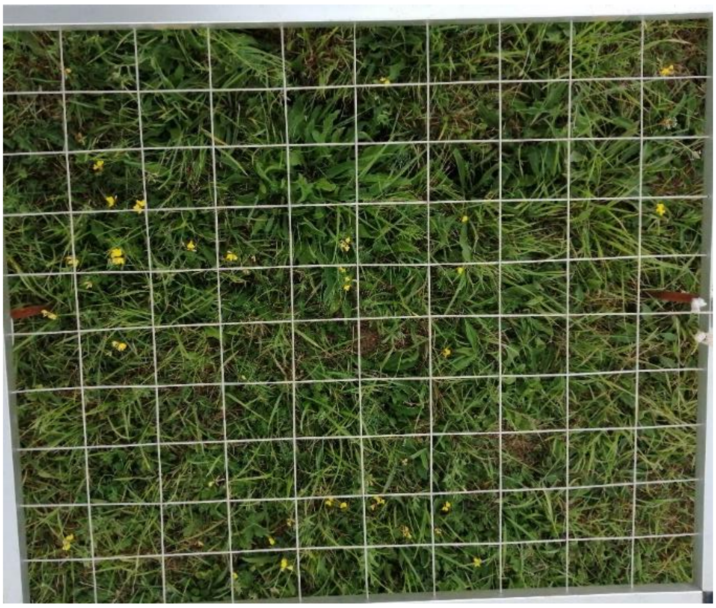
Plant biodiversity affects the relationship between vegetation height and light interception in a long-term fertiliser grassland experiment



MSc Thesis Plant Production Systems

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Contact office.pp@wur.nl for access to data, models and scripts used for the analysis.

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Summary
Currently, policy and research initiatives support extensification of grassland management, aiming for increasing biodiversity. Research on relationships between vegetation height, biomass production and interception of photosynthetically active radiation (PAR) are carried out often in intensively managed grasslands, but not yet in extensively managed grasslands. One aspect of extensification is decreasing nutrient inputs. In the long-term grassland experiment of the Ossekampen, the effects of nutrient application on biodiversity and productivity are already visible, allowing comparisons between more intensively and more extensively managed grasslands. Therefore, the effect of plant biodiversity on the relationship between vegetation height and PAR interception were investigated in the long-term fertiliser experiment of the Ossekampen.

The treatments were the application of calcium (Ca), phosphorus (P), potassium (K), nitrogen (N) and the combinations PK, NPK and PK+N. In PK+N, all N is applied in July, in N and NPK, 62.5% of N is applied in spring, the rest in July. Species richness, Shannon index of functional groups and the relative frequencies of graminoids, forbs and legumes were used as biodiversity indicators. Vegetation height and PAR interception were measured weekly during the growth of the second cut.

Application of NPK and PK+N resulted in the least biodiverse vegetation. Furthermore, it resulted in the highest fraction of graminoids, while application of PK led to the highest fraction of forbs. More biodiverse vegetations intercepted more PAR per mm of vegetation. Increasing fractions of graminoids decreased the PAR interception per mm of vegetation, while increasing fractions of forbs increased PAR interception per mm of vegetation. The difference in relationship between vegetation height and PAR interception was best explained by the species richness, while the proportion of forbs and graminoids and the Shannon index explained it equally well, but less than species richness. Thus, the time-consuming determination of species richness and relative frequency of occurring of each species cannot be replaced by obtaining the frequency of functional groups without decreasing the quality of predicting the relationship between vegetation height and PAR interception.

The expected transition of intensive to more extensive grassland management requires the inclusion of multiple species in grass-growth models. Therefore, new calibrations are required to include biodiversity in the relationship between vegetation height and PAR interception as well as vegetation height and standing biomass. Further research should be done to test whether extensively used grasslands can be grouped into types of grasslands that behave similarly in terms of PAR interception per mm of vegetation or dry matter yield per mm of vegetation height.

1. Introduction

In the following paragraphs, the historical development of grassland management and its effect on the environment are discussed. Subsequently, current and future grassland management strategies are covered.

1.1 Development of grassland management intensity

Grasslands cover approximately 40% of the European utilised agricultural area (Huyghe *et al.*, 2014). Grasslands are important not only because of their abundant presence in Europe, but also for providing a broad range of ecosystem services (Isselstein *et al.*, 2005; Van Den Pol *et al.*, 2018). For instance, grass is one of the cheapest proteins sources for ruminant meat and dairy production and grasslands aid in preserving biodiversity (Van Den Pol *et al.*, 2018). According to Bengtsson *et al.* (2019), the biodiversity in natural and semi-natural grasslands enhance agricultural production in the surroundings by pollination and biological control. Furthermore, grasslands in Europe affect processes such as regulation of water supply and purification of fresh water (Bengtsson *et al.*, 2019; Minns *et al.*, 2001). Additionally, soil erosion rates in grasslands are lower compared to the rates in orchards, vineyards and arable lands (Cerdan *et al.*, 2010). Cultural values of grasslands include the preservation of the aesthetic value of traditional landscapes (Bengtsson *et al.*, 2019; Van Den Pol *et al.*, 2018).

Early in the 20th Century, most grasslands in Europe were extensively managed and they were botanically very diverse (Park, 1987). This changed shortly after the Second World War, since measures were taken to increase food supplies to combat impending hunger (European Union, 2022). The vision of Sicco Mansholt, the Commissioner for Agriculture in the first European Commission, was that Europe should be self-sufficient and that a stable supply of affordable food should be guaranteed for all (European Union, 2022). This was achieved by establishing the first common agricultural policy (CAP) in 1962 (European Parliament, 2022). The CAP promoted agricultural intensification, which is defined as the increase of productivity per unit of land due to increased inputs such as labour, fertiliser or pesticides (Struik & Kuyper, 2017). Old and very biodiverse grasslands were converted to arable land or temporary grasslands meant for feed production for ruminants (Hopkins & Wilkins, 2006). In short time, food shortages disappeared (European Union, 2022).

Agricultural intensification led to problems related to the environment and human health (Al-Khudhairy, 2000; Egmond *et al.*, 2002; Volterra *et al.,* 2002). Excessive application of N and P from fertilised agricultural areas caused shifts in species compositions in aquatic bodies and ultimately led to eutrophication (Egmond *et al.*, 2002). In case of eutrophication, no light reaches aquatic plants, which impairs photosynthesis and therefore leads to a lack of oxygen. This results in blooms of the anaerobic cyanobacteria, which can produce toxins that are dangerous to human health (Volterra *et al.,* 2002). Moreover, agricultural intensification resulted in reduced plant biodiversity (Hopkins & Wilkins, 2006), which reduces ecosystem services such as pollination and biological pest control (Bengtsson *et al.*, 2019).

The first reform of the CAP that included environmental considerations into agricultural policies took place in 1992 (Al-Khudhairy, 2000; European Commission, 2022a). From then onwards, the integration of environmental considerations and agriculture continued. Nowadays, policy and research initiatives increasingly aim at agricultural extensification (Isselstein *et al.*, 2005; Runhaar, 2021), inversing the trend of intensification. For example, the UK made a transition plan to focus more on ecosystem services and sustainable farming practices (DEFRA, 2020). Additionally, the European Union developed the European Green Deal: a set of policy initiatives to revert biodiversity losses and reduce environmental pollution (European Commission, 2022b). A similar trend is seen in The Netherlands where pressure from NGOs, provincial authorities and food processors increases towards implementing nature-inclusive agriculture (Runhaar, 2021). For instance, subsidies will be provided to farmers that include herbs in their grasslands, or that have permanent grasslands (RVO, 2022).

Although many terms and even more definitions exist, the core idea of extensification, nature-inclusive agriculture and sustainable farming practices is to reduce inputs such as fertiliser and pesticides and focus on multiple ecosystem services instead of only aiming at the service of feed production (Runhaar, 2021). In grasslands this results in generally a lower yield, but a higher plant biodiversity (Isselstein *et al.*, 2005; Korevaar & Geerts, 2015). Korevaar and Geerts (2015) investigated the effect of different nutrients on herbage yield and species composition at the long-term grassland experiment The Ossekampen (Box 1).

Box 1: The Ossekampen Grassland Experiment (Elzebroek, 1983; Korevaar & Geerts, 2015).

The Ossekampen Grassland Experiment was set up in 1958 to investigate the effect of single nutrient applications of nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) and combined as PK and NPK on plant species richness and biomass production. The application of the nutrients were compared with a control, which has not received any fertiliser. The experiment was set up on an extensively used hay meadow near Wageningen and initiated with similar productivity and species composition.

Currently, species richness is highest when in plots where only Ca is applied, while it is lowest in plots where N, P and K are applied together. Biomass production is not increased by the application of single nutrients N, P, K and Ca. The application of N, P and K together resulted in the highest biomass production, followed by the combined application of P and K.

1.2 Resource use

Nutrients are essential for the growth of plants, just as water and light. Plants use light with a wave length of 400-700 nm for photosynthesis (McCree, 1972). This is called photosynthetically active radiation (PAR) (Mottus *et al.*, 2012). A fraction of the incident PAR is reflected or transmitted, the rest is absorbed and can be used for photosynthesis. The absorbed light, often calculated by PAR interception multiplied by a constant absorbance coefficient, is the amount of PAR used for photosynthesis (Rosati *et al.*, 2001). The light energy is converted into chemical energy, which provides energy for the maintenance of the plant and the production of biomass. Since PAR absorbance is a constant fraction of PAR interception, PAR interception determines biomass production (Rayburn & Griggs, 2020).

Whereas biomass production refers to the total amount of biomass produced in one year, the forage mass, also called herbage mass or standing biomass, refers to the amount of biomass at the field at a specific time. The relationship between PAR interception and forage mass provides information for both theoretical and practical applications. Firstly, it can be used in grass production simulation models such as LINGRA (Schapendonk *et al.*, 1998). LINGRA is a grass growth model based on light interception, only applicable for perennial ryegrass (*Lolium perenne* L). It can be used to investigate the impact of climate change on grass production or it can forecast grass yields (Schapendonk *et al.*, 1998). Secondly, more practically, PAR interception can be used as a criterium for the timing of defoliation (Rayburn *et al.*, 2016). The optimal PAR interception for defoliation depends on the specific management goal (Rayburn *et al.*, 2016). For instance, in case of aiming at maximal grass growth, PAR interception should be maximised. On the other hand, in case of interseeding legumes, the seedlings need adequate light for germination, thus the optimal PAR interception can be lower.

However, determining PAR interception is time consuming and requires expensive devices. The relationship between vegetation height and PAR interception can be used to easily obtain information about optimal grass management options. For instance, the rising plate meter can be used to measure the forage mass (Martin *et al.*, 2005; Murphy *et al.*, 2021). The rising plate meter non-destructively measures the height at which the plate is carried by the canopy, the so-called compressed vegetation height. In this thesis, I refer to it as vegetation height. Following, the vegetation height can be used to estimate PAR interception (Rayburn *et al.*, 2016) (Fig. 1).

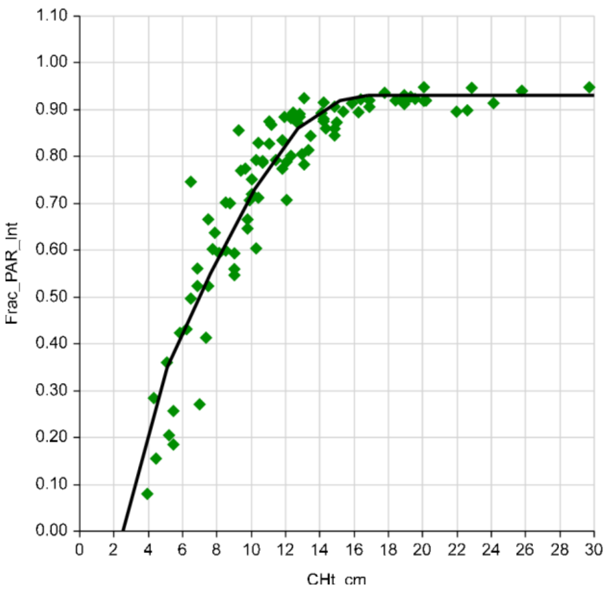

Fig. 1. Relationship between the fraction of photosynthetically active radiation (PAR) intercepted (Frac_PAR_int) and compressed vegetation height (CHt_cm) from Rayburn and Griggs (2020).

1.3 Plant biodiversity

The relationship between vegetation height and PAR interception is influenced by botanical composition (Martin *et al.*, 2005; Rayburn *et al.*, 2016). Botanical composition is an indicator of plant biodiversity, referring to the contribution of each species to the vegetation (Hooper & Vitousek, 1997). Species richness refers to the number of species in a certain area (Hooper & Vitousek, 1997) while the Shannon index combines richness and evenness, a measure how equally species are distributed in a certain area, resulting in a single indication while taking into account multiple groups (Sonkoly *et al.*, 2019).

Plant biodiversity can also be determined with functional groups rather than individual species to find more broad trends among treatments, instead of many differences between many species (Hooper & Vitousek, 1997). In this thesis, the plant species were grouped into graminoids, legumes, non-leguminous forbs and Equisetum. Graminoids contained all grasses and grass-like species, legumes contained all species from the family Fabaceae and non-leguminous forbs contained all broadleaved herbaceous plants that were not legumes. Equisetum is a vascular plant that reproduces sexually by spores and therefore did not fit in either of the abovementioned functional groups. When the Shannon index is calculated for functional groups, it takes into account the number and the evenness of the groups.

Many studies have investigated the effect of plant biodiversity on resource use efficiency (Anten & Hirose, 1999; Ashton *et al.*, 2010; Isselstein, 2005; Pontes *et al.*, 2015). Light is more effectively intercepted when plant biodiversity is increased (Spehn *et al.*, 2000). Anten and Hirose (1999) found that different species in a tall-grass meadow differed in the use of vertical space and time in the season to efficiently use the available light. This phenomenon of using resources at different times and in different spaces is called niche differentiation (Pontes *et al.*, 2015).

1.4 Objectives

Future grasslands are expected to be managed more extensively and to meet biodiversity and environmental goals. In the long-term fertiliser experiment of the Ossekampen, the effects of nutrient application on biodiversity and productivity are already visible, allowing comparisons between more intensively and more extensively managed grasslands. This leads to the following research question:

How does plant biodiversity affect the relationship between vegetation height and PAR interception in the long-term fertiliser experiment at the Ossekampen?

The research question was analysed in two steps. First, the effect of nutrient application on plant biodiversity was investigated. Second, the effect of plant biodiversity on the relationship between vegetation height and PAR interception was examined.

2. Methods

2.1 Experimental site

The Ossekampen Grassland Experiment is located on the westside of Wageningen (51°85'15'' N: 5°83'18'' E) (Pierik *et al.*, 2011). The heavy clay layer that is found on the experimental site (Elzebroek, 1983) consists of small particles that are sedimented on old layers of peat (Buringh, 1951). In the period between 1991 and 2021, the average precipitation was 848 mm per year, with an average temperature of 10.6 °C (Climate-data.org, 2022).

The Ossekampen Grassland Experiment consists of 16 plots of 2.5 x 16 m. The experiment has eight treatments that are divided into two blocks. The control does not receive any fertiliser. The other treatments receive a single nutrient or nutrients applied in combination with each other (Fig. 2). The nutrients that are applied are nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca). The combinations of nutrients applied are PK, NPK and PK+N. The difference between NPK and PK+N is that for NPK, 62.5% of the N-fertiliser is applied in spring and the rest after the first cut in July, while in PK+N, all N-fertiliser is applied in July after the first cut. Treatment N also receives its N-fertiliser partly in spring and partly after the first cut, like treatment NPK. All other nutrients are fully applied in spring. Mineral fertiliser is used for the nutrient application. Super 45 (45% P_2O_5) is applied for P-fertilisation, K60 (60% K_2O) for K-fertilisation, KAS 27 (27% N in form of ammonium nitrate) for N-fertilisation and lime marl for Ca-fertilisation. The yearly nutrient application rates are 160 kgN/ha, 357 kgCa/ha and depending on the treatment 22 or 33 kgP/ha and between 108 and 311 kgK/ha. The exact fertilisation scheme is placed in Appendix 1 (Korevaar & Geerts, 2015).

Originally, the experiment contained six treatments in two completely randomised blocks. However, in 1966 the treatments N and PK+N were added to the experiment. The original plots kept their location within the experiment. Therefore, it is now a partial random block design. In this thesis, I analysed the data as a complete randomised block design.

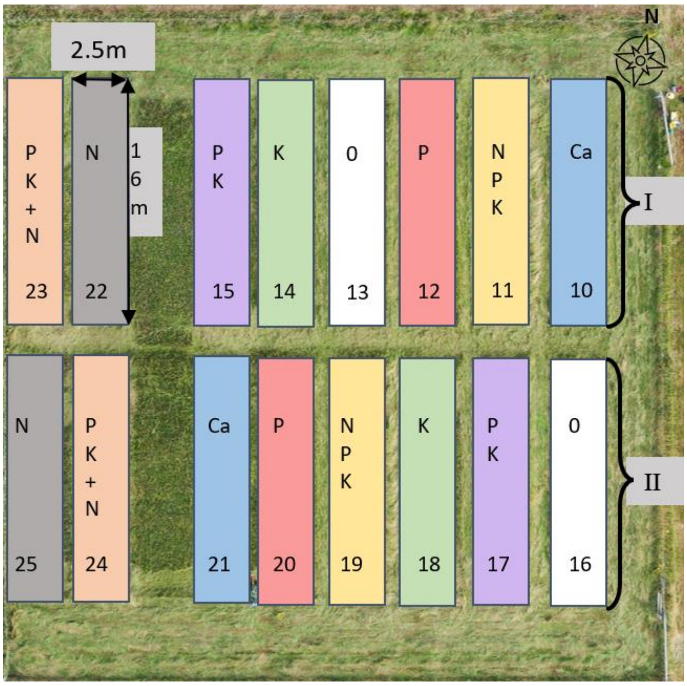

Fig. 2. Experimental design of the Ossekampen Grassland Experiment. It consists of 16 plots of 2.5 x 16 m in 2 blocks (upper number in rectangle). 8 treatments with nutrient application of calcium (Ca), phosphorus (P), potassium (K), nitrogen (N) and combinations of these nutrients PK, NPK and PK+N. In PK+N, all N is applied in July, in N and NPK, 62.5% of N is applied in spring, the rest in July.

2.2 Data collection and processing

The relationship between the intercepted PAR and vegetation height was investigated in the period between 20/07/2022 and 21/09/2022. This measurement period is indicated with week numbers. Week one refers to the first week of measurements (18/07/2022 to 24/07/2022) and week ten is the last week of measurements (19/09/2022 to 23/09/2022). The measurement period covers a large part of the growth period of the second cut, which was from 05/07/2022 to 27/09/2022.

During the growth period of the second cut, I determined vegetation height, PAR interception and ground cover weekly, just as rainfall and groundwater level. Soil temperature and soil moisture were measured continuously with data loggers. Vegetation samples were taken in the last week of measurements to obtain dry weight contributions per functional group. Furthermore, total biomass production was determined by measuring the mown vegetation per plot one week after the last measurements.

2.2.1 Vegetation height

The vegetation height was measured with a rising plate meter (NZ Agriworks ltd, 2022) Since I consistently walked the same route around the perimeter of each plot while taking approximately 20 measurements per plot, I approximately measured at the same locations each week. The smallest measurable interval was 1 mm. The measurements were averaged to obtain one data point per week per plot.

2.2.2 PAR interception

During the growth period of the second cut, I measured the amount of PAR intercepted by the vegetation with the SS1 SunScan Canopy Analysis System for ten weeks (Delta-T-Devices, 2022). The smallest measurable interval was 0.1µmol/m^2/s. 20 measurements per plot were taken every week. The measurements were carried out approximately 80 cm apart from each other, with the measuring rod consistently in the same direction. The measurements were generally taken between 12:30 and 13:30, with one exception. In week 3, it started to rain around the measurement time; measurements were taken between 14:30 and 15:15.

The SunScan Canopy Analysis System measures the incoming PAR intensity above the vegetation with a reference sensor and the PAR intensity that passed through the canopy with a measuring rod. The measuring rod measures the PAR interception at 1.5 cm above the surface. The reference sensor was set up in the middle of the field and the measuring rod was placed under the canopy for each measurement. For enabling comparison between measurements from the reference sensor and the measuring rod, a few measurements with the measuring rod above the vegetation were taken each week. The fraction of PAR that is intercepted by the vegetation was calculated by:

$$fraction\ PAR\ intercepted = \frac{reference\ PAR\ intensity - PAR\ intensity\ under\ canopy}{reference\ PAR\ intensity} \quad \textit{(eq. 2)}$$

PAR interception was averaged to obtain one data point per week per plot.

2.2.3 Plant biodiversity

Proportions of functional groups were obtained by three different methods. Proportions of functional groups were determined with using relative ground cover contribution, contribution to aboveground dry weight and frequency of occurring in the vegetation. The three methods are explained below.

Contribution to ground cover per functional group

Ground cover was obtained by assessing whether vegetation is present directly below the intersect of the wires of a grid (Korva, 1996). This was assessed every week, starting from week 3. The grid had 81 intersects and cells of 75 x 90 mm (Fig. 3). The grid was placed 2 cm above the highest point of the vegetation at the same spot every week. Per plot, I took one picture at the northern side and one at the southern side. A picture was made in such a way that the grid was fitting as well as possible in the picture (Fig. 3).

From the images, I noted per intersection whether a graminoid, a legume, a non-leguminous forb or an Equisetum was present exactly under that intersection. When multiple functional groups were present, all present groups were noted. The plant needed to be green in order to be marked as present. Per picture, the number of intersections with presence of vegetation of a specific functional group under it were counted per functional group, and the number of intersects with presence of vegetation in general were counted.

The total ground cover was calculated as a percentage by:

$$GC\% = \frac{Number\ of\ intersects\ at\ which\ vegetation\ was\ present}{81} * 100\ (eq.\ 1)$$

The proportions of functional groups were obtained by dividing the number of intersections of the functional groups by the number of intersections of present vegetation in general. The average contribution of the functional groups to ground cover of in the growth period of the second cut was used for analysing the effect of long-term nutrient application on fractions of functional group (n=2).

Fig. 3. Grid used to obtain ground cover fraction of the vegetation.

Contribution to aboveground dry weight per functional group

The fractions of dry weight per functional group were obtained by taking samples in the last week of the measurements. Vegetation clippings were taken along two parallels per plot, with two meter distance between clippings on one parallel, resulting in approximately 16 clippings per plot. This is similar to how it is done with species determination at the Ossekampen once every three years (Pierik *et al.*, 2011; Ministerie van Landbouw en Visserij, 1934). However, the determination of the vegetation in the samples was done differently compared to other years. I placed the clippings in a bag per plot and labelled the bag. All plants were sorted into functional groups and the fresh and dry weight per group was obtained in the laboratory. Brown plants were considered as a separate group. I weighed the empty bags before drying, the bags with fresh material, the bags with dry materials and the empty bags after drying. Two empty dry bags were lost before weighing. Therefore, the mean loss of the weights of the bags was subtracted from the fresh weights of the lost bags. For the other bags, I used the measured weight of the dry bags. The proportional contribution to the aboveground dry weight were calculated by dividing the dry weight of the sample of the functional group by the sum of the dry weights of all functional groups (including the dry weight of the dead material) (n=2).

Relative frequency of functional groups

Once every three years, 50 samples per plot are taken according to the De Vries method (Ministerie van Landbouw en Visserij, 1934). All plants in the samples are determined and the frequency of samples in which this species occurs is noted. I used the database with all species occurring per plot at the Ossekampen and their corresponding frequencies of occurring (Geerts & Bufe, 2022). In the database, the occurring species were assigned to functional groups already. For the calculations of the relative frequency per functional group I used the frequency of species from the samples in the years 2014, 2017 and 2020.

The vegetation frequency database of Geerts and Bufe (2022) was used to obtain the relative frequency of a functional group occurring in a sample. The relative frequencies of graminoids, forbs and legumes were calculated for 2014, 2017 and 2020. This was done by adding the frequencies of species belonging to the same functional group from all fifty samples. The relative frequency of the functional group per year was used for the analysis (n=6).

The Shannon index was calculated by taking the sum of the relative frequency of a functional group multiplied by its natural logarithm, including Equisetum (Shannon & Weaver, 1964). Additionally, the species richness was taken from the vegetation frequency database of Geerts and Bufe (2022) and was used as indicator for plant biodiversity.

2.2.4 Dry matter yield

Dry matter yield of the second cut was determined at 27/09/2022 by cutting a strip from the middle of the plot, thus excluding borders. The dimensions of the area that was cut was noted down clearly, so production in form of kgDM/ha could be calculated. The fresh weight from the vegetation that was cut was weighed onsite. From the freshly cut grass, samples were taken and analysed for dry matter content (Korevaar, 2022). The same procedure was done for the first cut this year (at 05/07/2022).The average dry matter yield of 2005-2014 from Korevaar and Geerts (2015) was used for comparison with previous years.

Furthermore, the vegetation height (Section 2.2.1) was compared with dry matter yield. This was done in two ways. First, vegetation height from the last week of measurements was compared with the dry matter yield. Second, the vegetation height of the remaining vegetation was subtracted from the vegetation measured just before mowing, which is referred to as harvested vegetation height in this thesis.

2.2.5 Environmental conditions

The groundwater level, amount of rainfall, soil temperature and soil moisture were monitored between July and October for comparisons among treatments.

Temperature

Continuous measurements were done with logging sensors for soil temperature (Thermochron, 2022) and soil moisture (Sensoterra, 2022). The soil temperature sensors were placed at 10 cm depth and measured the temperature at intervals of 2 hours. The smallest difference possible to detect was 0.5°C. Air temperature data measured at the Bilt for 20-07 to 26-09 in the years 2017 to 2022 were used (KNMI, 2022a). The smallest measurable air temperature interval was 0.1°C.

The average soil temperature during the growth period of the second cut over all treatments was 19 °C, while no differences were observed in average soil temperature among treatments. The lowest and highest measured soil temperature were 11°C and 26°C respectively. The mean air temperature during the growth period of the second cut of 2022 was 18.1 °C, while the average temperature in the same time period in the years 2017 to 2021 was 17.3 °C (KNMI, 2022a). Especially in August and in the first half of September, the daily mean temperature in 2022 was higher than the average of 2017 to 2021 (Fig. 4). Thus, growth period of the second cut in 2022 was warm compared to the previous five years. Daily mean soil temperature and air temperature during the growth period of the second cut in 2022 are shown in Appendix 2.

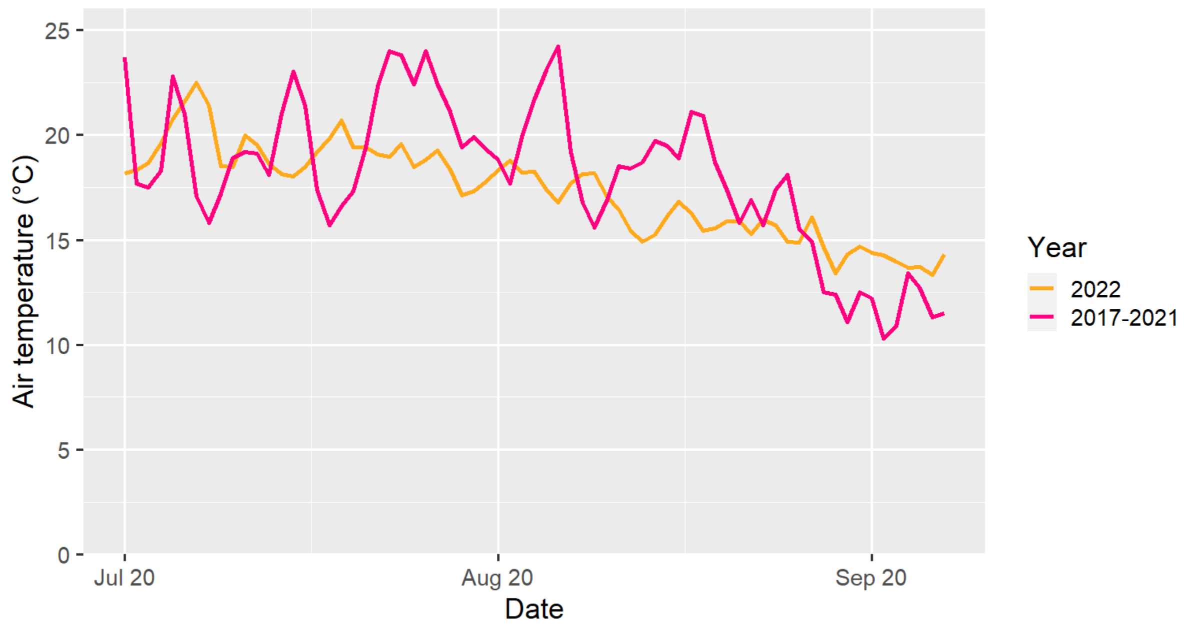

Fig.4. Daily mean air temperature (°C) during the growth of the second cut (20/07 to 26/09) in 2022 (pink) and averaged over 2017-2021 (orange). Data from (KNMI, 2022a)

Moisture

Soil moisture sensors were placed at 20 cm depth and measured at one hour intervals. The smallest measurable interval was 0.1%. The groundwater level was determined weekly with a measuring tape attached to a cylindrical immersion bell (Groentechniek, 2022) and rainfall was collected and noted every week with a rain gauge. For comparison with previous years, rainfall data from the weather station in Wageningen PD was used (KNMI, 2022b). Rainfall data from 20-07-2022 to 26-09-2022 from the datafile was compared with rainfall data from the same period in 2017 to 2021.

The average soil moisture content at the Ossekampen during the growth period of the second cut was 9.7%, while the smallest average soil moisture content during the growth period of the second cut was 7.3% in the P-fertilised plots and the largest 12.7% in the K-fertilised plots (Appendix 3A). One sensor in a Ca-fertilised plot measured soil moisture contents that were much higher than those from all other sensors, especially in the beginning of the growth period of the second cut (Appendix 3B). A possible cause was a mistake when placing the sensors. Therefore, the results from this sensor were excluded for the average soil moisture content.

The soil moisture content measured by all sensors showed similar trends during the growth of the second cut (Appendix 3B). Therefore, the average daily soil moisture content over all sensors was used to show trends (Fig. 5). This average daily soil moisture content slightly decreased until the end of August, but increased suddenly around 01-09-2022. This could be linked to the rainfall data, measured by the KNMI (2022b) in Wageningen (51° 59’ N: 5°39’ E) (KNMI, 2022b) (Fig. 5). It was especially dry in August, but more rain was collected in September.

During the period of data collection, 130 mm of rainfall was collected at the Ossekampen, while the weather station in Wageningen determined 146 mm during the same period (KNMI, 2022b). Data from KNMI (2022b) was used, because this allowed comparison of rainfall data with previous years and the rainfall data did not deviate much from the rainfall data from the Ossekampen. Averaged over 2017 to 2021, the sum of rainfall in this period was 170 mm (KNMI, 2022b). Thus, compared to the previous years, it was relatively dry during the measuring period. The groundwater level started at 100 cm in the beginning of July, reached its maximum depth of 127 cm in the start of September and subsequently lowered to 78 cm at the end of the growth period of the second cut (Appendix 3C).

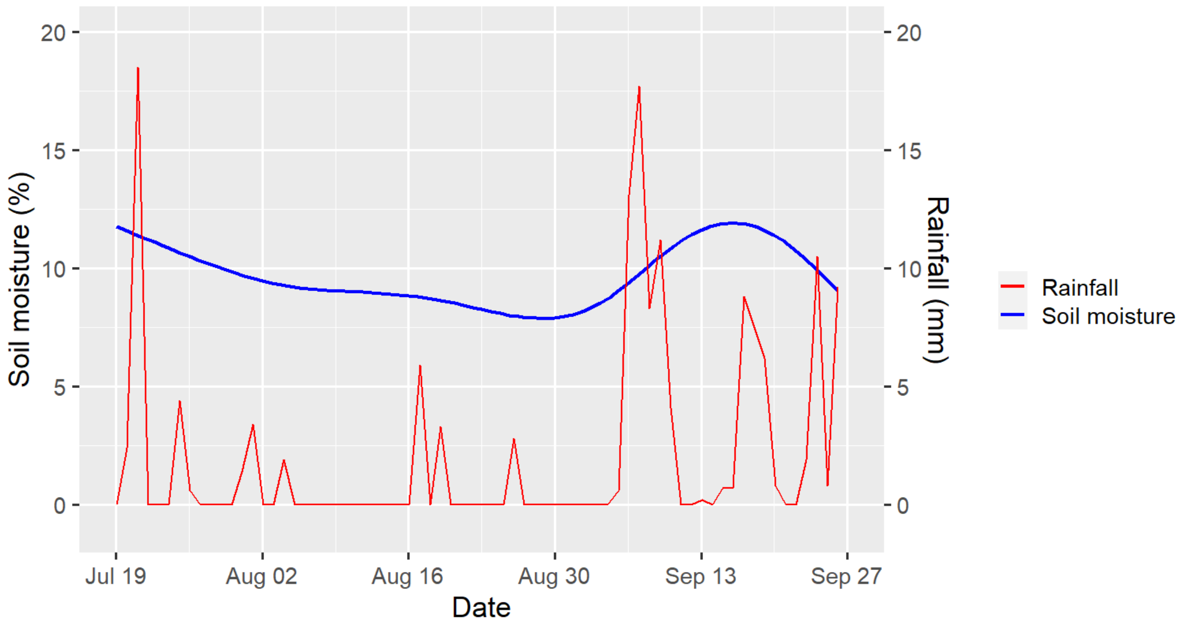

Fig. 5. Soil moisture (%) at 20 cm depth, measured every hour and daily rainfall (mm) during the growth period of the second cut. Daily rainfall data from (KNMI, 2022b)

2.3 Statistical analysis

2.3.1 Plant biodiversity indicators

For the analysis of treatments on plant biodiversity indicators, assumptions for using the analysis of variance (ANOVA) were tested first. The Shapiro-Wilk test was used for testing normal population's distributions (α=0.05) (Shapiro & Wilk, 1965) and equality of variances was tested by Levene's test (α =0.05) (Levene, 1960). The assumptions for ANOVA were only satisfied for species richness. The two-way ANOVA revealed that there was no significant interaction effect between treatment and block, but there was a significant effect of the main effects of treatment and block for species richness (Girden, 1992). Subsequently, a Tukey HSD test was performed with correction for the block effect to find which treatments caused differences in species richness (α=0.05) (Tukey, 1977).

For all other plant biodiversity indicators, the Scheirer-Ray-Hare test was used to test whether a significant interaction block-treatment effect was found (α=0.05) (Sokal & Rohlf, 1969). The Scheirer-Ray-Hare test is a non-parametric test that can be used for a two-factorial design. In all cases, no block-effect nor a significant block-treatment interaction was found. Therefore, the non-parametric post-hoc Dunn's test was used with treatment as single explanatory variable and the Benjamini-Hochberg method as p-adjustment method (Dinno, 2017).

2.3.2 Vegetation height and PAR interception

The vegetation height and dry matter yield were compared using a linear regression model and subsequently, R-squared was computed.

Linear mixed effect regression (lmer) from the package lme4 was used for analysis of the vegetation height and PAR interception during the measurement period, and for the relationship between vegetation height and PAR interception affected by treatment, species richness, Shannon index and proportions of graminoids, forbs and legumes (α=0.05) (Bates *et al.*, 2015). The fixed effects depended on what was analysed and the random effects were always Block per Plot to correct for repeated measurements. For the analysis of all fixed effects, plotting of the residuals was done to assess whether the lmer model satisfied the assumptions of linearity, normal distribution of residuals and homogeneity of variance.

<u>Vegetation height and PAR interception during the measurement period</u>

The analysis of vegetation height and PAR interception over time was done with the fixed interaction effect of treatment and the continuous variable week. The restricted maximal likelihood concept (REML) was used in the lmer. In case of a significant effect of the fixed effects, emtrends from package emmeans was used for pairwise comparisons (α=0.05) (Lenth, 2022). Moreover, the differences among treatments in vegetation height and PAR interception per week were analysed by using the fixed effects of treatment and the factor week. Pairwise comparison between treatments per week was done with emmeans from package emmeans (α=0.05).

<u>The relationship between vegetation height and PAR interception affected by treatment</u>

The relationship between vegetation height and PAR interception affected by treatment was analysed with the fixed interaction effects of vegetation height and treatment, with the REML concept (α=0.05). In case of a significant effect of the fixed effects, emmeans from package emmeans was used for pairwise comparisons (α=0.05).

The relationship between vegetation height and PAR interception affected by biodiversity indicators

The relationship between vegetation height and PAR interception affected by biodiversity was analysed with the fixed interaction effects of vegetation height and a biodiversity indicator. The biodiversity indicators that were used were species richness, Shannon index of functional groups and the fractions of graminoids, forbs and legumes. The maximum likelihood (ML) concept was used to allow comparisons between the models. The lmer models that used the plant biodiversity indicators were compared to each other with the Akaike information criterion as prediction error of the statistical methods.

In case of a significant effect of the fixed effects, three moderating variables of the biodiversity indicator were used for pairwise comparisons of the slope between vegetation height and PAR interception, done with emtrends from package emmeans (α=0.05). The three moderating variables that were used were the standard deviation subtracted from the mean, the mean and the standard deviation added to the mean of the biodiversity indicators. The moderating variables were also used to fit three lines of predicted values of PAR interception with all possible values of vegetation height, carried out with ggpredict from the package ggeffects (Lüdecke, 2018).

3 Results

Firstly, vegetation height, PAR interception and plant biodiversity and the effect of treatments on the aspects are treated separately. Subsequently, in paragraph 3.4, all aspects are integrated and the effect of treatments and plant biodiversity on the relationship between vegetation height and PAR interception are studied.

3.1 Vegetation height

The growth period of the second cut started two weeks after the first cut. At that moment, the plots contained mostly stubbles and visually, the vegetation height was similar in all plots. The vegetation in the plots with application of only N was red-brownish, while the plots with NPK and PK+N contained yellow and rigid stubbles. Over time, more greenness was observed in all plots.

Besides visual assessments of the vegetation, vegetation height measurements were carried out. Vegetation height increased significantly more per week in the plots treated with NPK, than in all other treatments ($P<0.05$) (Fig. 6). The slope of vegetation height per week in the plots with treatment PK+N increased significantly less than in treatment NPK, but still more than in all other treatments ($P<0.05$) (Table 1). Vegetation height increased least when no fertiliser was applied at all, but this was only significantly smaller than when Ca was applied, or N, P and K in combination.

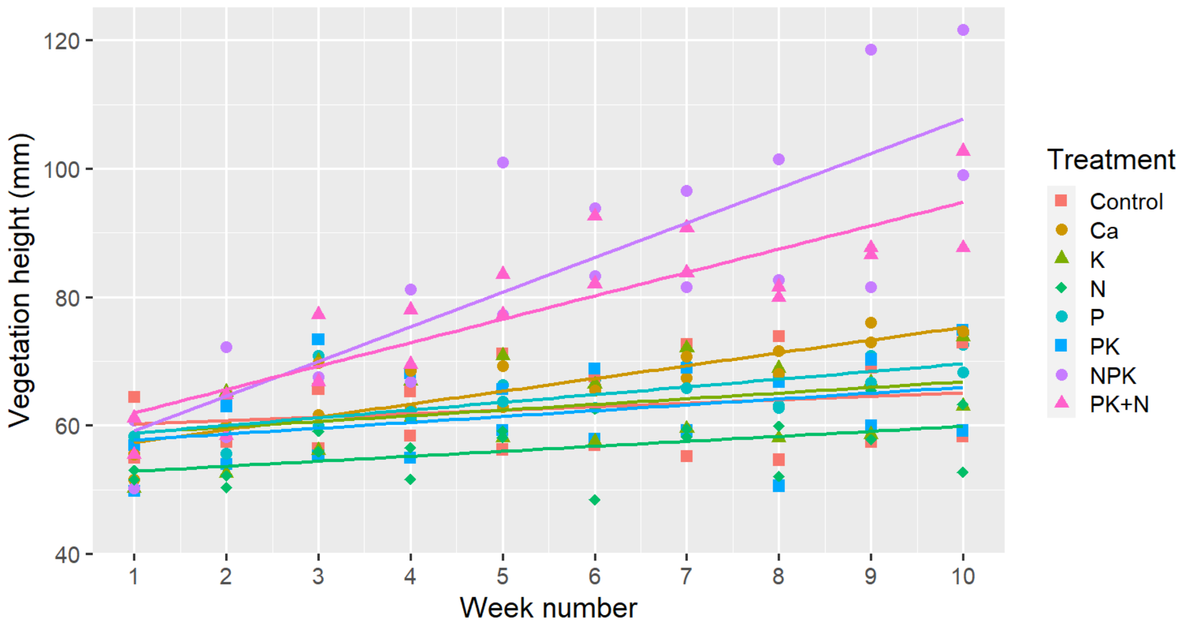

Fig. 6. Vegetation height (mm) per treatment measured during the growth period of the second cut.

At the beginning of the measuring period, vegetation height in all treatments was 55 mm on average, with no significant differences among treatments ($P>0.05$). The first significant differences among treatments occurred in week 4, where the vegetation height in the plots treated with NPK and PK+N was significantly higher than in plots treated with N ($P<0.05$). In week ten, the last week of the measurements, the vegetation height was largest in the plots that received NPK or PK+N (Table 1).

Table 1. Slope of vegetation height increment per week (mm/week) and vegetation height (mm) measured in week ten. Different letters represent significant differences in vegetation height between the treatments.

Treatment	Slope between vegetation height and weeks (mm/week)	Vegetation height in week ten (mm)
Control	0.5 a	75 a
Ca	2.0 b	66 a
K	0.9 ab	68 a
N	0.8 ab	58 a
P	1.2 ab	70 a
PK	0.9 ab	67 a
NPK	5.4 d	110 b
PK+N	3.6 c	95 b

The dry matter yield from this cut and the harvested vegetation height did not significantly differ among treatments (*P*>0.05) (Table 2). However, clear tendencies are visible. A reason why it was not significantly different could be the low sample size (n=2). In both the harvested vegetation height and dry matter yield, most vegetation was harvested in the treatments NPK and PK+N. The dry matter yield of the vegetation in treatment N was second smallest compared to other treatments, but treatment N resulted in the third highest harvested vegetation height (Table 2).

Table 2. Harvested vegetation height (mm) is the vegetation height measured just after mowing subtracted from the vegetation height measured before mowing. Dry matter yield (kgDM/ha) is the actual dry matter of the vegetation removed from the plots (n=2).

Treatment	Harvested vegetation height (mm)	Dry matter yield (kgDM/ha)
Control	15	365
Ca	22	965
K	16	454
N	24	456
P	25	708
PK	19	688
NPK	56	1697
PK+N	52	1655

The best-fit regression line between dry matter yield and vegetation height measured in week ten in the growth period of the second cut (R^2=0.837) explained the variation in dry matter yield better than the best-fit regression line between dry matter yield and harvested vegetation height (R^2=0.721). The slopes in both relations were similar. For the data from the first cut this year, taken place at 05-07-2022, a similar comparison was done. The slopes of dry matter yield and vegetation height were in the same order of magnitude, but the vegetation height and dry matter yield were less related. During the growth period of the first cut, R^2=0.672 for dry matter yield and vegetation height measured just before harvest and R^2=0.674 for dry matter yield and harvested vegetation height (Appendix 4).

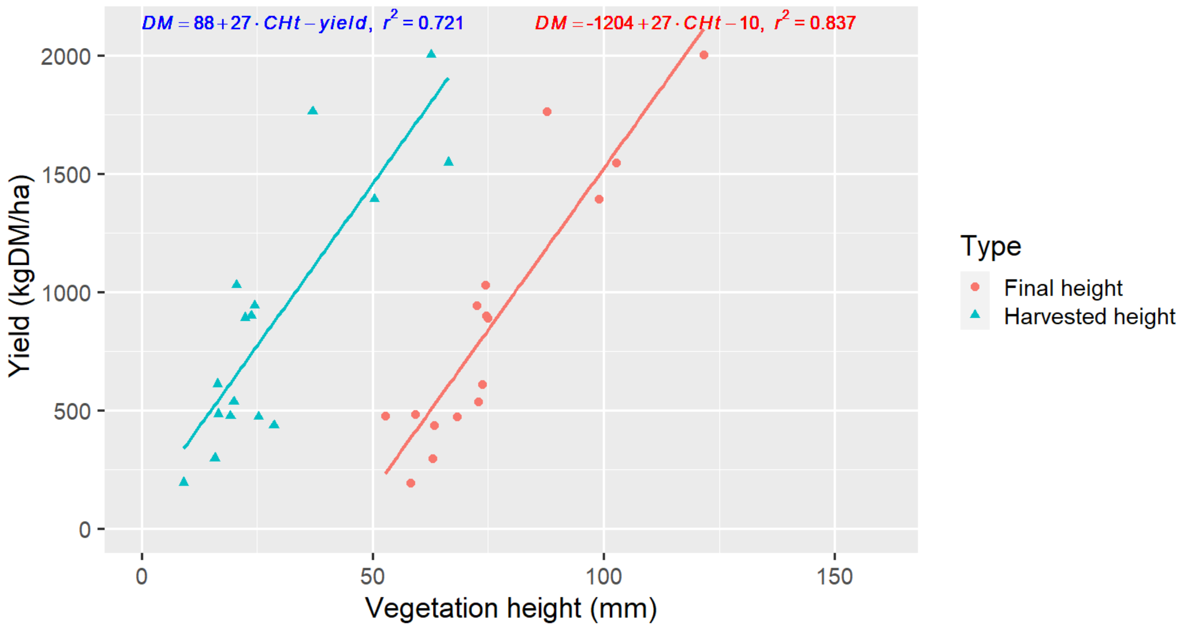

Fig. 7. Linear regression between dry matter yield (kgDM/ha) and harvested vegetation height (mm) (blue) and dry matter yield (kgDM/ha) and vegetation height measured in week ten (mm) (red).

3.2 PAR interception

PAR interception increased for all treatments during the growth period of the second cut, but the extent to which the PAR interception increased, differed among treatments ($P<0.05$) (Fig. 8). The increments of PAR interception per week in the plots treated with NPK and PK+N were significantly higher than in the control and plots treated with N (Table 3).

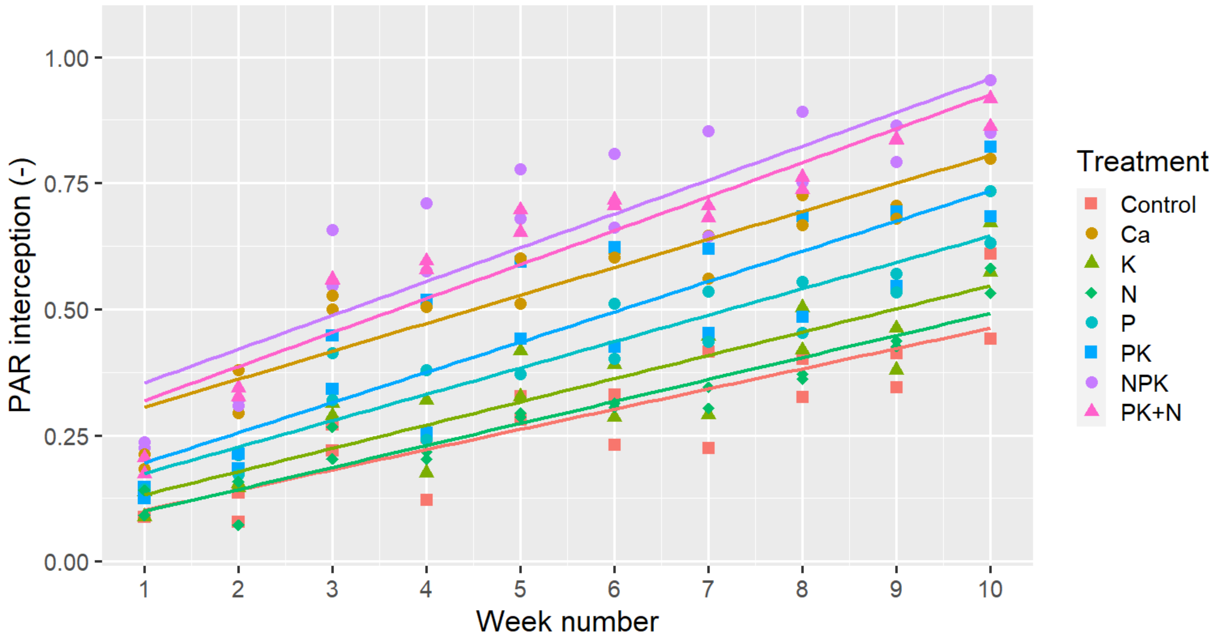

Fig. 8. Fraction of PAR interception per treatment measured during the growth period of the second cut.

The first differences among treatments in PAR interception occurred in the second week of the measurement period. In week two, the vegetation in the treatments NPK, PK+N and Ca intercepted more PAR than the vegetation in the treatments K, N and the control ($P<0.05$). In week ten, the last week of the measurement period, PAR interception in the control and treatments N, K and P were significantly lower than PAR interception in the treatments NPK and PK+N ($P<0.05$) (Table 3). Thus, interception of PAR increased most in the treatments NPK and PK+N and this resulted in the highest fraction of intercepted PAR at the end of the growth period of the second cut (Table 3).

Table 3. Increment of PAR interception per week (week^{-1}) and total fraction of PAR intercepted by the vegetation in week 10, the last week of measurements (-). Slopes and fractions of PAR interception followed by different letters in the same column differ significantly ($P<0.05$).

Treatment	Slope between PAR interception and time (week^{-1})	Fraction of PAR intercepted in week ten
Control	0.04 a	0.53 a
Ca	0.06 ab	0.81 cd
K	0.05 ab	0.62 ab
N	0.04 a	0.56 a
P	0.05 ab	0.68 abc
PK	0.06 ab	0.75 bcd
NPK	0.07 b	0.90 d
PK+N	0.07 b	0.89 d

3.3 Plant biodiversity

Indicators of plant biodiversity obtained by the three methods, contributions to ground cover, aboveground dry weight and relative frequency, are discussed in sections 3.3.1, 3.3.2 and 3.3.3 respectively. Subsequently, one of the above-mentioned methods is selected in section 3.3.4 to test the effect of different indicators of plant biodiversity on the relationship between vegetation height and PAR interception.

3.3.1 Relative contribution of functional groups to ground cover

The average total ground cover in treatment N was significantly smaller than in all other treatments (P<0.05), while total ground cover was largest in treatment Ca and NPK (Table 4). The proportion of ground cover by graminoids slightly increased over time in the control, but decreased in the plots treated with PK+N (Appendix 6). The average ground cover contribution of the graminoids during the growth period of the second cut did not differ significantly among treatments. This is likely due to low statistical power of the tests. Therefore, visible trends in aboveground dry matter contributions by functional groups are described anyway. In the plots treated with NPK and PK+N, the ground cover contributions of graminoids were higher than in all other treatments, while lowest proportions of graminoids were found in the control and in the treatments N and K (Table 4). The sum of ground cover contributions of all functional groups is less than one, because the functional group Equisetum is not included in the table.

Ground cover contribution of forbs increased slightly during the growth period of the second cut for all treatments and no significant interaction between treatment and week was found (Appendix 6). The average ground cover contribution by forbs during the growth period of the second cut did not differ significantly among treatments, but the proportions of forbs in the treatments NPK and PK+N were less than halve than proportions found in the treatments K, PK, P, the control and Ca (Table 4).

No significant effect of treatment or time was found in ground cover contributions by legumes. This is probably caused by the small contributions of legumes to ground cover and there were treatments in which no legumes were found. Therefore, ground cover contributions by legumes in week ten could not be tested. Without using statistical tests, it can be mentioned that no legumes were found in the treatments PK+N and N, while a very small fraction of ground cover was covered by legumes in treatment NPK. Larger contributions to ground cover by legumes were found in the control and treatments Ca, K, PK and P (Table 4).

Table 4. Average ground cover contributions of the functional groups graminoids, forbs and legumes and total ground cover from week 3 until week 10 during the growth of the second cut. Ground cover contributions of the same functional group or total ground cover followed by different letters represent significant differences (P<0.05) (n=2).

Treatment	Average ground cover contribution of graminoids	Average ground cover contribution of forbs	Average ground cover contribution of legumes	Average total ground cover
Control	0.23	0.63	0.13	0.63 b
Ca	0.46	0.45	0.20	0.87 e
K	0.27	0.55	0.18	0.63 b
N	0.24	0.68	0.02	0.44 a
P	0.40	0.57	0.10	0.75 d
PK	0.36	0.56	0.15	0.71 c
NPK	0.87	0.16	0.01	0.83 e
PK+N	0.78	0.25	0.00	0.80 d

3.3.2 Relative contribution of functional groups to above-ground dry weight

No significant differences among treatments or blocks occurred in proportional contribution to aboveground dry weight in any of the functional groups (P>0.05) (Table 5). However, some visible trends are mentioned. The aboveground dry matter contributions of graminoids were largest in the plots treated with NPK and PK+N, in which more than half of the aboveground dry weight belonged to graminoids. Smallest graminoid contributions to aboveground dry matter were found in the plots treated with K and Ca.

On the contrary, treatment Ca contained the largest proportion of forbs, followed by PK and N respectively (Table 5). Just as with the method of using ground cover, the forbs in NPK and PK+N contributed hardly to the aboveground dry weight. The contribution of legumes to aboveground dry weight was very low in general, with no legumes at all in the plots treated with N. Legume contribution to aboveground dry matter was largest in PK and P (0.23 and 0.13 respectively). The contributions of the functional groups to aboveground dry weight do not add up to one, because the functional group of Equisetum and the brown material are excluded from the table.

Table 5. Proportional contributions of graminoids, forbs and legumes to aboveground dry weight. No significant differences occurred in proportions of graminoids, forbs and legumes (P>0.05) (n=2).

Treatment	Relative contribution of graminoids to BM	Relative contribution of forbs to BM	Relative contribution of legumes to BM
Control	0.40	0.16	0.06
Ca	0.23	0.56	0.05
K	0.12	0.29	0.04
N	0.23	0.44	0.00
P	0.37	0.26	0.13
PK	0.14	0.49	0.23
NPK	0.71	0.01	0.00
PK+N	0.64	0.09	0.00

3.3.3 Relative frequency of functional groups

The vegetation frequency database from Geerts and Bufe (2022) was used to obtain the relative frequency of the functional groups. Graminoids were significantly more present in the plots treated with K, N, NPK and PK+N, than in the plots treated with Ca, P and PK ($P<0.05$). However, none of the treatments significantly changed the frequency of graminoids compared to the control ($P<0.05$) (Table 6). The relative frequency of forbs in the vegetation in the plots treated with Ca, P and PK was significantly higher compared to other treatments ($P<0.05$). The lowest amount of forbs were found in the most enriched plots; treatments NPK and PK+N (Table 6). In these enriched plots, no legume was found in the samples in 2014, 2017 and 2020 (Table 6), just as no aboveground biomass was allocated to legumes in 2022 (Table 6).

Table 6. Relative frequency of graminoids, forbs and legumes. Average frequency from 2014, 2017 and 2020. Adapted from (Geerts & Bufe, 2022) (n=6).

Treatment	Relative frequency of graminoids	Relative frequency of forbs	Relative frequency of legumes	Species richness	Shannon index
Control	0.55 ab	0.31 b	0.09 c	30.3 c	1.04 b
Ca	0.44 a	0.40 c	0.10 c	37.7 d	1.12 c
K	0.56 b	0.31 b	0.08 bc	32.8 cd	1.04 b
N	0.65 b	0.25 ab	0.01 a	22.8 b	0.87 ab
P	0.48 a	0.41 c	0.04 b	29.7 c	1.02 b
PK	0.49 a	0.39 c	0.09 c	31.8 c	1.03 b
NPK	0.78 b	0.20 a	0.00 a	15.8 a	0.57 a
PK+N	0.78 b	0.18 a	0.00 a	18.7 ab	0.63 a

From the same database, the number of species occurring at the Ossekampen in 2014, 2017 and 2020 were used to obtain the species richness and the Shannon index of functional groups (Table 6.). Liming resulted in the largest species richness, although it was not significantly larger than when K was applied ($P>0.05$). Similarly, the plots that were treated with NPK contained the lowest number of species, although it was not significantly different from treatment PK+N (Table 6).The Shannon index ranged from 0.61 in NPK to 1.12 in Ca (Table 6). Significantly smaller Shannon indices were found in the treatments NPK and PK+N compared to the other treatments, except for N. Treatment Ca resulted in the highest Shannon index.

The relationship between the species richness and the Shannon index of the functional groups was visualised in Fig. 9. Approximately 70% of the variance in species richness could be explained by the Shannon index.

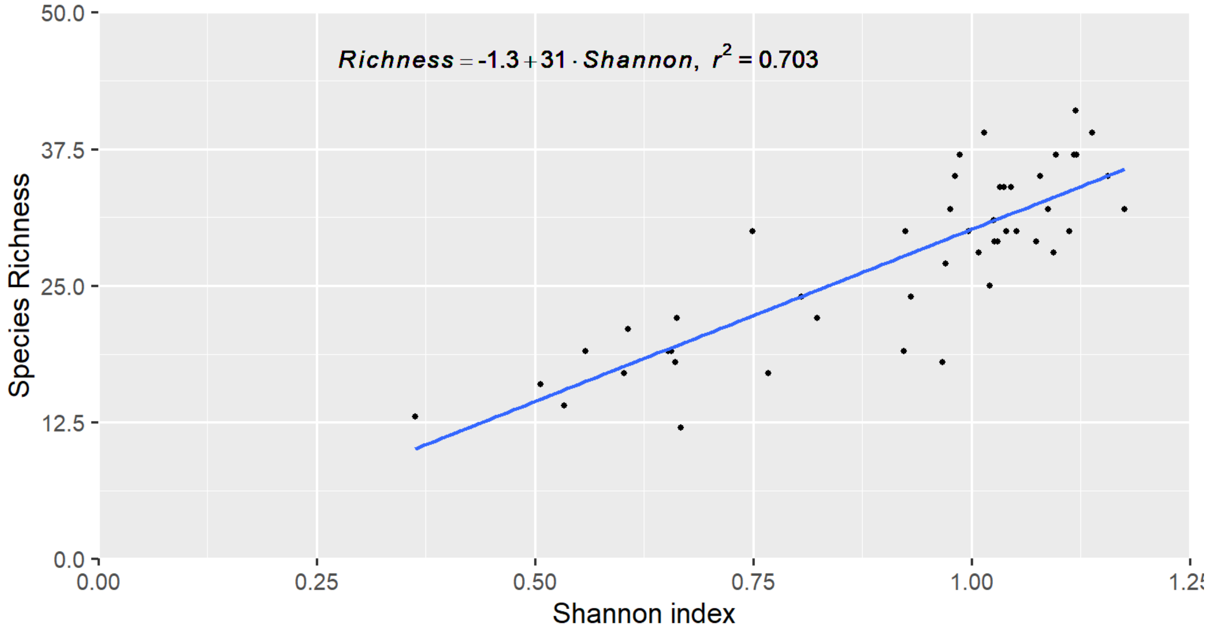

Fig. 910. Relation between Shannon index of functional groups and species richness.

3.3.4 Selection of the method for representing plant biodiversity

To test whether plant biodiversity affects the relationship between vegetation height and PAR interception, the relative frequency method (Section 3.3.3) is selected. This method was chosen to assess the proportions of the functional groups, because it was expected to be the least influenced by errors, compared to the other methods. For the relative contribution of functional groups to aboveground dry weight, the sample size was probably too small, which gives an increased risk of over- or underestimating a functional group. In addition, the dry masses of the functional groups were very different; a small measuring error had a smaller effect in graminoids than in legumes. For the relative contribution of functional groups to ground cover, pictures were made to prevent that changing the position of the head would influence the assessment of the ground cover. However, this reduced the visibility of the vegetation, which made the assessment of which functional group was present subject to bias. Comparisons between the methods are shown in Appendix 7.

3.4 The relationship between vegetation height and PAR interception

3.4.1 Effects of treatment

A significant effect of treatment on the relationship between PAR interception and vegetation height was found (Fig. 10). The vegetation in the treatments that received Ca, K, PK and P intercepted significantly more PAR per mm of vegetation height than the vegetation in the treatments NPK, PK+N and N (*P*<0.05) (Table 7). As seen in Fig. 6 and 8, treatments NPK and PK+N contained the highest vegetation and intercepted most light, even with the gentlest slope.

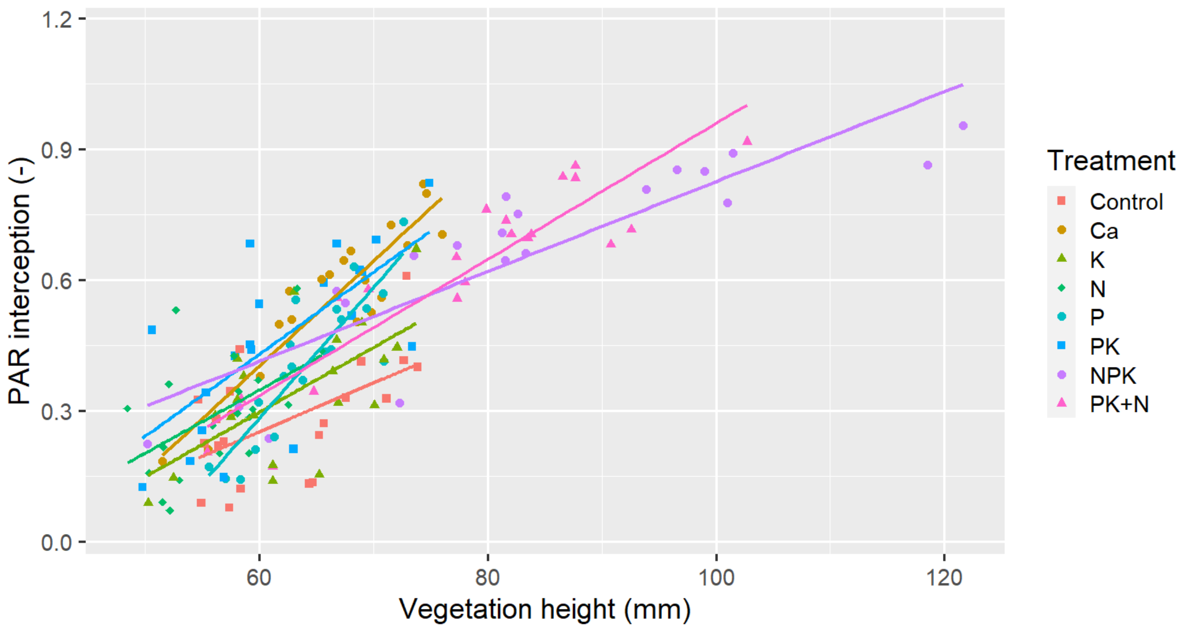

Fig. 110. The relationship between vegetation height and PAR interception (mm) affected by treatment.

Table 7. The slope for PAR interception per mm of vegetation height affected by treatment.

Treatment	Slope Treatment
0	0.022 ab
Ca	0.025 b
K	0.026 b
N	0.020 a
P	0.032 b
PK	0.028 b
NPK	0.012 a
PK+N	0.017 a

3.4.2 Effects of plant biodiversity

The relationship between vegetation height and PAR interception was significantly affected by species richness (Fig. 11A) and Shannon index of functional groups (Fig. 12A). With a higher number of species and a higher Shannon index, more PAR was intercepted per mm of vegetation. However, the largest fraction of PAR interception and highest vegetation height and thus the highest productivity occurred with least species richness (Fig. 11B) and lowest Shannon index (Fig. 12B).

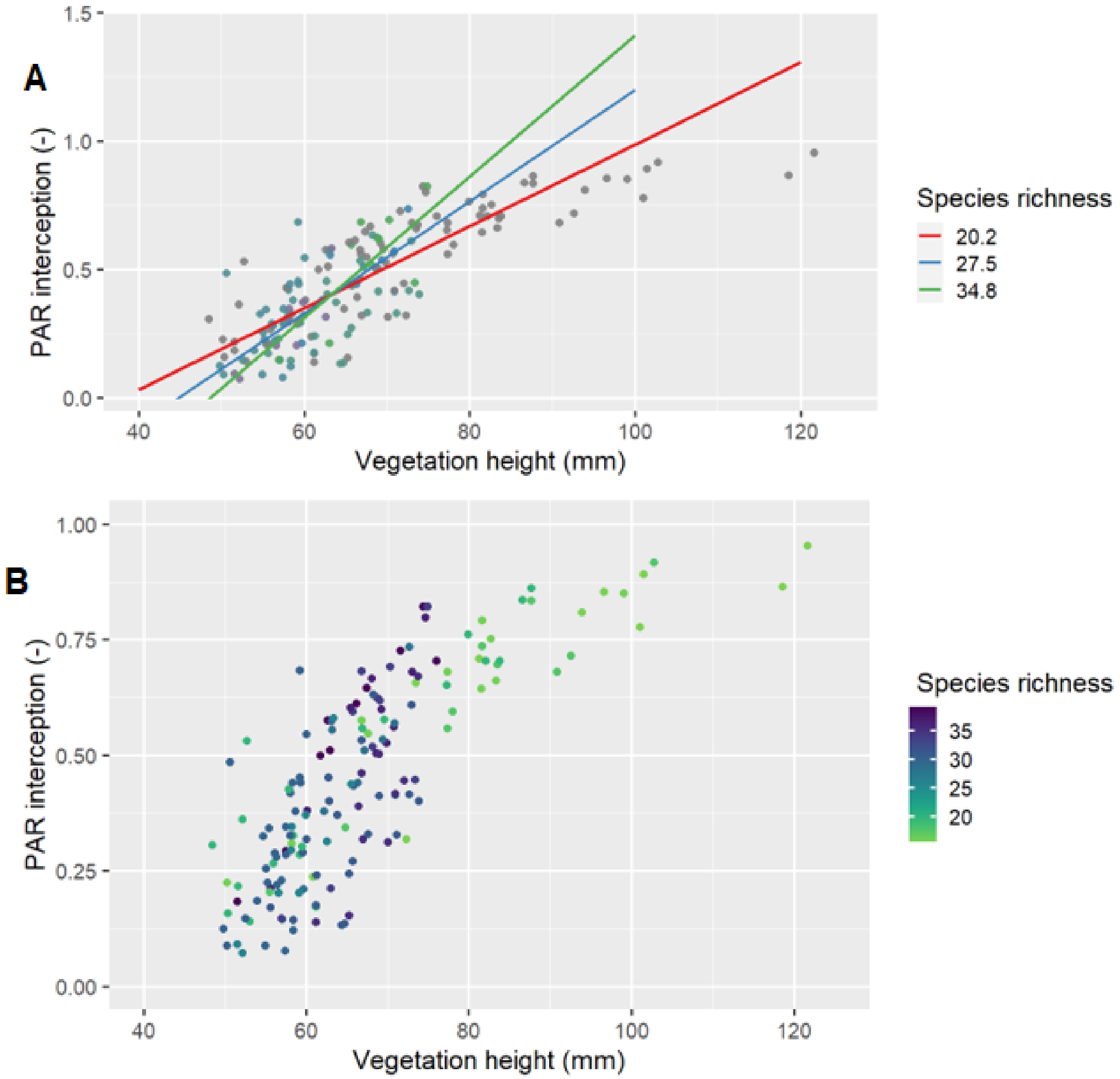

Fig. 11. Relationship between vegetation height (mm) and PAR interception affected by species richness. The three lines are predictions from the linear mixed effects regression model, using the moderating variables of species richness (A) (Section 2.3.2). The higher the species richness, the darker the points (B).

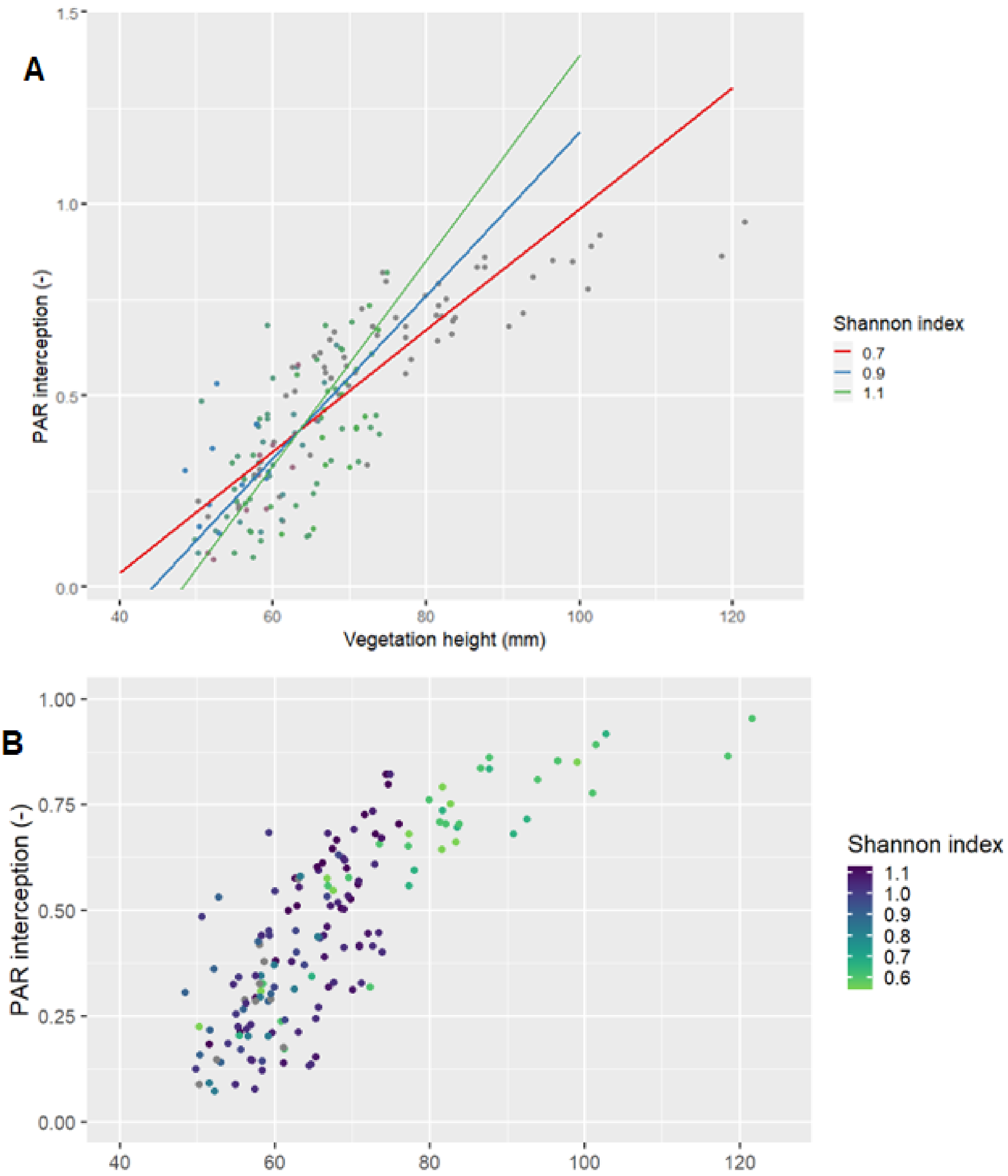

Fig. 12. Relationship between vegetation height (mm) and PAR interception (-)affected by the Shannon index of functional groups. The three lines are predictions from the linear mixed effects regression model, using the moderating variables the Shannon index (A) (Section 2.3.2). The higher the Shannon index, the darker the points (B).

The fraction of intercepted PAR per mm of vegetation decreased with increasing proportions of graminoids in the vegetation (Fig. 13A). In contrast, increasing proportions of forbs resulted in increasing PAR interception per mm of vegetation (Fig. 14A). A significant effect of the proportion of legumes on the relationship between PAR and vegetation height was found, but due to the small fractions of legumes, no specific trends could be determined and therefore, no lines could be fit. The largest fraction of intercepted PAR and the highest vegetation height were found when the proportion of graminoids were highest (Fig. 13B) and forbs and legumes were lowest (Fig. 14B and 15).

The Akaike information criteria of the linear mixed effect regression models using the different plant biodiversity indicators were compared. The species richness explained the relationship between vegetation height and PAR interception best. The relative frequencies of graminoids and forbs and the Shannon index of functional groups explained the relationship between vegetation height and PAR interception equally well, but all less than species richness. Appendix 8 contains the assessment of the relationship between PAR interception and vegetation height influenced by plant biodiversity determined with the ground cover method.

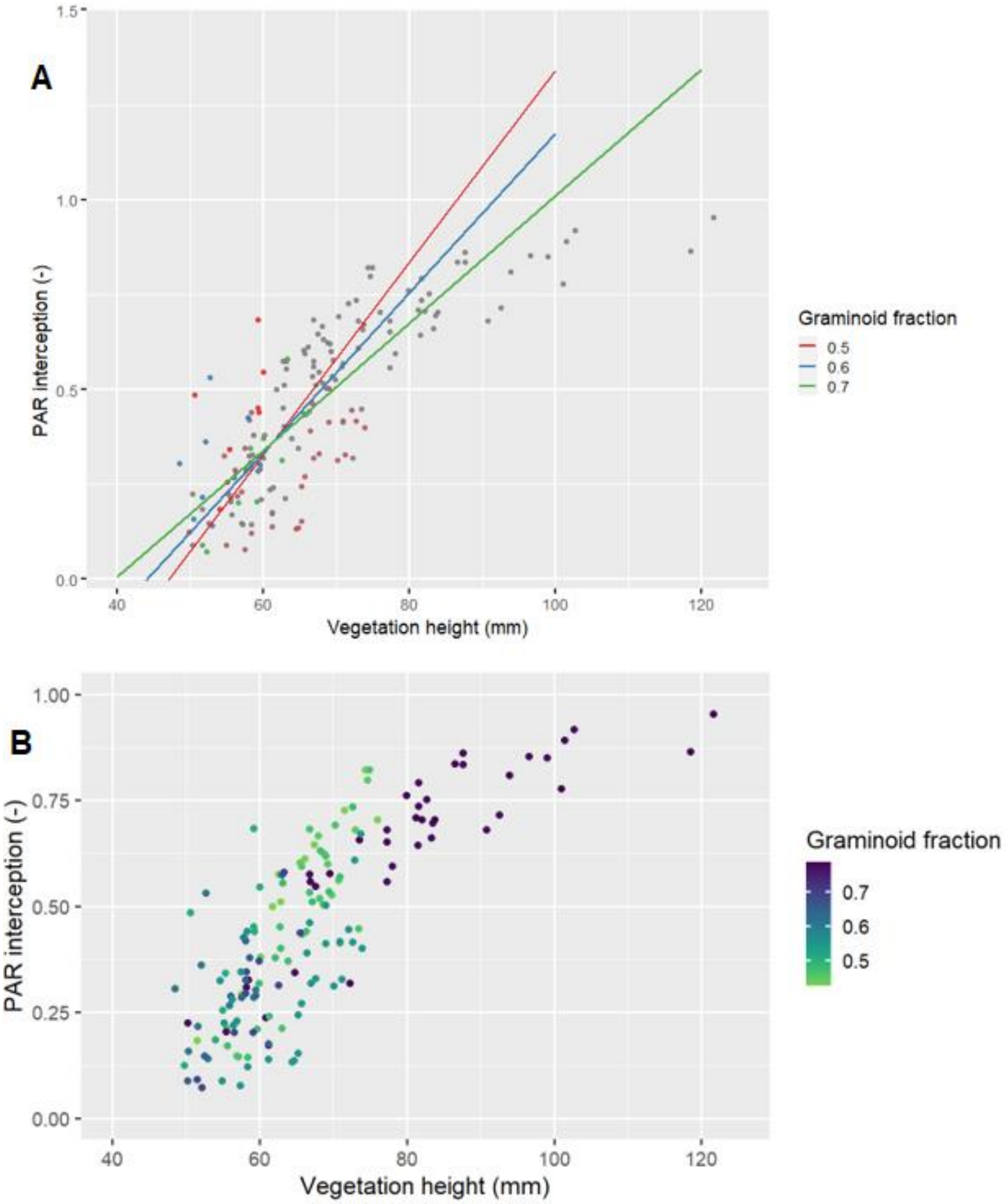

Fig. 123. The relationship vegetation height (mm) and PAR interception (-) affected by the proportion of graminoids. The three lines are predictions from the linear mixed effects regression model, using the moderating variables of the proportions of graminoids (A) (Section 2.3.2). The colours in B represent the fractions of graminoids.

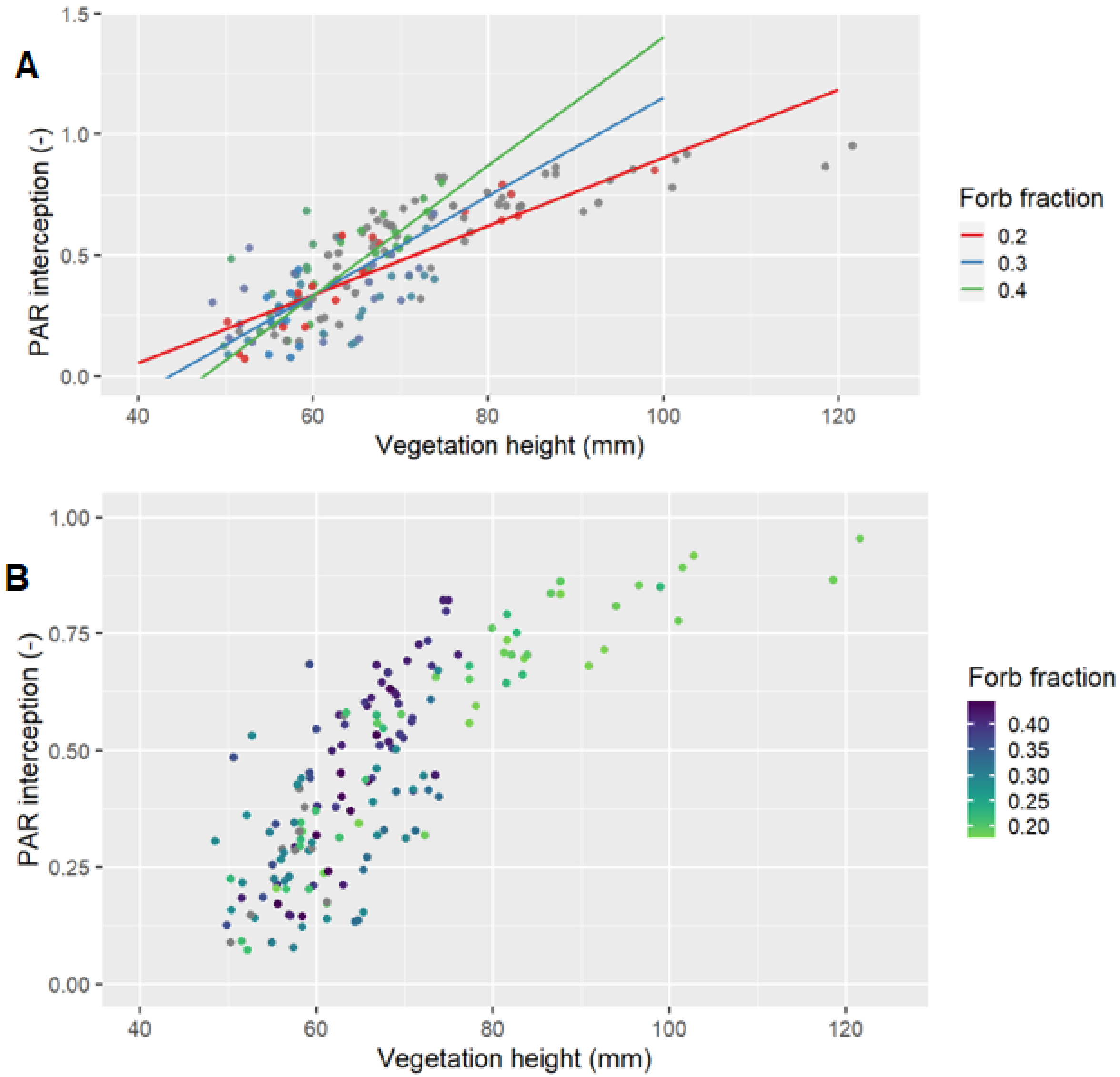

Fig. 14. The relationship vegetation height (mm) and PAR interception (-) affected by the proportion of forbs (A & B). The three lines are predictions from the linear mixed effects regression model, using the moderating variables of the proportions of forbs (A) (Section 2.3.2).. The colours in B represent the fractions of graminoids.

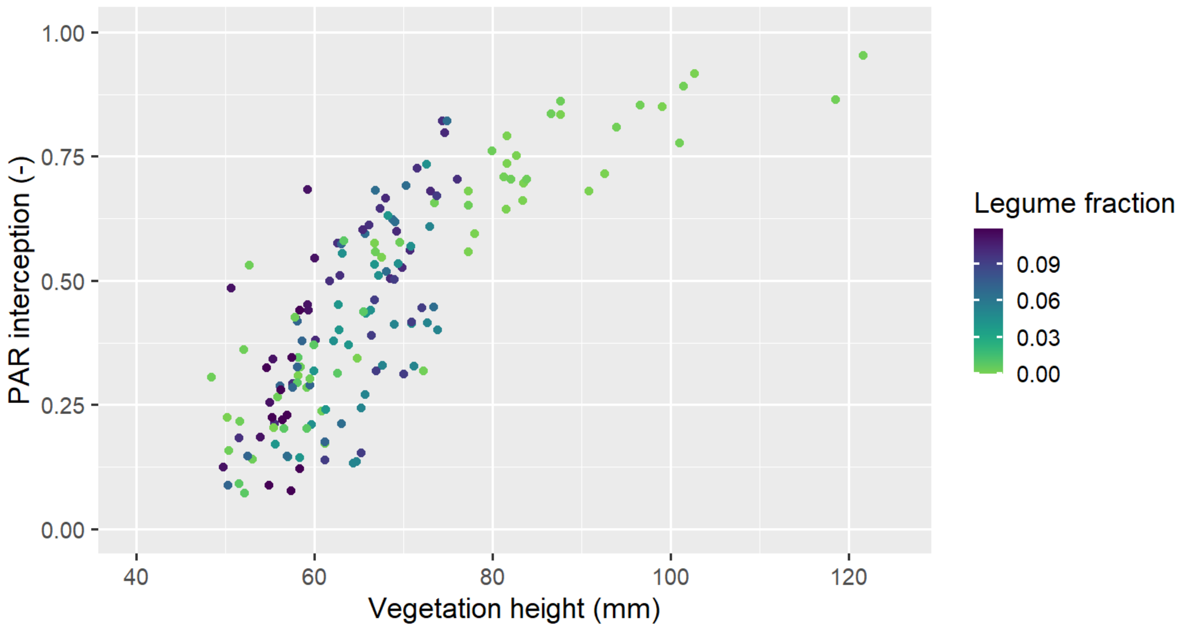

Figure 15. The relationship vegetation height (mm) and PAR interception (-) affected by the proportion of legumes. The colours represent the fractions of graminoids. Due to the small proportions of legumes, no lines could be fit.

4. Discussion

Plant biodiversity induced by long-term nutrient application at the Ossekampen affected the relationship between vegetation height and PAR interception. This effect is analysed in two steps. Firstly, the effect of long-term nutrient application on the indicators of plant biodiversity is discussed in paragraph 4.1. Secondly, the indicators of plant biodiversity affected the relation between vegetation height and PAR interception. This is discussed in paragraph 4.2.

4.1 Nutrient application and plant biodiversity

Long-term nutrient application affected plant biodiversity at the Ossekampen. Application of NPK and PK+N reduced plant biodiversity, while liming increased it, irrespective of whether it was expressed as species richness or Shannon index. Species richness and the Shannon index did not indicate changes in plant biodiversity equally with applications of only N. Species richness was significantly lower than the control when N was applied, while the Shannon index was not different for both treatments. In line with that, Shannon index only explained the variation of species richness for approximately 70%. Therefore, the indicators were not completely interchangeable.

The biodiversity indicators were probably all interrelated. For instance, the domination of graminoids in the nutrient-rich plots caused a lower Shannon index than in other plots. Furthermore, the proportion of forbs in treatment Ca was higher than in most other treatments. Pokorny *et al.* (2004) found that the majority of the species present in grasslands in Montana, the United States, were forbs. Also at the Ossekampen, forbs represented most of the present species. Thus, high species richness could be a result of the preference of forbs in the Ca-fertilised plots. Another explanation of the high species richness would be that the relative high pH in the Ca treatment enabled a larger range of plant species to occur (Pierik *et al.*, 2011).

Graminoids generally have narrower leaves than forbs and legumes but grow taller (Kull & Aan, 1997). Therefore, graminoids can outcompete other functional groups if light is the limiting factor and not nutrients (Smilauer & Smilauerová, 2012). This is in line with our results, in which graminoids dominated in the treatments NPK and PK+N. Moeneclaey *et al.* (2022) investigated the responses of temperate grassland species to phosphorus availability with a pot experiment. Tissue phosphorus concentrations in graminoid-dominated pots were lower than in pots dominated by forbs. Halsted and Lynch (1996) explain that graminoids require less phosphorus for their leaf growth than forbs and legumes because leaf growth in graminoids occurs only in the basal meristem, subsequently reducing phosphorus requirements in the rest of the leaf. Therefore, graminoids are more phosphorus-efficient and are less responsive to phosphorus application. This implies that the occurrence of forbs and legumes depended on the application of phosphorus and is therefore higher in the treatments P and PK. Legumes indeed require P and K for biological N-fixation (Divito & Sadras, 2014), which gives legumes an advantage over graminoids in areas with low availability of N.

The proportions of functional groups in the vegetation had similar tendencies among treatments, irrespective of which method was used to obtain the proportions. However, proportions of graminoids were higher with the frequency method, while the ratios of forbs were highest with the ground cover method. This could be explained by which property of the functional groups was used to obtain the proportions. Graminoids generally have narrower and more erectile leaves (Kattenborn *et al.*, 2019), which results in a higher relative frequency compared to ground cover or aboveground dry matter. Diversity indicators are often calculated for proportional data approached by biomass, frequency and ground cover (Chiarucci *et al.*, 1999). As shown in this thesis, when comparing vegetations with a biodiversity index such as the Shannon index, it is important to use the same method to obtain proportional data on biodiversity.

4.2 Plant biodiversity and PAR interception

4.2.1 Effects of plant biodiversity on PAR interception

The interception of PAR per mm of vegetation increased with a higher plant diversity. The difference in relationship between vegetation height and PAR interception was best explained by the species richness, while the proportion of forbs and graminoids and the Shannon index explained it equally well, but less than species richness. This implies that the time-consuming determination of species richness and relative frequency of occurring of each species cannot be replaced by obtaining the frequency of functional groups without decreasing the quality of predicting the relationship between vegetation height and PAR interception.

The increased PAR interception per mm of vegetation height with increased species richness and Shannon index could be explained by niche differentiation. With a higher number of species in the vegetation a larger chance exists that PAR interception in space, wave length or time differs among plants (Pontes *et al.*, 2015). This would result in increased interception of PAR per mm of vegetation. Similarly for the Shannon index; resource requirements are most likely distributed more evenly with more even distribution of functional groups.

However, Petersen and Isselstein (2015) did not find an effect of species richness on total PAR interception within the same nutrient application treatment. It concerned a grassland experiment in Germany that obtained differences in botanical composition by removing plants. The botanical composition in this removal-type biodiversity experiment is not in equilibrium with available resources, but the management-induced botanical composition in the Ossekampen experiment is (Wrage *et al.*, 2011). Therefore, comparisons between results from the Ossekampen and from the experiment performed by Petersen and Isselstein (2015) should be made with caution. Furthermore, as further discussed in section 4.4.1, fertilisation and botanical composition cannot be separated in the Ossekampen experiment, since the management itself caused the changes in botanical composition.

The effect of legumes on the relationship between vegetation height and PAR interception was hard to asses, because the overall occurrence of legumes in the vegetation was very low. For increasing proportions of graminoids, however, the interception of PAR per mm of vegetation decreased. An explanation could be the differences in growth forms of the functional groups. Graminoids have a lower leaf inclination than forbs (Kattenborn *et al.*, 2019), which results in less PAR interception per leaf area (Tappeiner & Cernusca, 1989). However, Elberse and Berendse (1993) carried out a pot experiment with graminoids that are frequently present at the Ossekampen to investigate which plant properties change as a result of nutrient application. The specific leaf area, the leaf area per leaf mass, was higher in nutrient-rich environments (Elberse & Berendse, 1993). With a higher specific leaf area, leaves are thinner and therefore intercept more PAR than leaves with a smaller specific leaf area. That means that nutrient application also has an influence on the relationship between vegetation height and PAR interception; in the NPK and PK+N treatments, graminoids would intercept more PAR per mm of vegetation than in the other treatments. The results in this thesis contradict that. Thus, the effect of plant biodiversity is stronger than the effect of nutrient application on the amount of PAR interception per mm of vegetation.

Understanding the changes in the relationship between vegetation height and PAR interception can contribute to modelling vegetation growth in species-rich grasslands. In this thesis, an exploration is done how plant biodiversity would change the processes used in grass-growth models such as LINGRA. It shows that the proportion of graminoids or forbs can be used to modify the relationship between PAR interception and standing biomass (vegetation height). Furthermore, it can aid in proposing optimal management strategies for species-rich grasslands. In intensively used grasslands with only perennial ryegrass, vegetation height can be measured to obtain the required fraction of PAR interception. With changing plant biodiversity, the required vegetation height would change as well. This thesis sheds a light how exactly that is changed with specific changes in occurrence of functional groups.

Although obtaining the proportion of graminoids or forbs for each field is less time-consuming than species richness, it is still not feasible in practice. Therefore, further research should be done whether extensively used grasslands can be grouped into types of grasslands that behave similarly in terms of PAR interception per mm of vegetation.

4.3 Atypical situations

Coincidences and situations that clearly deviate from what is typically observed in other years are explained and their potential effects on the results of this thesis are discussed.

4.3.1 Drought

The yield in PK+N in 2022 was lower than the average from 2005-2014 (Appendix 5). After the first cut, the weather was extremely dry and warm, possibly causing water to be the limiting factor instead of the missing nutrients in the treatments. The ground water table at the Ossekampen was around 100 cm in August, implying that water was indeed scarce at the Ossekampen during the measurement period. In the treatment PK+N, all nitrogen was applied after the first cut, which was probably not efficiently converted to biomass because of the drought. Therefore, there was no benefit of this extra application of N during the growth period of the second cut in treatment PK+N, but there was a disadvantage of the lacking N in the first cut compared to treatment NPK. Yield of the second cut was equal between NPK and PK+N, but NPK had a higher yield in the first cut (Appendix 5).

In general, drought could have influenced the slope between PAR interception and vegetation height. According to Wellstein *et al.* (2017), the specific leaf area of graminoids in temperate grasslands in Germany decreased in response to extreme drought. The specific leaf area of forbs was not significantly affected. That means that the leaf area of especially graminoids was likely smaller this year than it would have been in years with sufficient water availability. The amount of PAR interception per mm of vegetation might not decrease as it did this year (Fig. 16A). Therefore, it is important to conduct this experiment in other years with different environmental conditions before conclusions can be drawn.

4.3.2 Fertiliser scorching

At the beginning of the growth period of the second cut in 2022, nitrogen was applied to NPK, PK+N and N just before a hot and dry period. Wilting and subsequently necrosis occurred in all plots treated with nitrogen, but it was especially apparent in treatment N. The entire plot turned red-brownish (Fig 8). Burning of vegetation due to nitrogen application occurs in conditions with dry soils, hot weather, direct sunlight and excessive N availability (Ritchey *et al.*, 2003). Due to excessive salt accumulation, the osmotic pressure outside the crop exceeds the osmotic pressure inside the crop. Subsequently, the water flow reverses and the plant dries out (Richards & Wolton, 1976). In the treatments NPK and PK+N, fertilizer scorching occurred to a much lesser extent. The vegetation in these treatments could have been less sensitive to fertiliser burns because it benefited from of a stronger rooting system (Elberse & Berendse, 1993), which allowed more water uptake to reduce the impact of fertiliser burns. Furthermore, regarding Liebig's law of the minimum, nitrogen would not be the limiting nutrient in treatment N, but rather the nutrients P and K, which have not been applied for 64 years. In the treatments NPK and PK+N, N would more likely be the limiting nutrient. Therefore, a bigger surplus of nitrogen would exist in treatment N, whereas it can be taken up in NPK and PK+N.

The regrown vegetation was mainly brown knapweed (*Centaurea jacea* L.). Also, the total ground cover, vegetation height and PAR interception in the plots treated with N were low compared to other treatments. That is most likely because of the burnt vegetation and therefore not representative for this treatment in another year. Thus, the vegetation in treatment N is not resistant to nutrient or water stresses, while the vegetation in the treatments NPK and PK+N was less severely affected.

4.4 Reflection on methods

4.4.1 Nutrients as confounding factors

The experiment in the long-term grassland experiment of the Ossekampen had the strength that it concerned a semi-natural system where differences in species composition were management-induced (Korevaar & Geerts, 2015). In other studies concerning plant biodiversity, plant communities were sown (Spehn *et al.*, 2000) or the desired plant biodiversity was achieved and maintained by removing certain plants (Petersen & Isselstein, 2015). This does not represent natural systems, in which species composition is in equilibrium with the natural resources available (Wrage *et al.*, 2011). As a consequence, fertilisation and plant biodiversity cannot be separated in this experiment, since the fertilisation itself caused the changes in plant biodiversity. Nevertheless, in semi-natural grasslands, the effect of nutrients on plant biodiversity and PAR interception cannot be separated either. Therefore, this thesis contributes to better understanding the effects of management on biodiversity and processes related to productivity in semi-natural grasslands.

4.4.2 Reflection on statistical methods

As Pierik *et al.* (2011) mentioned, the experimental design of the Ossekampen experiment is not ideal, since it is only a partial random block design. The treatments N and PK+N were added later and are placed next to the already existing complete random block design. Therefore, possible gradients in the direction east-west in natural fertility, ground water table or other growing conditions could affect the results in treatment N and PK+N, while it cannot be accounted for in the block effect. A strength of this experiment is the exceptional duration, it adds understanding to the long-term effects of nutrient application.

Moreover, the average of multiple subsamples or measurements were used as single data points, which led to a low statistical power (n=2). Ground cover was determined twice per plot per week, vegetation height and PAR interception 20 times per week and 14 samples per plot were taken for contributions of the functional groups to aboveground dry weight. Taking averages per plot was done to decrease influence of the heterogeneity within the plots, but it did not increase statistical power. Therefore, I have described tendencies, even if they were not supported by significant effects.

4.4.3 PAR interception measurements

As explained in section 2.2.2, the PAR interception was measured by a referencing sensor and a measuring rod. Every week, I additionally took around three measurements of the PAR above the canopy with the measuring rod in order to exclude differences between measurements from the referencing sensor and measuring rod. However, the PAR measured with the referencing sensor was different from the PAR measured with the measuring rod. The results of the measuring rod measured between 94% and 120% from the results of the referencing sensor, while it was mainly above 100% . This could have led to an under- or overestimation of PAR interception by the vegetation. However, from the limited measurements I did above the canopy, it cannot be said whether it would be a constant underestimation among all light conditions and all vegetation heights.

4.4.4 Vegetation height by rising plate meter

This thesis is based on the assumption that the compressed vegetation height measured by the rising plate meter is related to the standing biomass, which determines productivity (Martin *et al.*, 2005; Murphy *et al.*, 2021). With data obtained in this thesis, the accuracy of vegetation height measurements can be assessed, which is done in the following paragraphs.

The vegetation height better explained dry matter yield when the remaining vegetation height was not subtracted (R^2=0.837 without subtracting and R^2=0.721 with subtracting). A likely explanation is that with low vegetation heights and uneven soils, the roughness of the soil causes overestimations of the actual remaining vegetation height (Murphy *et al.*, 1995). Holshof and Stienezen (2016) used the vegetation height, where the vegetation height was expressed in cm. The dry matter yield was obtained the following equation, adapted from Holshof and Stienezen (2016) and converted to a vegetation height in mm.

$Dry\ matter\ yield = -94.5 + 21.0 * vegetation\ height$ (eq. 3)

The difference between eq. 3 and the formula in Fig. 7 could be because the dry matter yield per vegetation height from all plots was used to obtain the formula in Fig. 7. Thus, it is assumed that the relationship would be equal in all treatments. Cudlín *et al.* (2018) found a large effect of fertilisation with N or P and with functional diversity on the relationship between dry matter yield and vegetation height. The dry matter yield per vegetation height indeed differed a lot depending on the treatment (Appendix 9). Therefore, the equation for dry matter yield per mm of vegetation should be calibrated depending on fertilisation or plant biodiversity and it should not be assumed to just add all points with different treatments in one graph, as I have done now.

Moreover, the rising plate meter is often used for intensive grasslands, where yield per cut is lower than in extensive grasslands such as the Ossekampen. Therefore, the usage of the rising plate meter is less compromised by decreased measurability at high vegetation heights. The correlation between dry matter yield and vegetation height just before the second cut was higher than before the first cut (R^2=0.837 and R^2=0.672 respectively). This was probably because the vegetation in the treatments NPK and PK+N was so high that it lodged, which caused an underestimation of the vegetation height. Holshof and Stienezen (2016) state that the rising plate meter predicts dry matter yield until 2.7 tonDM/ha. That would not be enough at the Ossekampen in the first cut.

Thus, further research is required on the usage of the rising plate meter in species-rich grasslands with high-standing biomass. Additionally, including aspects of plant biodiversity would be required to use the rising plate meter for dry matter production for species-rich grasslands accurately.

Conclusions

Plant biodiversity induced by long-term nutrient application affected the relationship between vegetation height and PAR interception at the Ossekampen. Application of NPK and PK+N resulted in the least biodiverse vegetation, irrespective whether it was expressed as species richness or Shannon index. Furthermore, it resulted in the highest fraction of graminoids, while application of PK led to the highest fraction of forbs. More biodiverse vegetations intercepted more PAR per mm of vegetation, irrespective whether biodiversity was expressed as species richness or Shannon index. Increasing fractions of graminoids decreased the PAR interception per mm of vegetation, while increasing fractions of forbs increased PAR interception per mm of vegetation.

The difference in relationship between vegetation height and PAR interception was best explained by the species richness, while the proportion of forbs and graminoids and the Shannon index explained it equally well, but less than species richness. Thus, the time-consuming determination of species richness and relative frequency of occurring of each species cannot be replaced by obtaining the frequency of functional groups without decreasing the quality of predicting the relationship between vegetation height and PAR interception.

The expected transition of intensive to more extensive grassland management requires the inclusion of multiple species in grass-growth models. Therefore, new calibrations are required to include biodiversity in the relationship between vegetation height and PAR interception as well as vegetation height and standing biomass. Further research should be done to test whether extensively used grasslands can be grouped into types of grasslands that behave similarly in terms of PAR interception per mm of vegetation or dry matter yield per mm of vegetation height.

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Appendices

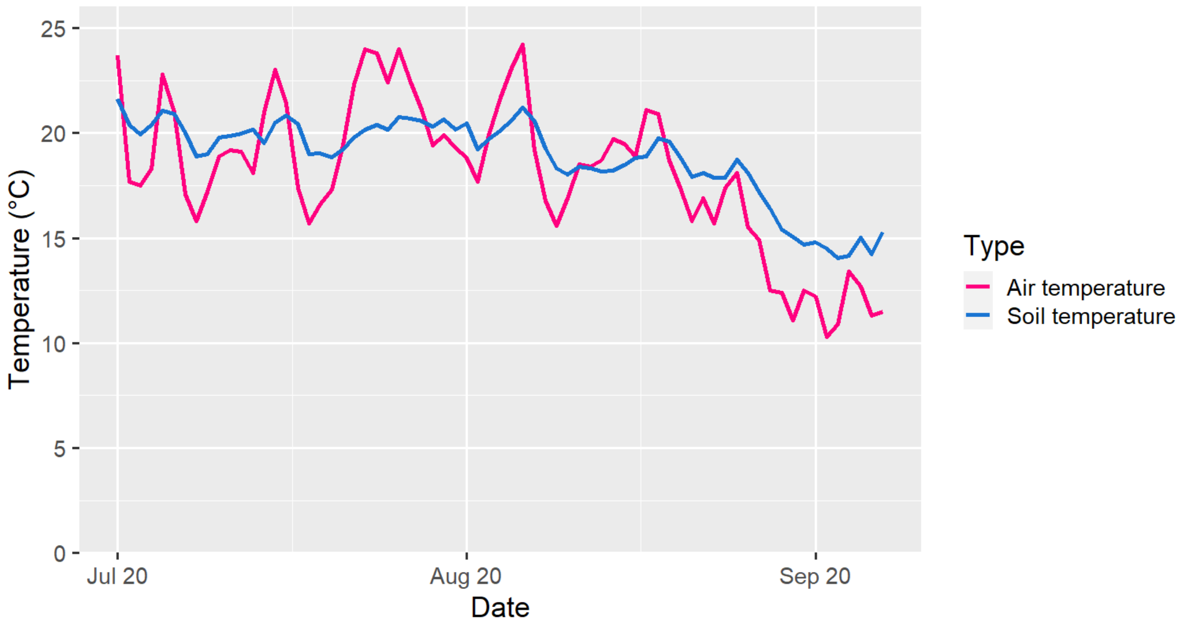
Appendix 1: Fertilisation scheme at the Ossekampen

Appendix 1. Annual nutrient application since 1986 adapted from Korevaar et al., (2015).

	Nutrient applied (kg/ha/ y)			
Treatment	N	P	K	Ca
Control	0	0	0	0
Ca	0	0	0	357
P	0	22	0	0
K	0	0	108	0
PK	0	22	166	0
N[1]	160	0	0	357
NPK[1]	160	33	311	0
PK+N[2]	160	33	311	0

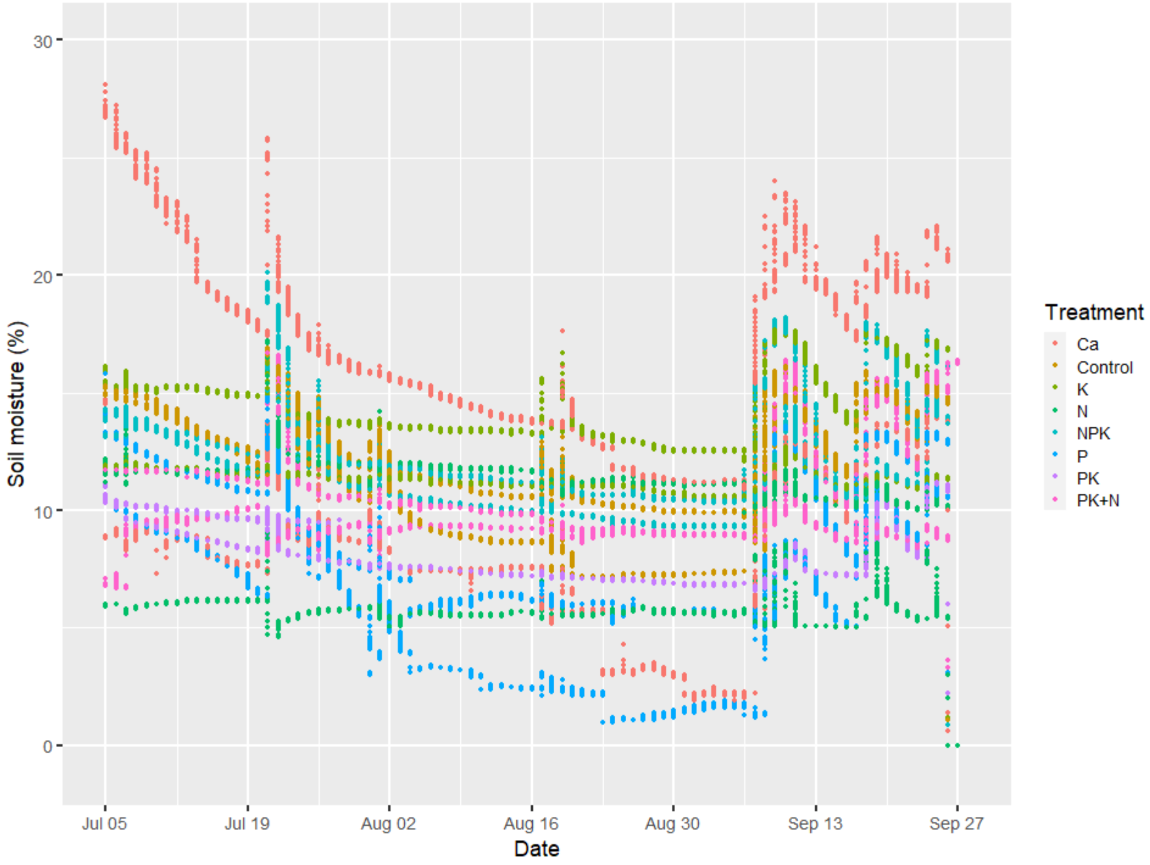
[1)]100 kgN/ha is applied in April and 60 kgN/ha is applied after the first cut in July.
[2)]160 kgN/ha is applied after the first cut in July.

Appendix 2: Soil and air temperature



Appendix 2. Daily soil temperature (°C) (blue) and daily air temperature (°C) (blue) during the growth period of the second cut. Daily air temperature from (KNMI, 2022a).

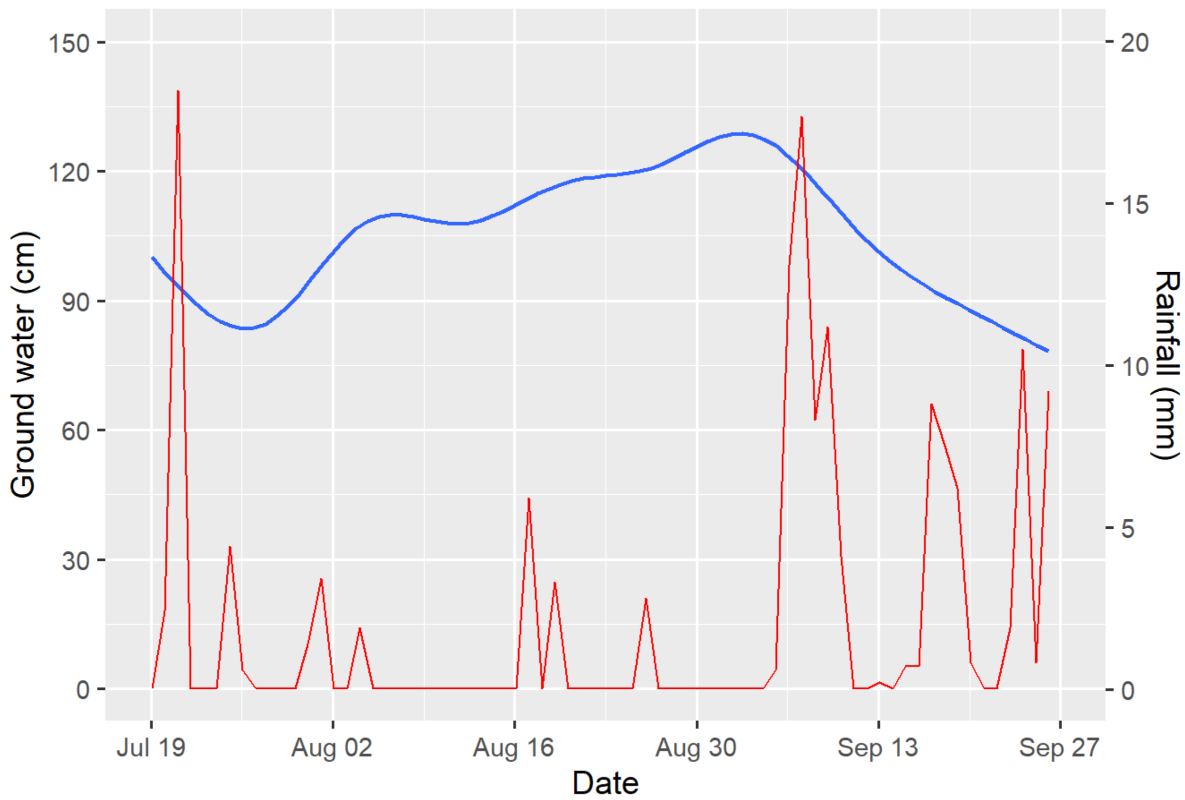
Appendix 3: Soil moisture, groundwater level and rainfall



Appendix 3A. Soil moisture content during the growth period of the second cut in 2022. The soil moisture content in the different blocks from the same treatment are shown separate.

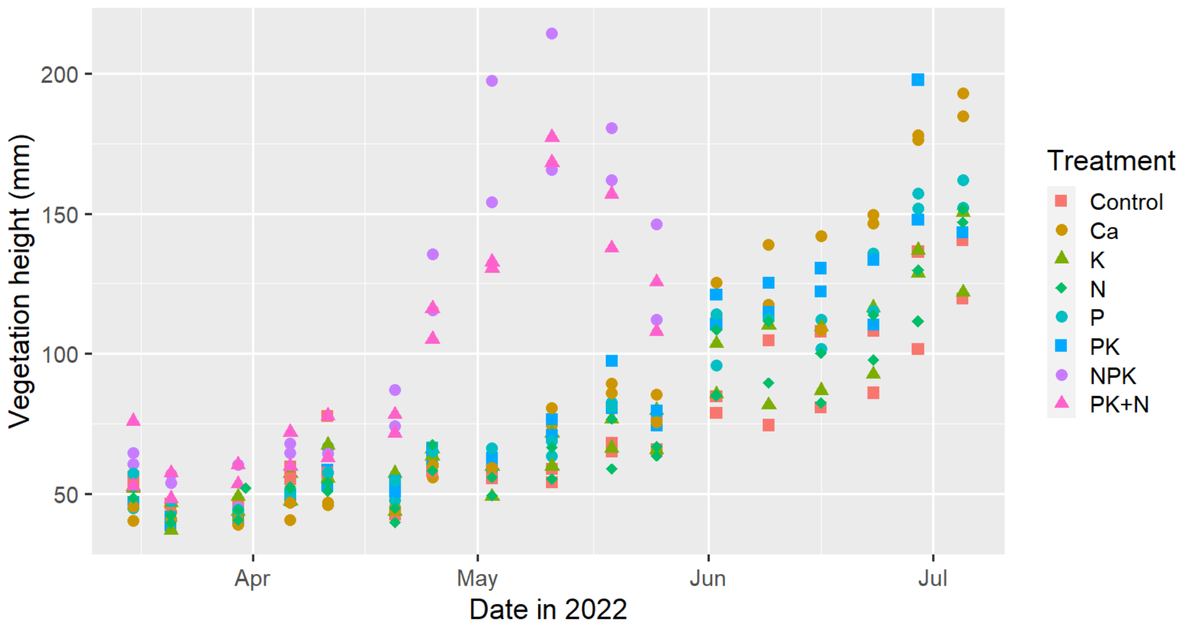
Appendix 3B. Mean soil moisture content measured at 20 cm depth in the second in 2022, reported per treatment.

Treatment	Soil moisture (%)
Control	11.7
Ca	8.0
K	12.7
N	8.6
P	7.2
PK	8.3
NPK	11.8
PK+N	10.1

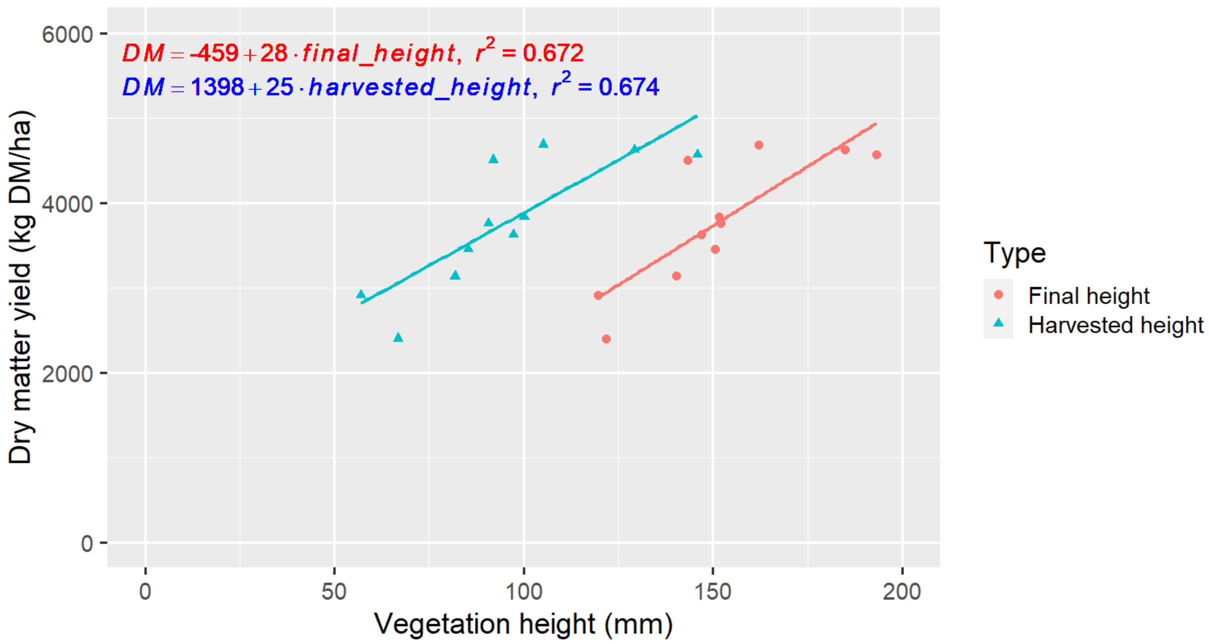


Appendix 3C. Ground water level (cm) that is determined weekly (blue) and rainfall (mm) that is obtained daily by KNMI (2022b) (red) during the growth period of the second cut in 2022.

Appendix 4: Vegetation height in the first cut



Appendix 4A. Vegetation height (mm) measured every week in the first cut.



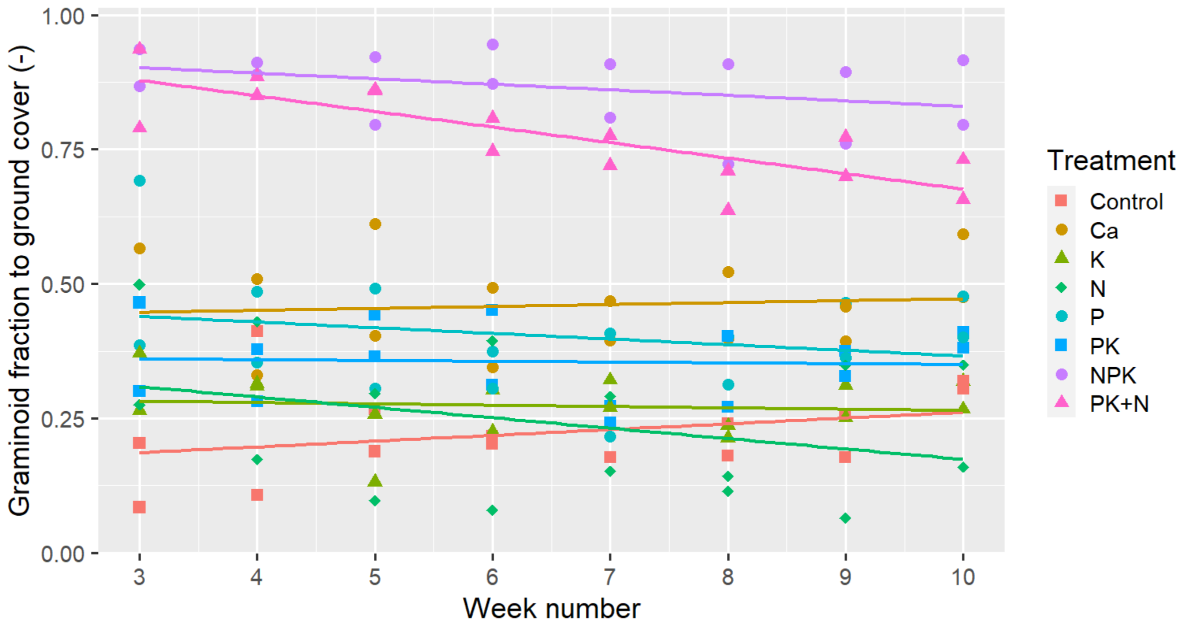
Appendix 4B. Regression between dry weight of mowed vegetation (kg DM/ha) and harvested vegetation height (mm) and Dry matter yield and final vegetation height (mm).

Appendix 5: Dry matter yields

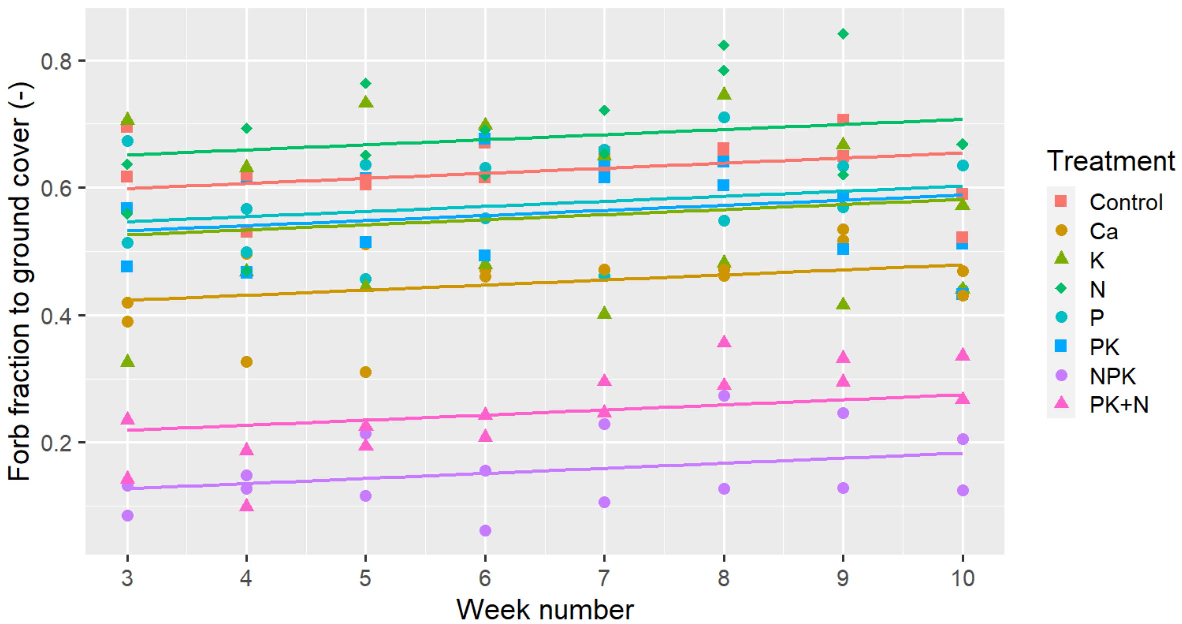
Appendix 5. Dry matter yield from first cut 2022, second cut 2022, total dry matter yield 2022 and average dry matter yield of 2005 to 2014, all in ton/ha (from Korevaar et al. 2015).

Treatment	Dry matter yield first cut (ton/ha)	Dry matter yield second cut (ton/ha)	Dry matter yield total 2022 (ton/ha)	Dry matter yield 2005-2014 (ton/ha)(Korevaar et al 2015)
Control	3.0	0.4	3.4	5.0
Ca	4.6	1.0	5.6	5.9
K	2.9	0.5	3.4	5.4
N	3.7	0.5	4.2	5.2
P	4.2	0.7	4.9	6.0
PK	4.8	0.7	5.5	6.7
NPK	7.8	1.7	9.5	8.7
PK+N	6.4	1.7	8.0	9.3

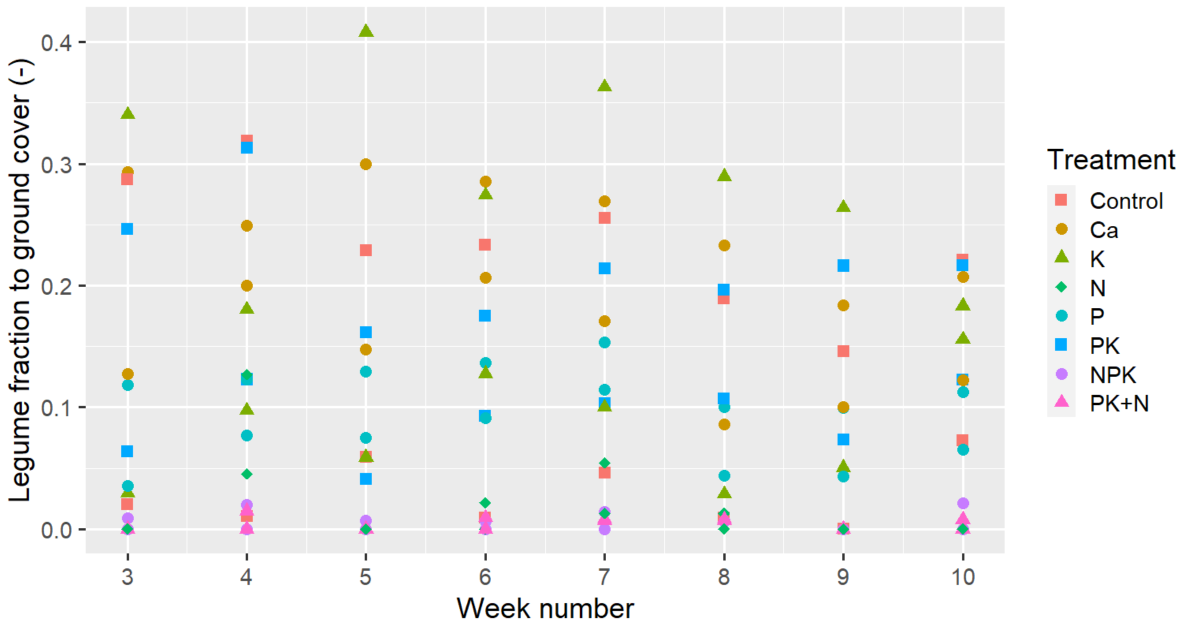
Appendix 6: Ground cover contributions of functional groups per week



Appendix 6A. Contribution of graminoids to ground cover during the growth period of the second cut. Points represent average relative contribution of graminoids to ground cover per treatment per block per week. A significant interaction effect of treatment and week was found for ground cover contribution of graminoids (lmer, P<0.05).

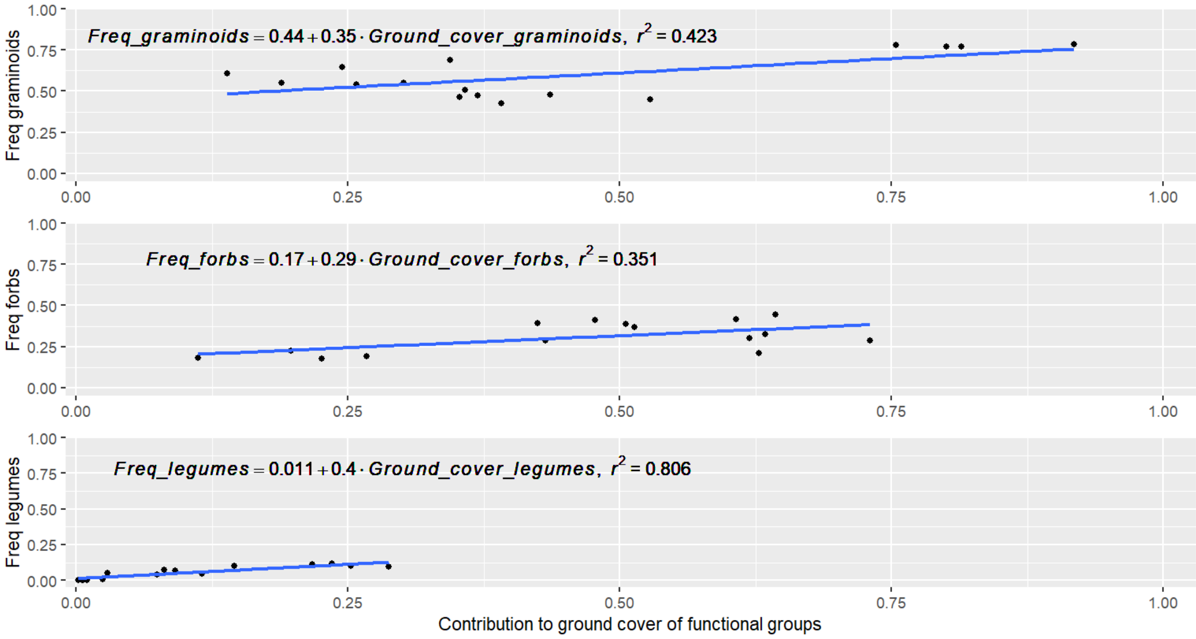


Appendix 6B. Contribution of forbs to ground cover during the growth period of the second cut. Points represent average relative contribution of forbs to ground cover per treatment per block per week. A significant main effect of treatment for ground cover contribution of forbs was found (lmer, P<0.05).

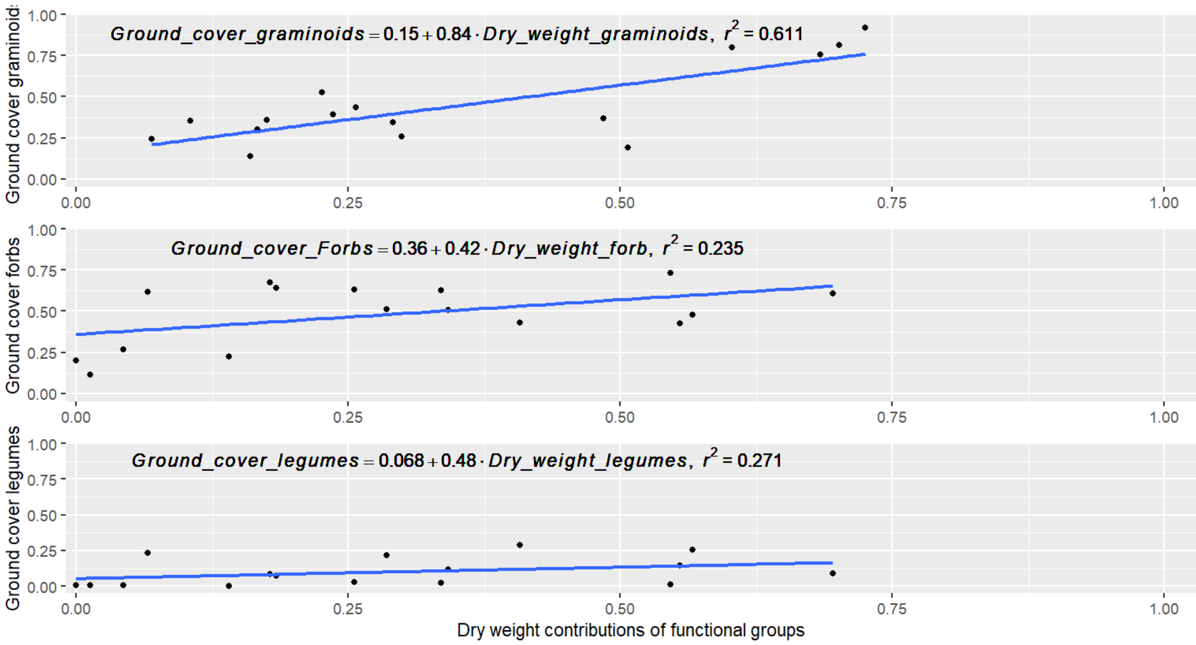


Appendix 6C. Contributions of legumes to ground cover per week. No clear trend was found for ground cover contribution of legumes during the growth period of the second cut (P>0.05)

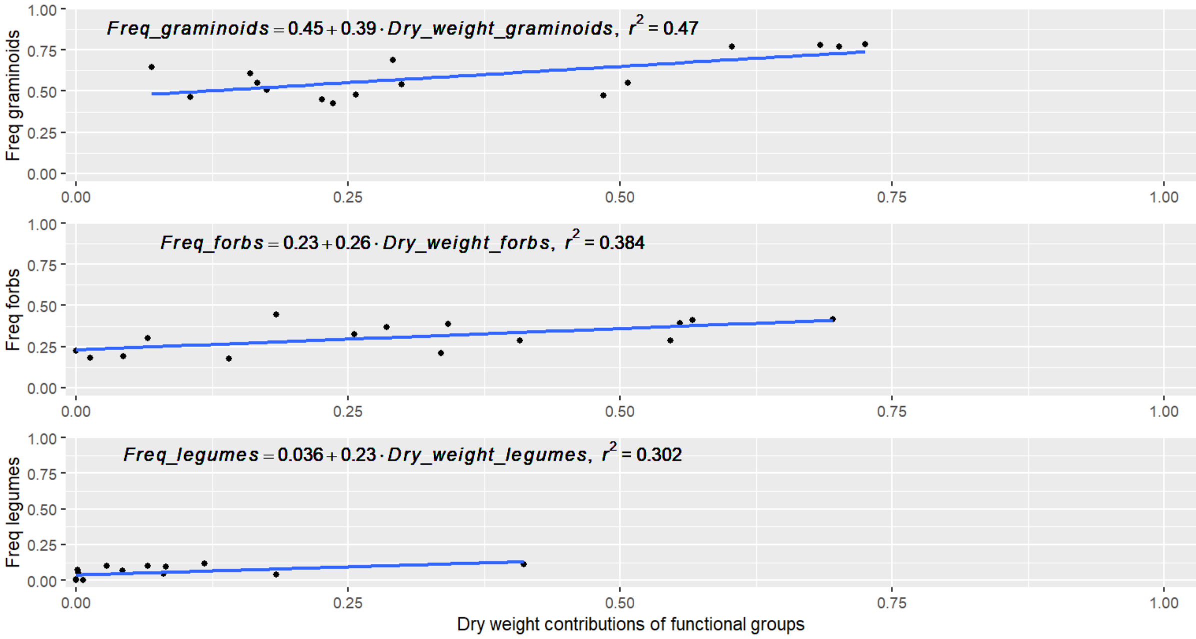
Appendix 7: Comparisons between methods in obtaining functional group proportions



Appendix 7A. Comparison between relative frequency of functional groups (Freq graminoids, forbs and legumes) and ground cover contributions of functional groups. The comparisons of the functional groups are graminoids, forbs and legumes from top to bottom.

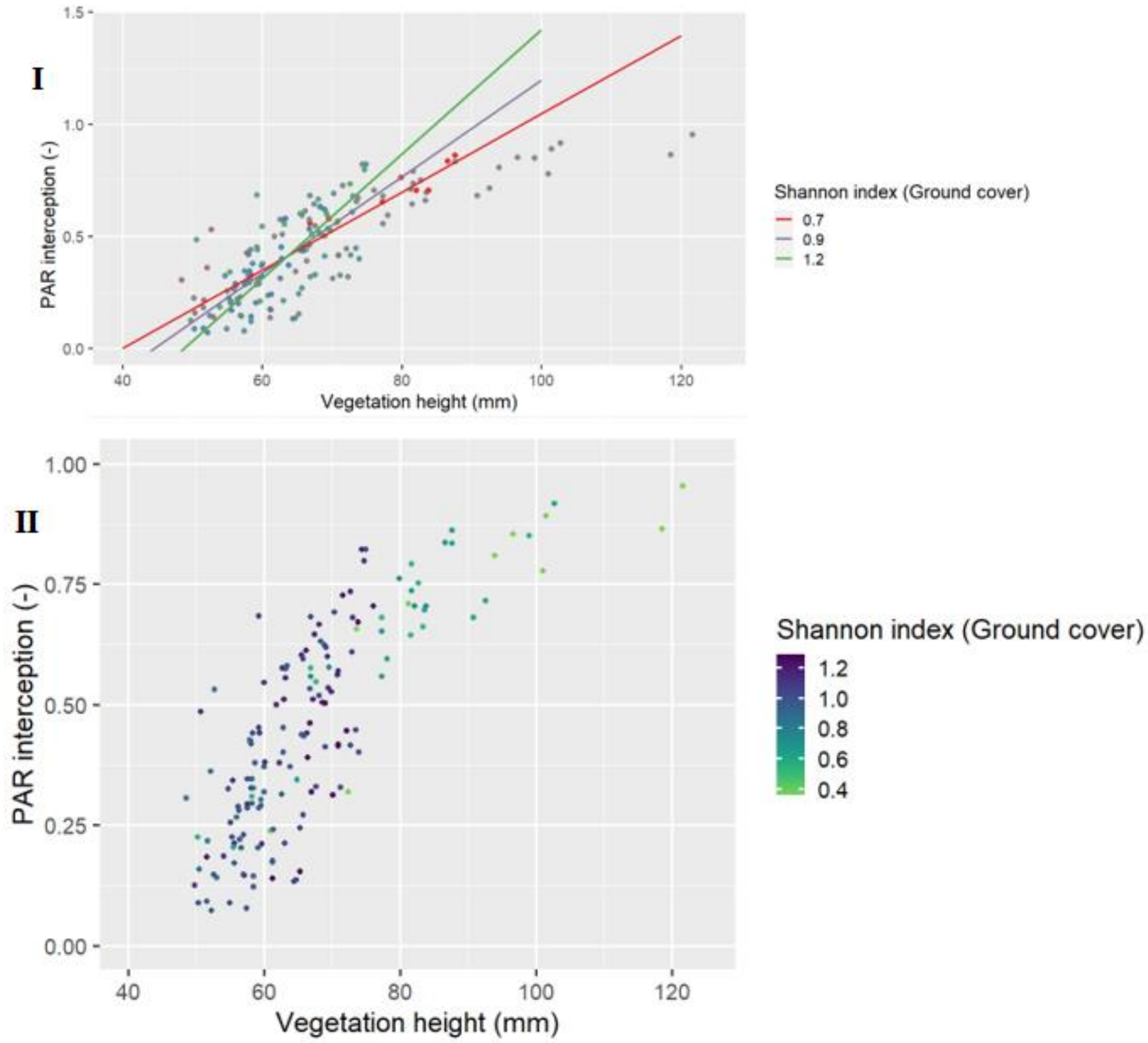


Appendix 7B. Comparison between contributions of functional groups to ground cover (ground cover graminoids, forbs and legumes) and relative contribution to aboveground dry weight. The comparisons of the functional groups are graminoids, forbs and legumes from top to bottom.

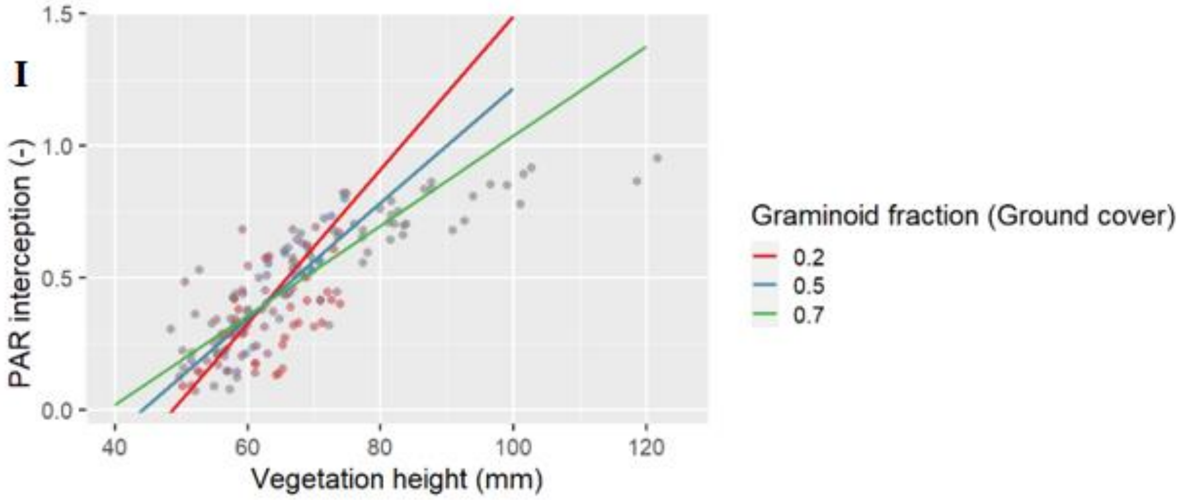


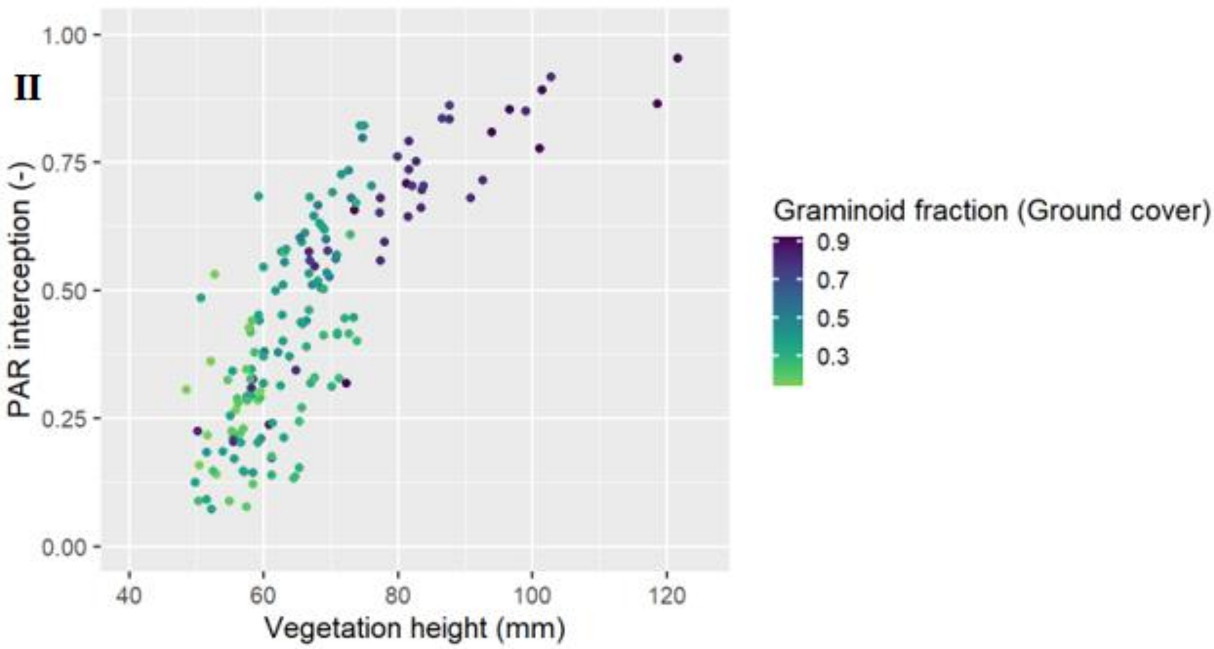
Appendix 7C. Comparison between relative frequency of functional groups (Freq graminoids, forbs and legumes) and contributions of functional groups to aboveground dry weight (fractions). The comparisons of the functional groups are graminoids, forbs and legumes from top to bottom.

Appendix 8: PAR interception per mm of vegetation affected by biodiversity indicators

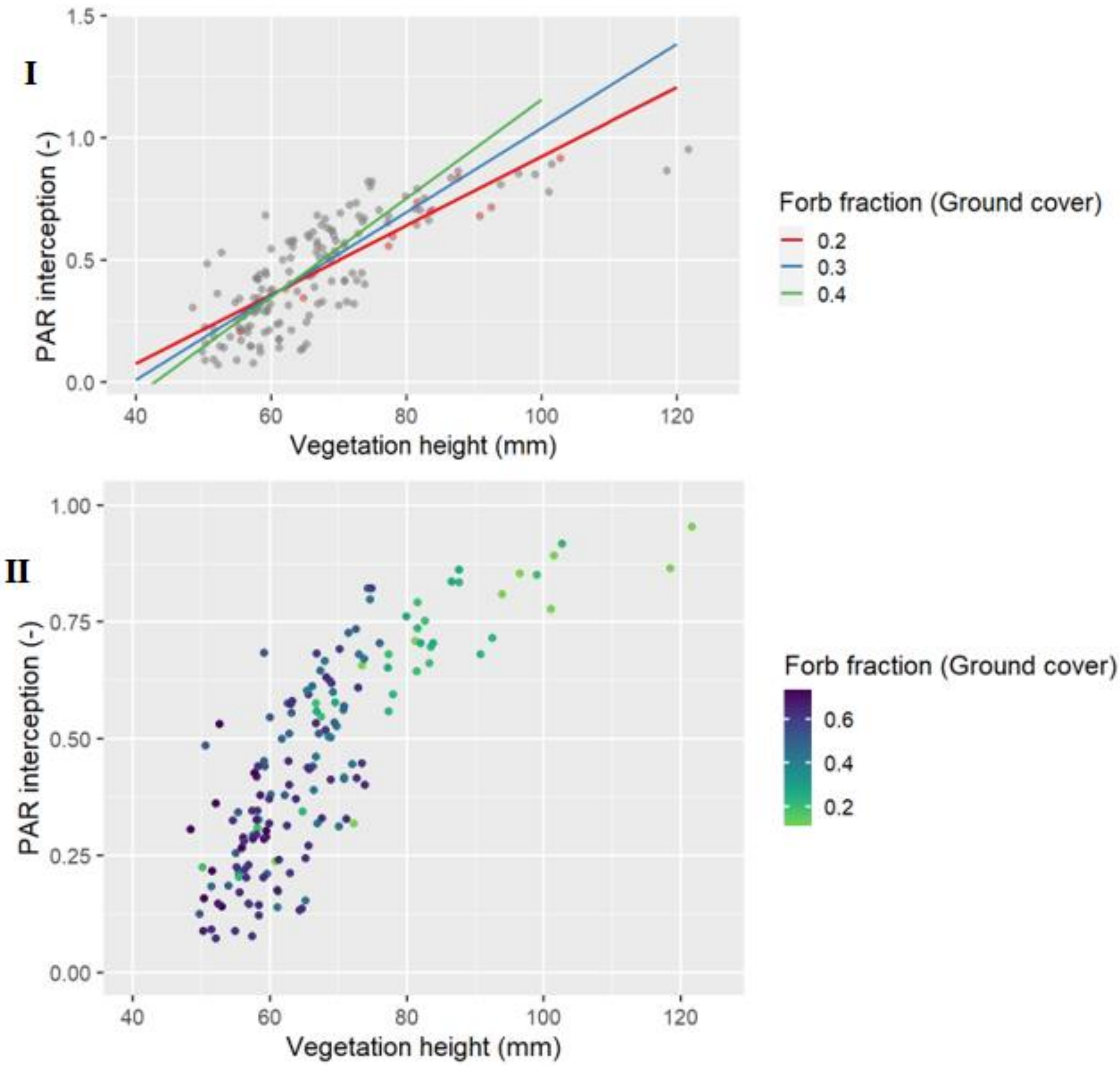


Appendix 8A. The relationship between vegetation height and PAR interception (mm) affected by Shannon index contribution of functional groups obtained with the ground cover method. The three fitted lines use the moderating variables of the Shannon index, as explained in section 2.3.2 (I). The higher the Shannon index, the darker the points (II).

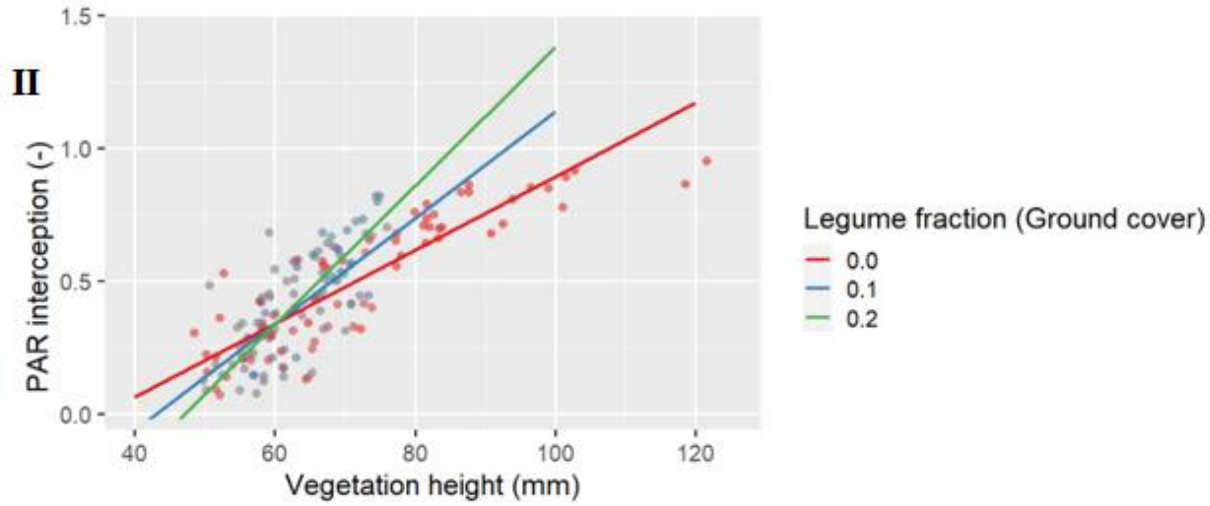


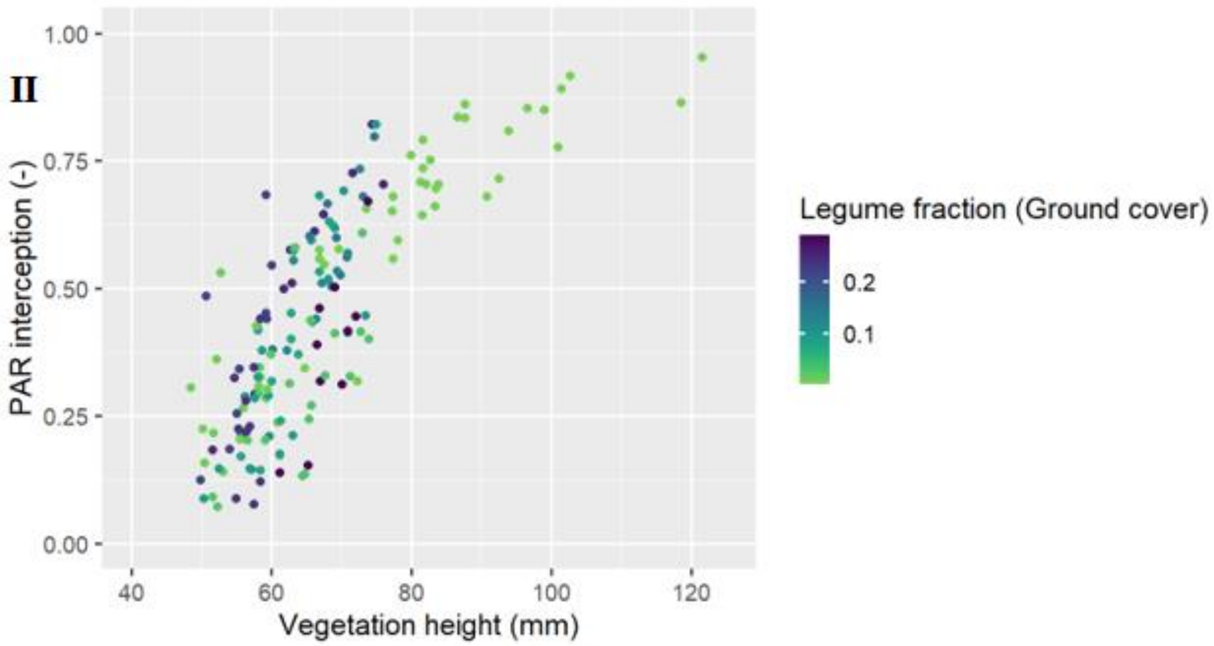


Appendix 8B. The relationship between vegetation height and PAR interception (mm) influenced by relative contribution to ground cover of graminoids. The three fitted lines use the moderating variables of the fraction of graminoids that cover the ground, as explained in section 2.3.2 (I). The higher the contribution of graminoids to ground cover, the darker the points (II).



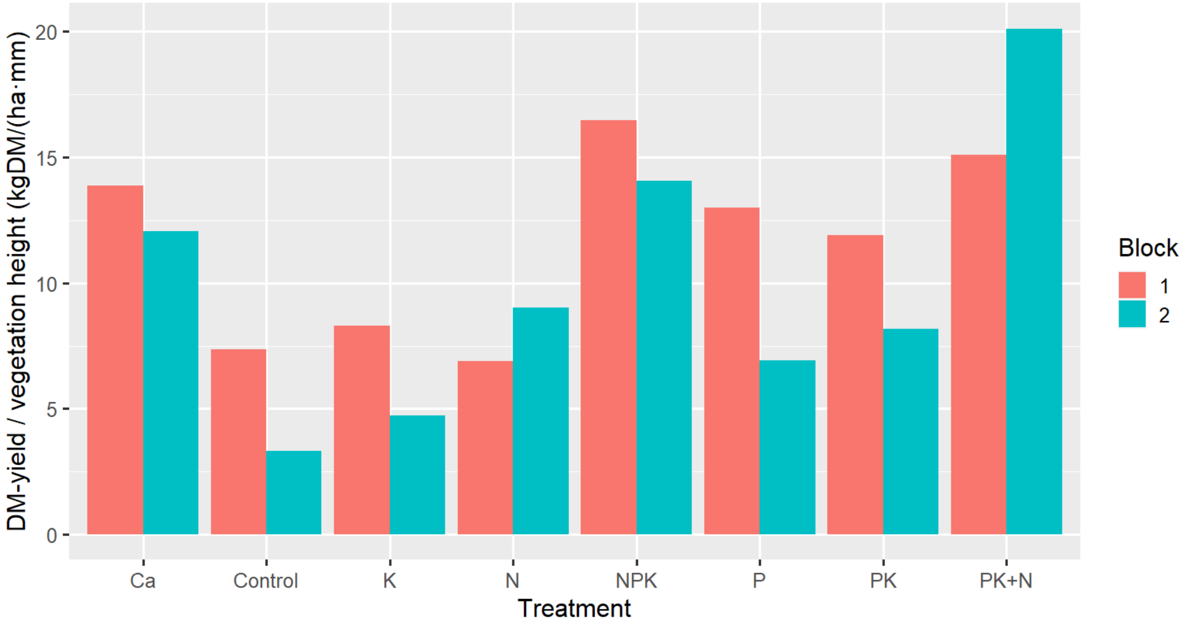
Appendix 8C. The relationship between vegetation height and PAR interception (mm) affected by relative contribution to ground cover of forbs. The three fitted lines use the moderating variables of the fraction of forbs that cover the ground, as explained in section 2.3.2 (I). The higher the contribution of forbs to ground cover, the darker the points (II).





Appendix 8D. The relationship between vegetation height and PAR interception (mm) affected by relative contribution to ground cover of legumes. The three fitted lines use the moderating variables of the fraction of legumes that cover the ground, as explained in section 2.3.2 (I). The higher the contribution of legumes to ground cover, the darker the points (II).

Appendix 9: Dry matter yield per vegetation height of the second cut



Appendix 9. Dry matter yield per mm of vegetation height at harvest (kg DM/(ha·mm)) per treatment.