversorgung und höher bei *F. arundinacea* als bei *L. perenne*. Die Grasart *L. perenne* zeigte bei hoher Stickstoffversorgung höhere Stickstoffkonzentrationen in der Gesamttrockenmasse als *F. arundinacea*, was auf Verdünnungseffekte zurückzuführen sein könnte, da *F. arundinacea* eine höhere Gesamttrockenmasse aufwies. Die NRE beider Grasarten variierte, wurde aber nicht durch die Höhe der Stickstoffversorgung beeinflusst. Generell wies *F. arundinceae* bei niedriger und hoher N-Versorgung eine höhere NRE und eine geringere N-Konzentration in den abgestorbenen Blättern auf als *L. perenne*.

Bäume verringern die Graslandproduktion und verändern die botanische Zusammensetzung, wobei Leguminosen am meisten von den Konkurrenzeffekten betroffen sind. Das Erreichen von hohen Erträgen mit gleichzeitig hoher Verdaulichkeit

des Grünlandfutters in der Nähe von Bäumen könnte eine Herausforderung darstellen. Eine extensive Bewirtschaftung der Grünlandbereiche in der Nähe von Bäumen kann eine Möglichkeit zur Förderung der Artenvielfalt sein. Nichtsdestotrotz sind Studien erforderlich, um mögliche langfristige Auswirkungen der baumbedingten Veränderungen der botanischen Zusammensetzung im Hinblick auf den Nährstoffkreislauf im Boden zu bewerten. Darüber hinaus deuten die Ergebnisse darauf hin, dass *F. arundinacea* aufgrund seiner Trockentoleranz und seiner Fähigkeit, Stickstoff effizient zu recyceln, eine geeignete Grasart für landwirtschaftlich genutztes Grünland in den gemäßigten Breiten unter den künftigen Klimaveränderungen sein könnte.

ACKNOWLEDGEMENTS

CURRICULUM VITAE

Positions and Education

since 12/2015	**Georg-August-Universität Göttingen, Germany.** PhD candidate „Grassland production in agroforestry systems" led by Prof. Dr. Johannes Isselstein
10/2013 – 02/2014	**Universität Hohenheim, Germany.** Research assistent "Strengthening drought resilience of the pastoral and agro-pastoral population in the lowland of Ethiopia" led by PD Dr. Anna Treydte
04/2010 – 09/2013	**Universität Potsdam, Germany.** Master of Science „Ecology, Evolution & Nature Conservation" (very good: 1.4). Thesis: „Long term effects of land use on vegetation structure in southern Kalahari savanna rangelands" led by PD Dr. Niels Blaum, PD Dr. Thilo Heinken
10/2009 – 03/2010	**Universität Wien, Autria.** Master of Science „Nature conservation and biodiversity management"
10/2005 – 10/2008	**Hochschule Anhalt (FH), Bernburg, Germany.** Bachelor of Science „Nature conservation and landscape planning" (good: 1.8). Thesis: „Status-quo-Erhebung von unbeweideten Kontrollflächen in sechs Untersuchungsgebieten des Projektes „Erhaltung von Offenland-Lebensräumen an pflegeproblematischen Steilhängen durch ganzjährige Ziegenbeweidung" im Unteren Saaletal sowie erste Datenvergleiche mit bereits beweideten Flächen" led by Prof. Dr. Sabine Tischew, Dr. Sandra Mann
0/1999 – 09/2005	**Universität Leipzig, Sportwissenschaftliche Fakultät, Germany.** Diploma „Sport science with focus of rehabilitation sports, sports therapy and disabled sports" (good: 1.7). Thesis: „Bestimmung der körperlichen Leistungsfähigkeit adipöser Kinder und Jugendlicher in der stationären Rehabilitation mittels mobiler Spiroergometrie" led by Dr. Hans-Dieter Jahn, Dr. Andreas W. A. van Egmond-Fröhlich
09/2001 – 02/2002	**Universidade de Tecnica de Lisboa, Faculdade de Motricidade Humana, Portugal.** Subject „Sport sciences" and „Dance"

Conference contributions and publications (since 2015)

02/2021   **Schmiedgen, A.**, Komainda, M., Kowalski, K., Hostert, P., Tonn, B., Kayser, M., & Isselstein, J. (2021). Impacts of cutting frequency and position to tree line on herbage accumulation in silvopastoral grassland reveal potential for grassland conservation based on land use and cover information. *Annals of Applied Biology*. 179(1) 75-84.

10/2019   **Schmiedgen, A.**, Sutterlütti, R., Komainda, M., Tonn, B., Kayser, M., Isselstein, J. (2019). Grassland tissue and litter production as affected by tree x grass sward interaction and grassland management - Does drought matter? 7. Forum Agroforstsysteme, Freising. Poster presentation – 10/2019

04/2017   Treydte, A. C., **Schmiedgen, A.**, Berhane, G., & Tarekegn, K. D. (2017). Rangeland forage availability and management in times of drought–A case study of pastoralists in Afar, Ethiopia. *Journal of arid environments.* 139 67-75.

12/16   **Schmiedgen, A.**, Kayser, M., Tonn, B., Isselstein, J. (2016). Grasland- und Streuproduktion unter dem Einfluss der Wechselwirkung Baum x Grasnarbe sowie der Bewirtschaftungsintensität. 5. Agroforstforum, Senftenberg. Poster presentation – 12/2016.

11/2016   **Schmiedgen, A.**, Kayser, M., Tonn, B., Isselstein, J. (2016). Grassland tissue and litter production as affected by tree x grass sward interaction and grassland management. BONARES Status-Seminar, Leipzig. Poster presentation – 11/2016.

DECLARATIONS

This dissertation is a cumulative work of three manuscripts, either published, submitted or to be submitted:

Schmiedgen, A., Komainda, M., Kowalski, K., Hostert, P., Tonn, B., Kayser, M., Isselstein, J. (2021). Impacts of cutting frequency and position to tree line on herbage accumulation in silvopastoral grassland reveal potential for grassland conservation based on land use and cover information. *Annals of Applied Biology*. 179(4). https://doi.org/10.1111/aab.12681.

Andrea Schmiedgen: Conduction of the experiment and data collection, Data curation and analysis, Writing – original draft, Writing – Review and Editing. **Martin Komainda:** Data analysis, Conceptualization, Writing – Review and Editing. **Katja Kowalski:** Methodology, Data curation and analysis, Writing – original draft, Writing – Review and Editing. **Patrick Hostert:** Methodology, Data curation and analysis, Writing – original draft, Writing – Review and Editing. **Bettina Tonn:** Conceptualization, Methodology. **Manfred Kayser:** Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition. **Johannes Isselstein:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Schmiedgen, A., Komainda, M., Tonn, B., Kayser, M., Isselstein, J. (2022). Trees in silvopastoral systems reduce legume proportion with no consequence for internal N resorption efficiency. *Journal of Plant Ecology*. https://doi.org/10.1093/jpe/rtac090.

Andrea Schmiedgen: Conduction of the experiment and data collection, Data curation and analysis, Writing – original draft, Writing – Review and Editing. **Martin Komainda:** Data analysis, Writing – Review and Editing. **Bettina Tonn:** Conceptualization,

Methodology. **Manfred Kayser:** Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition. **Johannes Isselstein:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

Schmiedgen, A., Komainda, M., Tonn, B., Isselstein, J. (to be submitted). Effects of nitrogen supply on internal nitrogen resorption of *Lolium perenne* and *Festuca arundinaceae*.

Andrea Schmiedgen: Conduction of the experiment and data collection, Data curation and analysis, Writing – original draft, Writing – Review and Editing. **Martin Komainda:** Writing – Review and Editing. **Bettina Tonn:** Conceptualization, Methodology, Writing – Review and Editing. **Johannes Isselstein:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision.

I confirm that I have composed the present scientific treatise (thesis) independently using no other sources and resources than those stated. I have accepted the assistance of third parties only in a scope that is scientifically justifiable and compliant with the legal statutes of the examinations. In particular, I have completed all parts of the dissertation myself; I have neither, nor will I, accept unauthorised outside assistance either free of charge or subject to a fee.

Furthermore, I have not applied for an equivalent doctoral examination elsewhere and submitted the present thesis as a whole or in parts at another university.

I am aware of the fact that untruthfulness with respect to the above declaration repeals the admission to complete the doctoral studies and/or subsequently entitles termination of the doctoral process or withdrawal of the title attained.

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(Signature)