# Ratio vegetation indices have the potential to predict extractable protein yields in green protein paludiculture

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**SUMMARY**

Paludiculture can be a tool to incentivise rewetting of agricultural peatlands with the option for biomass utilisation in green protein biorefineries. However, the economic feasibility for green protein paludiculture depends on product maximisation. This study explored the potential of a ratio vegetation index (RVI) model, with inclusion of climatic factors relevant for biomass growth, to predict crude protein (CP) contents in green protein precipitates from biorefining *Phalaris arundinacea* and *Festuca arundinacea* cultivated under different management intensities on a wet fen. Assessing yields for two years of cultivation, we found that timing of harvest was a key variable for CP extractability using the biorefinery technique. Biomass and protein yields were similar between management treatments and years, but extractability was enhanced in the dryer of the two years. This study highlighted the potential of an RVI model to predict, under varying climatic conditions, CP contents in the protein precipitate with good model performance ($R^2$ = 0.64, NRMSE = 0.23) and accuracy. In 92 % of occurrences, the model was able to predict statistically similar CP contents compared to measured CP in the protein product, with an average deviation between measured and predicted annual values of 1.7 % across species and management intensities. The findings highlight an option for maximising the overall efficiency of green protein paludiculture by determining the optimal timing of harvest, thereby demonstrating an economic potential to incentivise paludiculture farming.

**KEY WORDS:** biorefinery, fen, *Festuca arundinacea*, peatland, *Phalaris arundinacea*

## INTRODUCTION

Large-scale rewetting of drained peatlands to mitigate adverse climatic effects, and thus contribute to achieving climate neutrality by 2050 (Abel *et al.* 2019), is currently a hot topic on many regional and national agendas (e.g., Kreyling *et al*. 2021). Paludiculture is a sustainable utilisation option for wet peatlands that includes the productive land-use of rewetted peatlands for biomass cultivation (Wichtmann *et al.* 2016, Tanneberger *et al.* 2021) that has gained international popularity over the last few years owing to a vast array of associated ecosystem services (Bonn *et al.* 2016). However, marketability of the so-called ‘paludicrops’, i.e., flood-tolerant plants suitable for paludiculture, is still in its infancy (de Jong *et al.* 2021). Nonetheless, cultivation of biomass on rewetted peatlands may be a critical instrument in the restoration of formerly drained eutrophicated arable land, and thus a powerful tool in the transition towards a healthy ecological state (Vroom *et al.* 2022). In this context, biomass harvesting to remove excess nutrients such as nitrogen (N) and phosphorus (P) is necessary to meet additional environmental goals such as the mitigation of eutrophication (Hinzke *et al.* 2021), thereby improving biodiversity in the long term (Zak & McInnes 2022). An array of paludiculture plants is known from the ‘Global Database of Potential Paludiculture Plants’ (Abel & Kallweit 2022), offering market opportunities for e.g., bioenergy (Hartung *et al.* 2020) and building materials (de Jong *et al.* 2021). Besides typical wetland ‘paludicrops’ such as *Typha* spp. and *Phragmites australis*, an array of flood-tolerant perennial grasses, e.g., *Phalaris arundinacea* (Reed Canary Grass - RCG) and *Festuca arundinacea* (Tall Fescue - TF), can be cultivated successfully in paludiculture.

Better utilisation of grass biomass, irrespective of whether it originates from mineral or peat soils, is linked to the goal of enhanced sustainability in animal husbandry (Pulina *et al.* 2022), particularly with regard to reducing the use of imported protein feed. Grass protein products derived from perennial grasses and legumes through biorefinery processes have been shown to be potential substitutes for the commonly fed soy protein on the basis of amino acid composition and digestibility (Stødkilde *et al.* 2019). The primary feedstock for green biorefinery currently consists of plants adapted to mineral soil (e.g., *Medicago sativa, Trifolium* spp.*, Lolium* spp.), which yield up to 10 kg of protein precipitate with concentrations of up to 46 % crude protein (CP) per t of fresh crop (Santamaría-Fernández *et al.* 2017). While government ambitions regarding peatland rewetting may not influence farming practices in countries where arable land on mineral soil is abundant (e.g., in Denmark), the opposite could be the case in countries where paludiculture is the only future farming option. For instance, most farmers in large parts of The Netherlands and northwest Germany are dependent on peatland as the typical arable land available (van den Akker *et al.* 2012, Wittnebel *et al.* 2021). In these regions, which are characterised by significant outputs of animal products (European Commission 2022), protein extracted from paludiculture crops through green biorefining offers an opportunity to balance various ecosystem services in the categories of cultural heritage, regulation and provisioning.

Although previous studies have shown that high yields of biomass and CP are achievable from RCG and TF (Nielsen *et al.* 2021), questions remain regarding the variation of protein extractability in relation to environmental variability between years. Insights into mechanisms affecting protein extractability from biorefining are crucial in securing maximum utilisation and, thereby, the overall sustainability of the concept. Temperature and precipitation rate are known to influence grass yields directly through plant stress reactions (White 1985) and to affect soil biogeochemical factors, particularly N and P availability (Mariotte *et al.* 2020). Perotti *et al.* (2021) highlighted that grass yields and quality are significantly affected by soil N availability in addition to climatic conditions, although with differing dependencies across growth cycles. Furthermore, under appropriate soil nutrient conditions, plant rejuvenation by harvesting favours the regrowth of aboveground biomass and, therefore, N concentration in leaves (Walker *et al.* 2014, Tejera *et al.* 2022). In this context, plant maturity is another influential factor. Stødkilde *et al.* (2021) found that harvest time and dry matter (DM) content affected protein yields in green protein precipitates from legumes, with an increase in DM counteracting CP extractability.

In recent decades, statistical models based on local environmental conditions and spectral vegetation indices (SVI), e.g., the ratio vegetation index (RVI; Richardson & Wiegand 1977) and normalised-difference vegetation index (NDVI; Rouse *et al.* 1974), have been increasingly utilised to predict forage yields and quality (e.g., Biewer *et al.* 2009, Zhou *et al.* 2019, Peters *et al.* 2022, Han *et al.* 2022). However, while a variety of models can estimate CP contents in standing grass biomass with good or satisfactory precision, the potential for estimating protein extractability from biorefining, and therewith CP contents in green protein, is as yet unexplored. RVI models could provide the tool that is needed for determining the optimal balance between yield and quality under local climatic conditions and, eventually, boosting the economic feasibility of green protein biorefineries (Stødkilde *et al*. 2021). In this context, an optimisation of harvest time for grass biomass produced in paludiculture for utilisation in green protein biorefineries could fill the economic gap in the transition from traditional agricultural use of drained peatlands to agro-industrial utilisation of biomass from peatlands that have been restored by rewetting. Because the implementation of paludiculture and the accompanying facilitation of sustainable long-term environmental improvements depends on economic feasibility, the development of a model based on RVI and climatic variables to maximise yields of protein precipitate with adequate CP content has potential to promote the development of economic and environmental sustainability in green protein paludiculture.

This study aimed to explore the possibility of linking RVI and climatic variables of importance for grass development in a model to predict extractable CP contents in the biorefined green protein precipitate from paludiculture biomass. Furthermore, we aimed to determine biomass and CP yields for RCG and TF over two consecutive years with different climatic conditions - under the overall hypothesis that stable green protein yields from paludiculture grass biomass should be achievable from biorefining despite inter-annual variations in climatic conditions, because of the naturally higher soil moisture levels in peat soil.

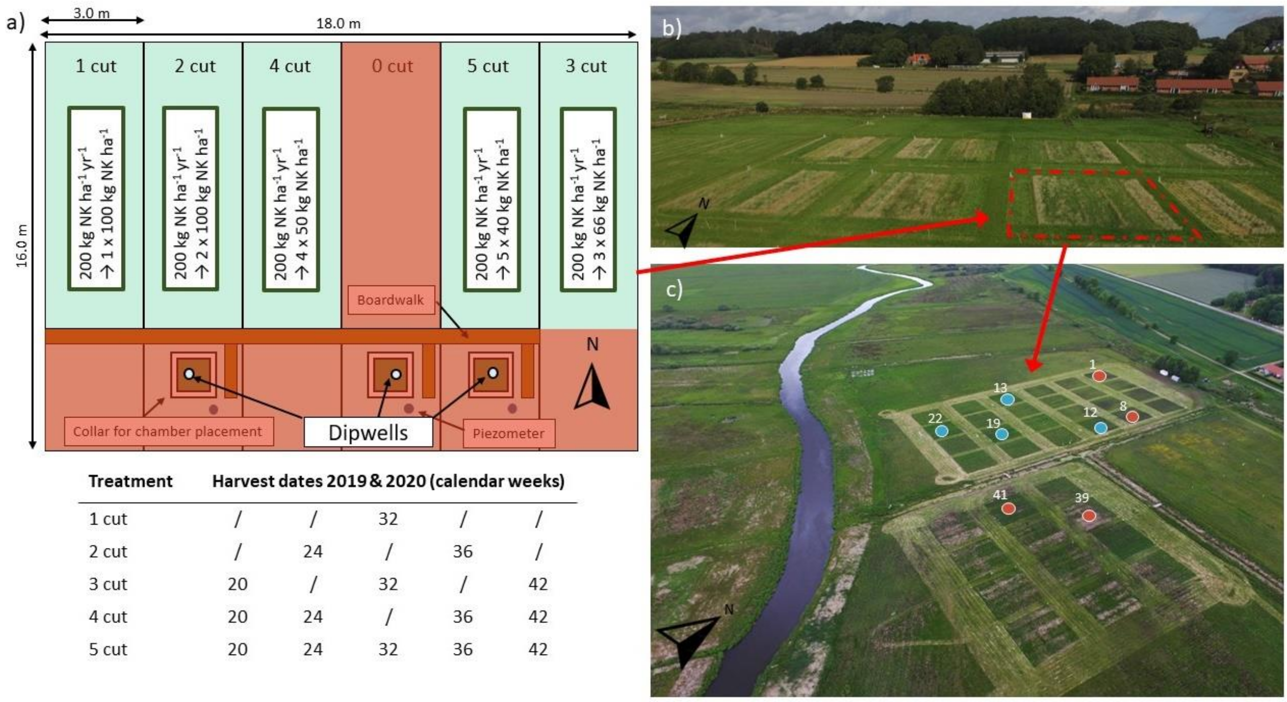
## METHODS

### Study site and experimental design

The study was performed in 2019–2020 at the Vejrumbro field site in Denmark (56° 26' 15.3" N, 9° 32' 44.1" E). The site is classified as a permanent grassland on a shallowly drained riparian fen peatland. Mean annual water table depth (WTD) was -10 cm during the period April 2020 to May 2021 (Figure A1 in the Appendix), with an average summer WTD of -25 cm (April to September 2020) and inundation (-1 cm) during winter (October 2020 to March 2021). For the growing period of April 2019 to March 2020, no records of WTD exist. From April

2020 onwards, WTD was logged automatically every hour in the three previously installed dipwells (Figure 1a; inserted to 1.0 m depth) on a total of four plots covering the spatial extent of the field site in a north–south direction (Figure 1c). The annual average temperature was 9.0 °C in 2019 and 9.5 °C in 2020. The year 2020 was not only warmer but, with a total of 704 mm of precipitation, also dryer than 2019 (892 mm) (Figures A2a and A2b). The difference in annual precipitation was also apparent in May–October during the study period, with a total of 328 mm in 2020 as compared to 550 mm in 2019. Long-term (1991–2021) average annual precipitation measured at the meteorological station Aarhus University Viborg was 675 mm and annual average temperature was 8.3°C. The daily average global radiation was similar for both years (Figure A2c).

In 2018, four plots were established with each of RCG (Reed Canary Grass, *Phalaris arundinacea*, cultivar: Lipaula) and TF (Tall Fescue, *Festuca arundinacea*, cultivar: Swaj) in a split-plot design, with species defining the plot and treatment the subplot. In both years, biomass was subjected to different harvesting frequency treatments, ranging from one to five annual cuts. The timing of harvests was predefined before the study commenced and is shown in calendar weeks in Figure 1a. The choice of harvest times was based on typical practice in forage production at different intensities. Biomass was harvested with a sickle bar mower (Grillo G107, Grillo SpA, Cesena, Italy) from biomass harvest areas of 1.8–3.6 $m^2$ within the sub-plots (Figures 1a, 1b). Harvested biomass was collected manually and brought to the laboratory for protein extraction and determination of dry matter (DM) and total nitrogen (TN) content in the unprocessed plant biomass. The sub-plots were fertilised with a combined NPK fertiliser (grade 14-3-15). The treatment with one annual cut received a single dose of 100 kg N and 100 kg P $ha^{-1}$ $yr^{-1}$ prior to the onset of growth in mid-April of both years. Other treatments received split-fertiliser applications in equal doses totalling 200 kg N and 200 kg P $ha^{-1}$ $yr^{-1}$, applied in mid-April as well as following each harvest. Details of site-specific soil properties, cultivation and harvest procedures are provided by Nielsen *et al.* (2021).




| Treatment | Harvest dates 2019 & 2020 (calendar weeks) | | | | |
|---|---|---|---|---|---|
| 1 cut | / | / | 32 | / | / |
| 2 cut | / | 24 | / | 36 | / |
| 3 cut | 20 | / | 32 | / | 42 |
| 4 cut | 20 | 24 | / | 36 | 42 |
| 5 cut | 20 | 24 | 32 | 36 | 42 |

Figure 1. a) Conceptual diagram of an example plot with sub-plots and the associated fertilisation rates. Piezometers, boardwalks and collars for chamber placements (for greenhouse gas flux measurements) within the red areas are part of the instrumentation of the split-plot design but are not relevant to this study. Harvest dates as calendar weeks for both years are tabulated below the diagram; b) drone image of the of the split-plot design as schematically illustrated in a), on c) the Vejrumbro field site, with blue dots identifying plots with *Phalaris arundinacea* and orange dots locating *Festuca arundinacea* plots. Photos: Jens Kjeldsen.

**Environmental variables and vegetation indices**

Hourly values for air temperature, precipitation, net radiation and global radiation were obtained from the meteorological station at Foulumgård, Aarhus University Viborg, approximately 6.5 km from the study site. Reflectance of red (656 nm) and near-infrared (NIR, 778 nm) bands was measured at monthly intervals during the growing season periods of March to September 2019 and April to October 2020 for each species, plot and subplot with three replicate measurements per location on the various plots (Figure 1c). Measurements were conducted with a Spectro Sense 2+ GPS 8-channel meter for NDVI (Skye instruments Ltd., Powys, UK). From these measurements, RVI (Equation 1) vegetation indices were calculated according to Tucker (1979) and averaged over the three measurements.

$$RVI = (NIR/RED) \qquad [1]$$

**Protein extraction**

This study was based on biomass and biorefinery yields harvested and processed in 2020. However, the various biorefinery outputs were compared to previously published data for the same study site in 2019, for which details on protein extraction are described by Nielsen *et al.* (2021). Harvested grass biomass was stored cool (2 °C) overnight and processed the day following harvest according to the lab-scale biorefinery approach described by Damborg *et al.* (2018, 2020). In this procedure, unchopped and unsorted grass biomass was separated into fractions of juice and pulp using a twin-screw press (Angelia 8500S Angel slow-juicer, Angel Co. Ltd, Busan, Korea). Grass protein was extracted from the juice fraction as described by Stødkilde *et al.* (2019). The juice was acidified to pH 4 using 14.8 mol $L^{-1}$ phosphoric acid and, after an overnight incubation at 4 °C, centrifuged at 2000×g at 4 °C. This resulted in precipitation of the protein concentrate from the residual brown juice fraction. All processed fractions were analysed for TN (Vario MAX CN; Elementar Analysesysteme GmbH, Hanau, Germany) to derive CP contents using an N-to-protein conversion factor of 6.25, justified by the predominant application in protein determination methods (Angell *et al.* 2016) despite the potential for overestimation (Yeoh & Wee 1994). No processing of biomass from the one-cut treatment was performed due to advanced maturity of the plants.

**Modelling of crude protein yield**

To explore the potential for modelling of CP yields in protein precipitates from paludiculture biomass under different harvest frequencies, we applied a generalised additive model (GAM) using restricted maximum likelihood (REML) in the package *mgcv* (Version 1.8-39; Wood 2022) in R (Version 4.1.2; R Core Team 2020). GAM was chosen for its capacity to simultaneously account for linear and non-linear relationships (Marra & Wood 2011, Wood 2011, Wood *et al*. 2016). Based on the derived values for CP yield in the protein precipitate from 2020, the possibility of fitting a RVI model, accounting for environmental temperature and precipitation variables known to affect biomass growth and senescence, was explored. Assessing the accuracy of using RVI and avoiding overfitting and concurvity/ collinearity resulted in the following model (Equation 2):

$$CP_i \sim N(\mu, \sigma^2)$$

$$\begin{aligned} CP_i = \alpha + f_1(RVI_i) + f_2\left(Prec_{10d_i}\right) \\ + f_3\left(10°Days_{30d_i}\right) \\ + f_4\left(Temp_{10d_i}, Days_{last_{cut_i}}\right) \\ + \beta_1\rho_1 + \beta_2 x_1 + \varepsilon_i \end{aligned} \qquad [2]$$

$$\varepsilon_i \sim N(\mu, \sigma^2)$$

where $CP_i$ is the CP yield in protein precipitate, $\mu$ is the overall mean, $\alpha$ is the intercept and $\sigma^2$ is the experimental error, affected by:

- $f_1 - f_4$ , which are the smooth functions of:
  - RVI of a certain day (*RVI*),
  - average precipitation over the last 10 days ($Prec_{10d}$),
  - the number of days above 10 °C over the last 30 days ($10°Days_{30d}$), and
  - the isotropic product smooth representing the marginal effects and interaction of the average temperature over the last 10 days ($Temp_{10d}$) and the number of days since the last harvest ($Days_{last\_cut}$),
  - each at the $i^{th}$ sample;
- $\beta_1\rho_1$ , the parametric variable of the number of days without precipitation over the last 30 days;
- $\beta_2 x_1$ , the categorical predictor variable of biomass species (RCG or TF).

**Statistical analyses**

The model residuals were inspected for normality and homoscedasticity. After calibration on data for the two-to-five-cut treatments from 2020, the model was applied to data from 2019 using the function predict in the R package stats (version 4.3.0). Model outputs included predicted values for each RVI measurement occasion, including confidence intervals at 95 % to determine the uncertainty in predictions. For all included values and parameters we reported observations as means, including standard errors (n = 4) to present the dispersion around the means, unless otherwise specified. Significance of differences between means for observations are reported as letters and were tested by one-way ANOVA with post-hoc Tukey's HSD at 95 % confidence level. The effects of harvest intensity, year, timing of harvest, and their interaction on CP yields in biomass and protein precipitates were assessed using a linear mixed effects model (Equation 3) using the *lmer* function in the R package *lme4* (Bates *et al*. 2015, Version 1.1.-23):

$$CP_i \sim N(\mu, \sigma^2)$$

$$\mu = \alpha_{j(i)} + \beta_1(Year) + \beta_2(Week) + \beta_3(Cuts) + \beta_4(Week * Year) \quad [3]$$

$$\alpha_j \sim N(\mu_{\alpha_j}, \sigma^2_{\alpha_j}), \qquad for\ Plot\ j\ =\ 1, \ldots, J$$

where $CP_i$ is the observed dependent variable, $\mu$ the overall mean, $\alpha$ the intercept, and $\sigma^2$ the experimental error. $\beta_{1\text{-}4}$ are the fixed effects of year (2019 and 2020), harvest timing (*Week*), harvest intensity (*Cuts*), and the interaction between harvest timing and year (*Week* * *Year*) for *j*, the random effect of plot, which defined the species.

## RESULTS

### Biomass dry matter yields were similar across years

For most interactions between species and harvest frequency, a numerically lower annual average DM production was found in 2020 as compared to 2019 (Nielsen *et al*. 2021). In detail, DM yields for RCG with two and four annual cuts were reduced by 3 t ha$^{-1}$ yr$^{-1}$, while DM biomass yield for TF with a 2-cut management was reduced by 4.4 t ha$^{-1}$ yr$^{-1}$. In contrast, an increase of approximately 3 t ha$^{-1}$ yr$^{-1}$ was observed for the RCG 3-cut and TF 5-cut treatments. However, none of the observed differences in annual cumulative DM yields were statistically significant, neither per species and treatment nor across treatments, where average DM yields were reduced from 2019 to 2020 by -0.8 (RCG) and -1.2 (TF) t ha$^{-1}$ yr$^{-1}$. It was found that biomass harvested in calendar weeks 24, 32 and 36 contributed the most to annual cumulative biomass DM yields (Figure 2). An early cut in calendar week 20 contributed 11 % to the annual yields on average across treatments and species, and a late cut in week 42 contributed no more than 12 % (Tables A1 and A2 in the Appendix).

### Higher relative CP contents in green protein precipitate

Biomass CP contents were numerically lower in 2020 compared to the values from 2019 (Nielsen *et al*. 2021), but the numerical differences were mostly insignificant due to large variations between replicates. Nonetheless, the reduction of CP content within biomass ranged from 11 % (RCG, 3-cut) to 50 % (TF, 2-cut) (Table 1). On two occasions (RCG 3-cut, TF 5-cut), lower CP yields were found in 2020 than in 2019 for treatments with numerically higher biomass DM yields per hectare. One example was TF with five annual cuts, where 0.4 t less CP within biomass was found despite a 2.7 t ha$^{-1}$ yr$^{-1}$ higher DM biomass yield compared to 2019. Generally, while the share of average CP in biomass across treatments ranged from 18 % (3-cut) to 22 % (2-cut) for RCG and 19 % (3-cut) to 23 % (4-cut) for TF in 2019, the relative CP contents within biomass decreased in 2020 to an average of 15 % for RCG and 16 % for TF across all treatments of harvest frequency. However, despite numerically lower DM and CP yields in 2020 as compared to 2019 (Nielsen *et al*. 2021), we found that CP extractability remained stable between the two years (Table 1). Expressed in terms of shares, the CP content in the 2020 protein precipitate accounted for 93 % of the CP content in biomass in 2019 across all treatments and species. Considering that the total CP content within biomass in 2020 accounted for only 68 % of the result for 2019, this indicated an increase in extractability. This increase in relative extractability was balanced both amongst species and across treatments.

### Crude protein yields were well predicted by an RVI model

Results from both assessed years regarding the protein extractability (expressed in t CP ha$^{-1}$) using biorefinery techniques indicated that grass maturity, as indicated by timing of harvest, significantly ($p < 0.001$) affected the CP contents in biomass and protein precipitate (Table A3). The ability to predict CP yields in the precipitated protein paste from RCG and TF paludiculture by RVI modelling was investigated and a positive regression was observed between predicted vs. measured yields (Figure 3). The exploration of whether an RVI model can be successfully applied to predict CP yields in the precipitated protein paste from paludiculture biomass showed good model performance ($R^2$ = 0.64, $p < 0.001$, NRMSE = 0.23) despite the observed spread in yields on a replicate plot basis (Figure A3). Averaged over all harvest occurrences, variances between the model-predicted and laboratory-measured values for the species and treatments ranged from 0.001 (TF, 5-cut) to 0.041 (RCG, 3-cut) t CP $ha^{-1}$, with no statistically significant difference (Figure 4). Overall, it was found that the RVI model predicted statistically similar yields for 92 % of the harvest occurrences per species and harvest intensity treatment for 2020 (Table 2). Thus, it was found that a simple RVI model was able to predict the CP content in biorefinery-derived protein precipitate from paludiculture biomass with a deviation of ± 0.021 t CP $ha^{-1}$ across all harvest occurrences. Applied to annual CP yields for the different treatments and species, the deviation between measured and modelled yields was 1.7 %.

Several factors were found to significantly influence the prediction (Table A4). Of these, the parametric coefficients (i.e., categorical variables) of species and the number of days without precipitation over the last 30 days before harvest were of high ($p < 0.001$) significance. Continuous variables regarding the average precipitation (in mm) over the last 10 days prior to harvest, as well as the average temperature over the last 10 days in interaction with the number of days since the last harvest, were equally significant ($p < 0.001$). RVI contributed to the prediction of CP within protein precipitate with $p < 0.01$. Overall, the model statistics showed that not only plant rejuvenation or manipulation by harvest but also, in particular, environmental conditions regarding temperature and precipitation, were of similar importance for measured CP contents in the protein precipitate and their accurate prediction.

## DISCUSSION

### Stability of green protein yields in paludiculture

Averaged across treatments, we found that annual DM yields, particularly for TF, were stable between the two years of our study, which is in agreement with earlier studies on yield stability for RCG and TF (Jansone *et al*. 2012, Chen *et al.* 2022). This also applied to CP contents within biomass, where only two instances of significantly lower CP content were found. Despite a dryer growing season in 2020 compared to 2019, which could have adversely affected biomass yields and N uptake, no effect on

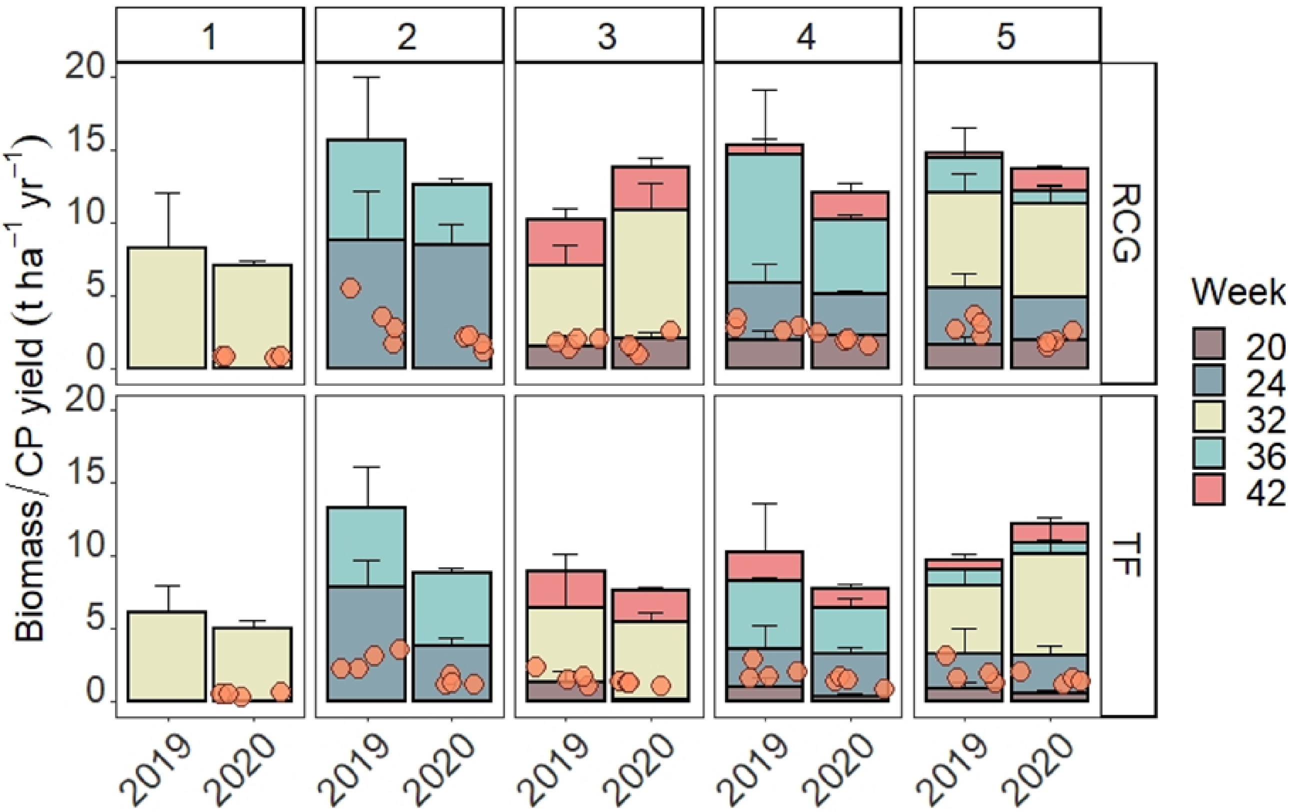



Figure 2. Cumulative annual yields of biomass (DM; columns) and crude protein (CP; dots), showing the mean contributions of different harvest occurrences (weeks) to the total. Error bars indicate the maximum deviation from the mean by showing the standard error. Compared horizontally, the panels (1 to 5) indicate treatments (different harvest frequencies), while vertically they show results for the two species *Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF). Data for 2019 were taken from Nielsen *et al*. (2021).

Table 1. Annual cumulative yields for dry biomass and all fractions resulting from green biorefining, including the respective crude protein (CP) yields, for *Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF) under harvest frequencies of 1 to 5 annual cuts, and for both assessed years. All values in t $ha^{-1}$ $yr^{-1}$ (as dry matter (DM) for biomass and biorefinery fractions). Standard error is given in brackets. Letters indicate differences between means of species and harvest for both years, where treatments with the same letter are not significantly different. **Bold** type indicates the (three) significant differences. Values for 2019 from Nielsen *et al*. (2021). Missing data (NA = not available) could not be determined due to technical difficulties. Values for 2019 from Nielsen *et al*. (2021).

| | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| RCG | | | | | | | | | | |
| Biomass (DM) | 8.29 (±1.88)a | 7.06 (±0.37)a | 15.66 (±3.77)a | 12.69 (±1.64)a | 10.25 (±1.25)a | 13.89 (±2.32)a | 15.06 (±2.14)a | 12.1 (±0.82)a | 14.45 (±1.35)a | 13.75 (±1.99)a |
| CP in biomass | NA | 0.88 (±0.04) | 3.44 (±0.80)a | 1.90 (±0.24)a | 1.88 (±0.16)a | 1.67 (±0.35)a | 2.97 (±0.19)a | 2.08 (±0.19)a | **2.99 (±0.30)a** | **2.02 (±0.22)b** |
| Protein paste (DM) | NA | NA | 1.68 (±0.31)a | 1.35 (±0.13)a | 1.19 (±0.15)a | 1.54 (±0.24)a | 1.91 (±0.19)a | 1.67 (±0.11)a | 1.51 (±0.09)a | 1.73 (±0.13)a |
| CP in protein precipitate | NA | NA | 0.61 (±0.11)a | 0.48 (±0.07)a | 0.40 (±0.04)a | 0.43 (±0.06)a | 0.65 (±0.03)a | 0.59 (±0.05)a | 0.56 (±0.05)a | 0.59 (±0.05)a |
| Brown juice (DM) | NA | NA | 1.83 (±0.44)a | 1.75 (±0.17)a | 1.40 (±0.22)a | 1.80 (±0.24)a | 1.98 (±0.40)a | 1.77 (±0.12)a | 1.64 (±0.10)a | 2.00 (±0.26)a |
| CP in brown juice | NA | NA | 0.31 (±0.08)a | 0.26 (±0.04)a | 0.19 (±0.03)a | 0.24 (±0.05)a | 0.29 (±0.03)a | 0.24 (±0.02)a | 0.22 (±0.03)a | 0.24 (±0.03)a |
| Pulp (DM) | NA | NA | 11.47 (±2.95)a | 9.07 (±1.22)a | 7.15 (±0.85)a | 9.42 (±1.32)a | 10.48 (±1.51)a | 7.97 (±0.55)a | 10.27 (±0.99)a | 8.03 (±0.93)a |
| CP in Pulp | NA | NA | 1.33 (±0.26)a | 0.80 (±0.10)a | 0.80 (±0.07)a | 0.94 (±0.12)a | 1.08 (±0.11)a | 0.92 (±0.05)a | 1.28 (±0.10)a | 0.98 (±0.10)a |
| TF | | | | | | | | | | |
| Biomass (DM) | 6.1 (±0.95)a | 5.03 (±0.59)a | 13.32 (±1.96)a | 8.9 (±0.64)a | 8.99 (±1.82)a | 7.67 (±0.76)a | 9.37 (±1.51)a | 7.74 (±1.17)a | 9.56 (±1.98)a | 12.27 (±3.45)a |
| CP in biomass | NA | 0.58 (±0.07) | **2.86 (±0.32)a** | **1.43 (±0.16)b** | 1.74 (±0.26)a | 1.31 (±0.08)a | 2.13 (±0.29)a | 1.41 (±0.18)a | 2.07 (±0.42)a | 1.61 (±0.18)a |
| Protein paste (DM) | NA | NA | 1.27 (±0.19)a | 0.93 (±0.07)a | 1.07 (±0.24)a | 0.93 (±0.05)a | 1.07 (±0.20)a | 1.00 (±0.15)a | 1.12 (±0.28)a | 1.26 (±0.24)a |
| CP in protein precipitate | NA | NA | 0.46 (±0.06)a | 0.34 (±0.05)a | 0.35 (±0.07)a | 0.31 (±0.04)a | 0.40 (±0.08)a | 0.36 (±0.04)a | 0.42 (±0.11)a | 0.45 (±0.09)a |
| Brown juice (DM) | NA | NA | 1.77 (±0.40)a | 1.23 (±0.08)a | 1.31 (±0.31)a | 0.89 (±0.06)a | 1.22 (±0.26)a | 1.06 (±0.18)a | 1.25 (±0.32)a | 1.43 (±0.33)a |
| CP in brown juice | NA | NA | 0.27 (±0.02)a | 0.22 (±0.06)a | 0.21 (±0.03)a | 0.16 (±0.01)a | 0.21 (±0.02)a | 0.17 (±0.01)a | 0.18 (±0.04)a | 0.20 (±0.03)a |
| Pulp (DM) | NA | NA | **9.54 (±1.37)a** | **5.76 (±0.33)b** | 6.08 (±1.20)a | 5.55 (±0.67)a | 6.22 (±0.91)a | 5.03 (±0.76)a | 6.67 (±1.27)a | 6.61 (±0.95)a |
| CP in pulp | NA | NA | 1.09 (±0.10)a | 0.57 (±0.05)b | 0.75 (±0.12)a | 0.59 (±0.06)a | 0.79 (±0.09)a | 0.61 (±0.08)a | 0.95 (±0.18)a | 0.84 (±0.10)a |

biomass or CP yields was found. A similar observation was reported by Meisser *et al.* (2018), who found a constant CP content despite drought conditions. Reduced precipitation is not necessarily accompanied by an increase in temperature. In our study, differences in precipitation between the two assessed years were pronounced but the difference in average annual temperature did not exceed + 0.5 °C. In a long-term study on drought effects, Cantarel *et al.* (2012) found significantly reduced yields under similar reductions of precipitation although these were accompanied by a temperature increase of 3.5 °C, and Wu *et al.* (2021) highlighted the decline of grassland net primary productivity (NPP) as a result of climate warming. Reduced precipitation during the growing season could potentially affect biomass growth and N accumulation and has been shown to significantly affect grass and legume species cultivated in mineral soils (Perotti *et al*. 2021, Baral *et al*. 2022). However, soil moisture is rarely a limiting factor in wet or rewetted peatlands, even under conditions of lower water table or precipitation, owing to capillary rise in the peat substrate (Irfan *et al*. 2020, Dai *et al.* 2022); and in our study reduced precipitation did not compromise the resilience of paludiculture biomass. This indicated a relative stability of biomass yields despite annual variation in climatic conditions. In addition, no effects of mowing frequency on biomass yields, CP contents or the yields of fractions resulting from biorefining and their CP concentrations were found. This was true for both years of observation, which indicated a resilience of protein yield stability for paludiculture grasses across stages of plant maturity. However, our study only assessed yields for the grass leys two years after establishment. Considering that highest productivity is frequently obtained for younger grass stands (Robbins *et al.* 1987), there is a need for the resilience to changes in annual average precipitation or temperature of older leys to be evaluated in long-term studies.

**High stability of CP content in protein products**

Interestingly, while no statistical differences were found in biomass DM yields or CP contents in biomass and the precipitated protein, numerical differences were apparent. Relative to the production of biomass per ha and considering the CP content within biomass input for biorefining, an increased extractability of CP in the protein precipitate was detected for the year 2020. However, this relative increase in extractability was best described as a high stability of CP content in the protein precipitate. Considering that 2020 was characterised by reduced precipitation, the result of high stability of CP contents in protein product yields contrasts with

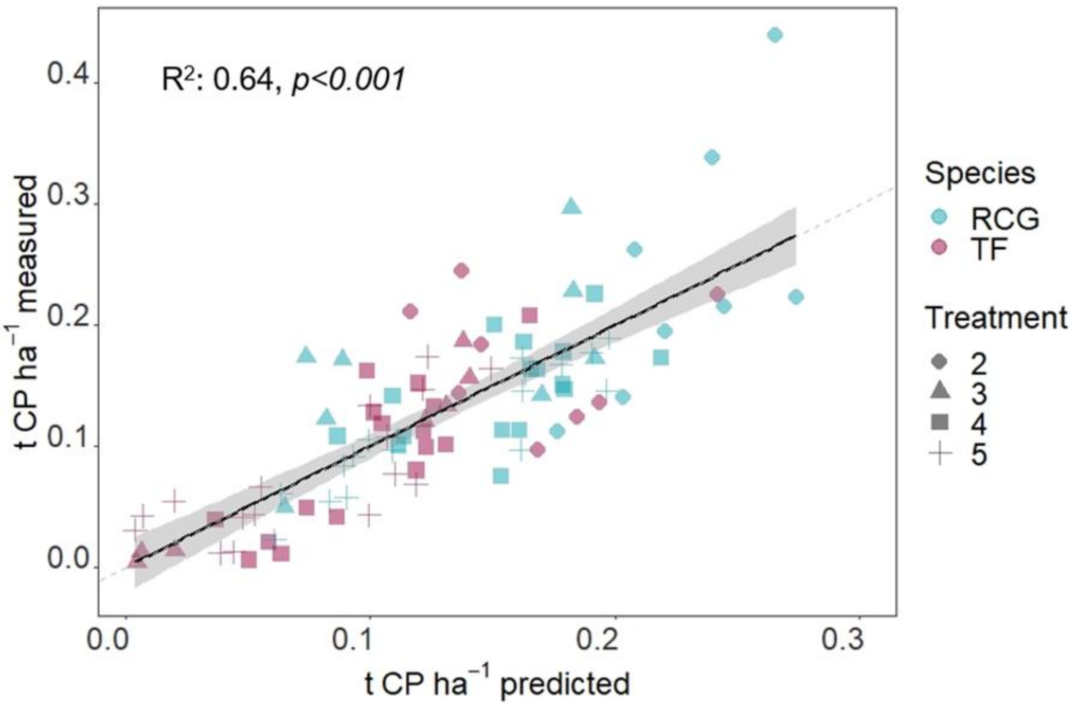



Figure 3. Regression plot of measured vs. model derived (predicted) yields of crude protein (CP) in protein precipitate (t $ha^{-1}$) across treatments of harvest frequencies (2 to 5 harvest occurrences per year) and the two species *Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF).

findings from reported studies on forage quality, where decreasing CP content was correlated with reduced water availability (e.g., Melo *et al*. 2022). However, as already mentioned, while soil moisture declines rapidly in soil types with large pore spaces or little organic carbon (Minasny & McBratney 2018), this effect may not be so pronounced on a wet peatland. Here, water table drawdown or reduced soil moisture might pose stress conditions on the grasses (Xu & Zhou 2011) without severely affecting their functioning due to a sufficient water supply from the peat substrate (Moskal *et al*. 2001). In addition, the biogeochemical response of peatlands to reduced soil moisture during periods of reduced precipitation is likely to partly explain the low variation of protein extractability. Núñez *et al*. (2022) found nutrient availability as a result of fertilisation and soil water content to be the key driver of CP content in grass leaves. A large proportion of fen peatland is nutrient-rich, like our study site. Typically, wet peatlands have low rates of N mineralisation due to anoxic conditions. However, reduced soil moisture during dry periods leads to increased soil oxidation, enhanced N mineralisation and consequent increases in plant-available inorganic forms of N (Minkkinen *et al*. 2020). This 'natural fertilisation' by enhanced N availability from peat mineralisation may have led to the accumulation of more readily extractable N within plant tissue, causing the observed relative increase in extractability per input unit of biomass. Hence, the stability of CP content within green protein from paludiculture biomass is notable, not only despite climatic variations between years, but also despite varying biomass management intensities.

**RVI models have the potential to maximise biorefinery yields**

The exploration of whether a simplified RVI model, with inclusion of climatic factors relevant for biomass growth, can be used to predict CP content within the biorefinery-derived protein precipitate resulted in a positive outcome. In 92 % of occurrences, the model was able to predict CP contents that were statistically similar to measured CP in the protein product, showing a deviation of only 1.7 %. Until now, crop simulation models have been used mainly to predict grass growth rates (e.g., 'CropSyst', Stöckle *et al*. 2003), leaf N content (Ma *et al*. 2022), or forage quality(e.g., 'GrasProg', Peters *et al*. 2022). Since, to the best of our knowledge, an

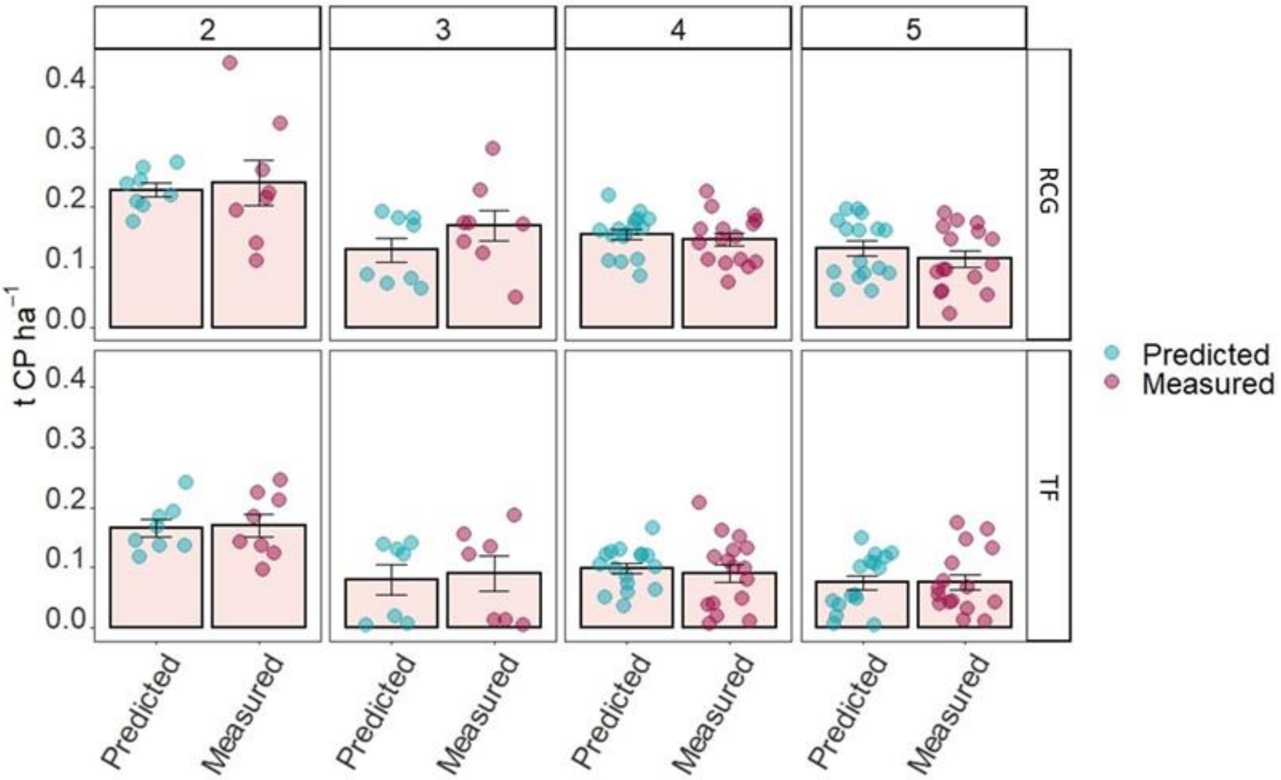



Figure 4. Model derived (Predicted) and actual (Measured) values of crude protein (CP; t ha$^{-1}$) in protein precipitate across harvest frequencies of one to five occurrences per year, for *Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF). The (pink) columns represent mean values and the error bars show standard error. Dots indicate the individual observations.

estimation of the CP content of protein products has not been performed previously, our results provide a strong indication that we have identified a hitherto under-utilised opportunity to improve green protein product yields. With a model performance of $R^2 = 0.64$, the approach shows promise for increased accuracy with further adjustments of model parameters, in particular soil moisture content and soil N, both of which can provide key information regarding the variation in CP content (Styczen *et al.* 2020) and, thus, extractability. In addition, the incorporation of integrated time-series SVI (satellite) data from Sentinel-2 or MODIS, which are already commonly utilised in other applications (e.g., Sharifi 2020, Matłok *et al.* 2021), might enable the capture of daily to periodical dynamics of plant response to growth conditions on larger fields that are of appropriate quality for inclusion in high-resolution data. In addition, it was found that the timing of harvest is a key variable for both CP content in the protein precipitate and its extractability, which is in line with previous studies (e.g., Olszewska 2021, Stødkilde *et al.* 2021) and a decisive criterion for the economic potential of green protein biorefineries. In this context, the results of the present study indicated the importance of achieving an equilibrium between maximum biomass yield and maximum CP content for total yield improvements.

Table 2. Crude protein yields (t ha$^{-1}$) in the protein precipitate for all annual harvest frequencies as indicated by the treatments (2-cut to 5-cut) and both species (*Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF)) per harvest week as predicted by the model and as measured. Standard error (n = 4) is shown in brackets. Letters indicate differences between means; treatments with the same letter are not significantly different and **bold** type highlights (the two) significant differences. Missing data could not be determined due to technical difficulties, resulting in omitted values for the 3-cut and 5-cut treatments at harvest week 32 owing to insufficient data.

| Treatment | Species | Week | Predicted | Measured |
|---|---|---|---|---|
| 2-cut | RCG | 24 | 0.256 (±0.008)a | 0.304 (±0.053)a |
| | | 36 | 0.202 (±0.009)a | 0.177 (±0.033)a |
| | TF | 24 | 0.197 (±0.016)a | 0.145 (±0.028)a |
| | | **36** | **0.134 (±0.006)b** | **0.196 (±0.021)a** |
| 3-cut | RCG | 20 | 0.077 (±0.005)a | 0.129 (±0.029)a |
| | | 42 | 0.182 (±0.004)a | 0.210 (±0.034)a |
| | TF | 20 | 0.010 (±0.005)a | 0.010 (±0.003)a |
| | | 42 | 0.133 (±0.004)a | 0.149 (±0.014)a |
| 4-cut | RCG | 20 | 0.105 (±0.006)a | 0.114 (±0.009)a |
| | | 24 | 0.189 (±0.011)a | 0.185 (±0.014)a |
| | | 36 | 0.170 (±0.005)a | 0.149 (±0.015)a |
| | | 42 | 0.157 (±0.004)a | 0.138 (±0.028)a |
| | TF | **20** | **0.052 (±0.006)a** | **0.019 (±0.007)b** |
| | | 24 | 0.123 (±0.015)a | 0.151 (±0.020)a |
| | | 36 | 0.125 (±0.003)a | 0.103 (±0.011)a |
| | | 42 | 0.095 (±0.010)a | 0.091 (±0.028)a |
| 5-cut | RCG | 20 | 0.093 (±0.002)a | 0.085 (±0.010)a |
| | | 24 | 0.191 (±0.005)a | 0.170 (±0.009)a |
| | | 36 | 0.079 (±0.011)a | 0.058 (±0.015)a |
| | | 42 | 0.162 (±0.000)a | 0.144 (±0.017)a |
| | TF | 20 | 0.046 (±0.003)a | 0.027 (±0.009)a |
| | | 24 | 0.119 (±0.011)a | 0.118 (±0.020)a |
| | | 36 | 0.021 (±0.012)a | 0.048 (±0.008)a |
| | | 42 | 0.114 (±0.006)a | 0.110 (±0.030)a |

From the perspective of maximum protein precipitate yields, the results highlighted the potential for an extensive to intermediate management system with two to three annual harvests to balance the economic cost-effectiveness of green protein paludiculture. However, to increase the overall economy of paludiculture for green protein biorefinery, a holistic context for fractions and their applications should be considered. For instance, the fibre fraction has demonstrated excellent quality for ruminant roughage (Damborg *et al.* 2018), and the sugar-rich brown juice fraction has an ideal composition for production of biogas (Feng *et al.* 2021). The inclusion of all fractions resulting from the biorefinery concept may contribute to the economic and environmental sustainability of green protein paludiculture. Nonetheless, optimisation of harvest timing in relation to specific environmental conditions still holds potential for adding value. In areas or countries with large agriculturally used peatland areas, such as The Netherlands, Germany and the Baltic countries, sustainable farming practices are highly sought-after. In these situations, the possibility to maximise crude protein contents in the final protein product by determining the optimal timing of harvest using an RVI model might offer a simple method for increasing overall efficiency with little technological input. In this context, our study has demonstrated a potential route towards boosting cultural heritage, regulating and provisioning services through peatland rewetting and sustainable paludiculture biomass utilisation, by incentivising paludiculture farming for green protein biorefinery.

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## AUTHOR CONTRIBUTIONS

CN developed the study design, performed the experimental work and data analysis, and wrote the manuscript. LS contributed significantly to improvement of the manuscript. All authors contributed to the study design, the writing and proofreading of the manuscript, and approved the final manuscript.

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## Appendix

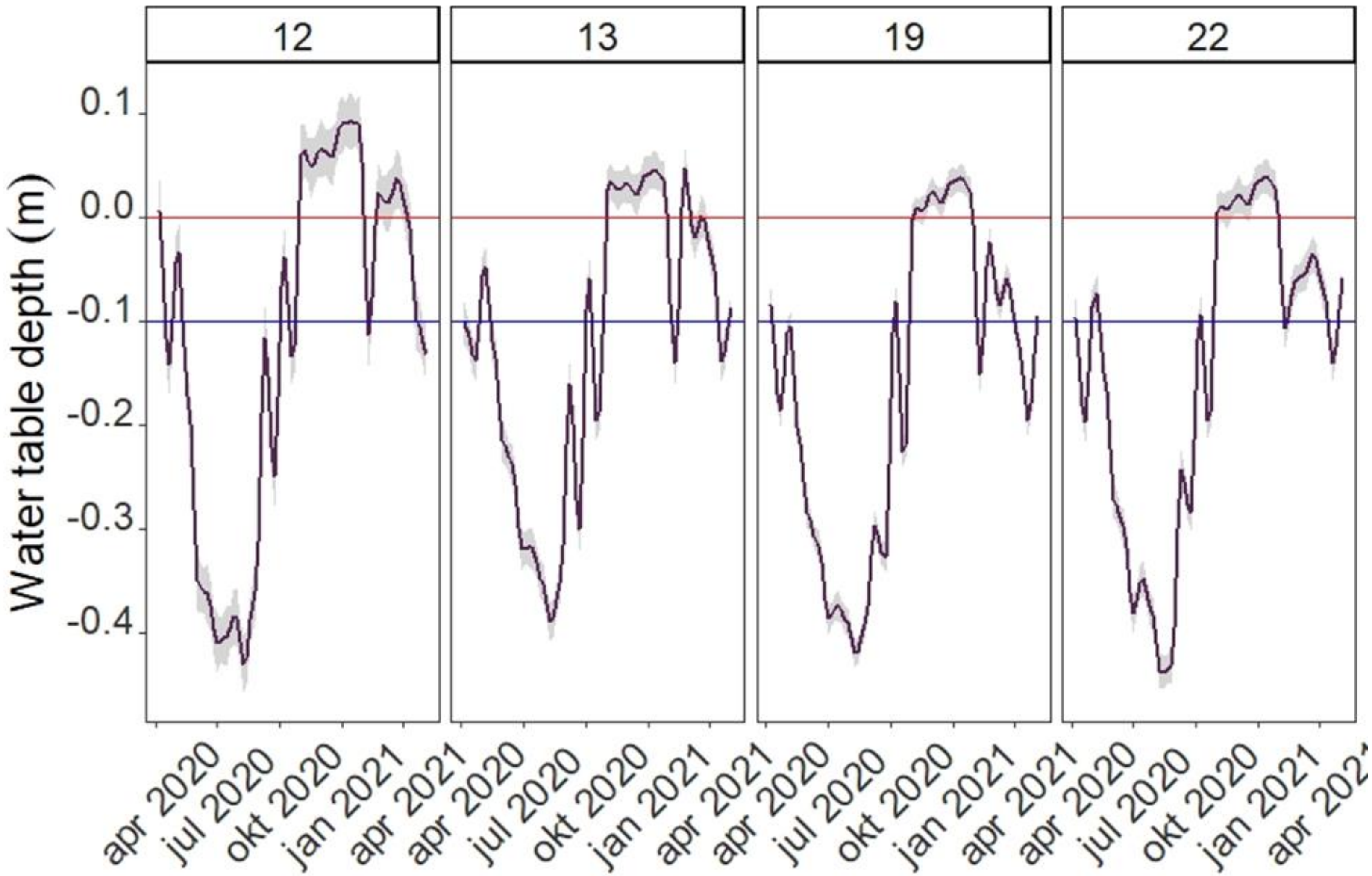


Figure A1. Records of hourly logged water table depth on four of the eight plots.

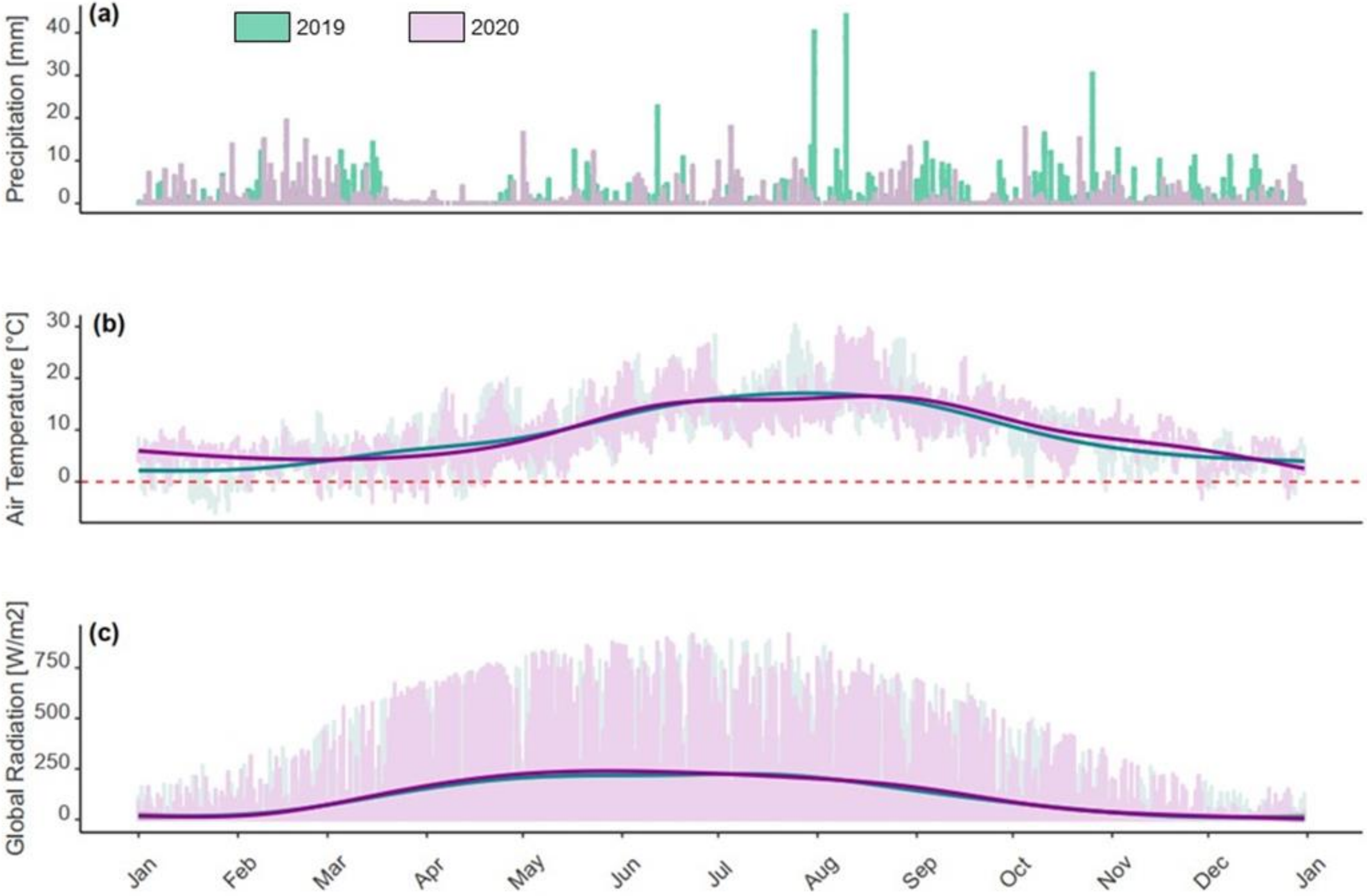


Figure A2. a) Daily precipitation totals; b) daily average air temperature; and c) daily average global radiation for the study site during the years 2019 (turquoise) and 2020 (violet).

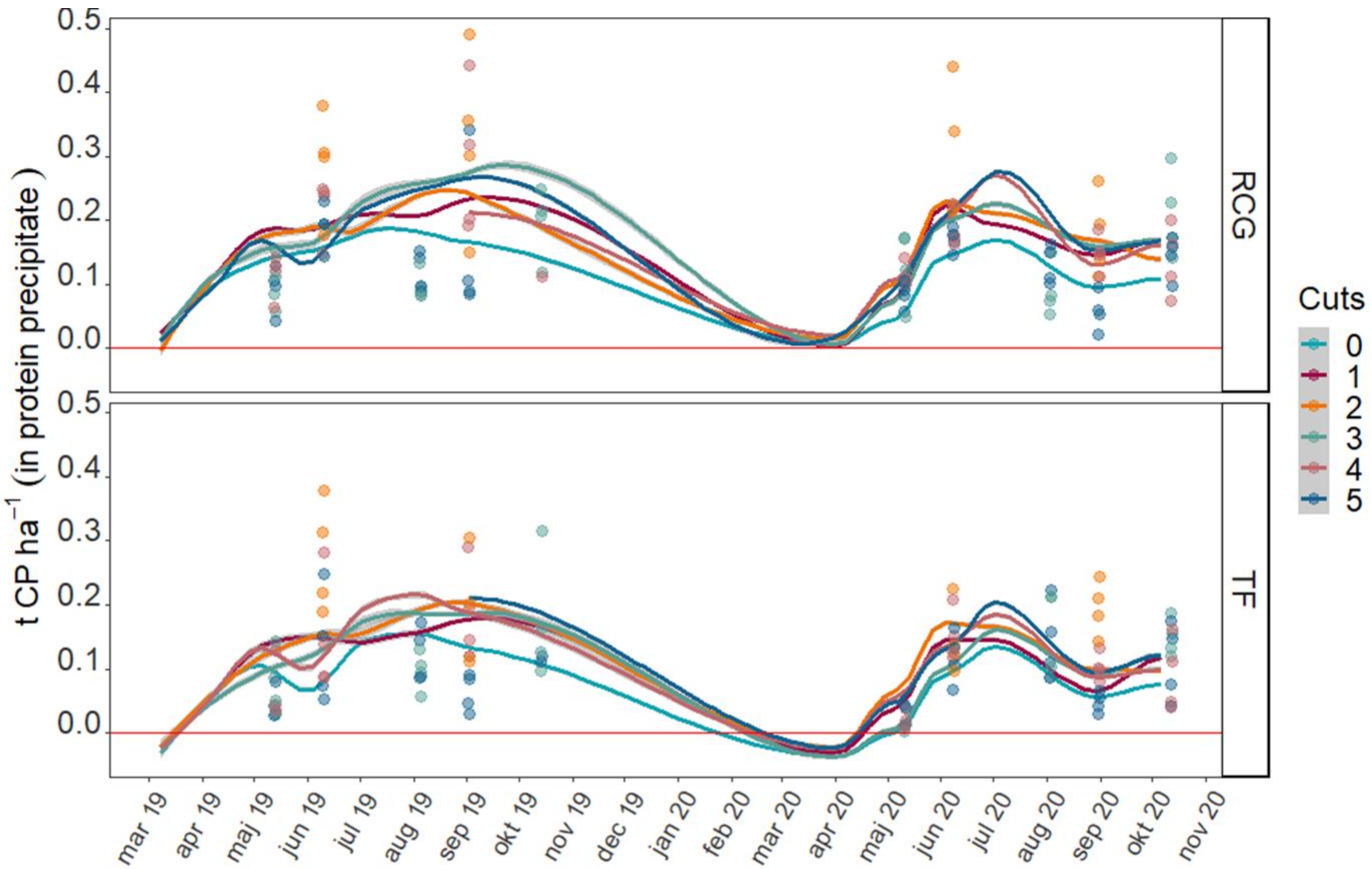


Figure A3. RVI model output for the species *Phalaris arundinacea* (RCG) and *Festuca arundinacea* (TF) over the years 2019 and 2020. Lines show modelled crude protein (CP) in protein precipitate yields (t ha$^{-1}$) per date and treatment. Harvest frequencies are indicated by 0–5 annual cuts and dots show measured yields.

Table A1. Average yields for biomass and all fractions resulting from green biorefinery, including the respective crude protein (CP) yields, for *Phalaris arundinacea* (RCG) under harvest frequencies of one to five annual cuts, for each harvest occurrence as indicated by calendar week and for both assessed years. Standard error is given in brackets. All values are expressed in t $ha^{-1}$ $yr^{-1}$ (DM or CP) except for nitrogen (N) (%). Missing data (NA = not available) could not be determined due to technical difficulties. Values for 2019 are taken from Nielsen *et al*. (2021).

| Year | Cuts | Week | Biomass (DM) | CP in biomass | Juice (DM) | Protein paste (DM) | CP in protein | Pulp (DM) | CP in pulp | Brown juice (DM) | CP in brown juice | Biomass N (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2019 | 1 | 32 | 8.29 (±1.88) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) |
| | 2 | 24 | 8.83 (±1.66) | 2.02 (±0.37) | 1.78 (±0.38) | 0.77 (±0.10) | 0.29 (±0.04) | 6.86 (±1.34) | 0.87 (±0.11) | 0.87 (±0.18) | 0.16 (±0.03) | 3.68 (±0.06) |
| | | 36 | 6.83 (±2.19) | 1.42 (±0.46) | 2.19 (±0.61) | 0.91 (±0.21) | 0.32 (±0.07) | 4.61 (±1.66) | 0.47 (±0.16) | 0.95 (±0.28) | 0.16 (±0.05) | 3.45 (±0.34) |
| | 3 | 20 | 1.58 (±0.36) | 0.33 (±0.08) | 0.54 (±0.12) | 0.30 (±0.06) | 0.10 (±0.02) | 1.02 (±0.25) | 0.16 (±0.04) | 0.20 (±0.04) | 0.03 (±0.01) | 3.30 (±0.04) |
| | | 32 | 5.50 (±0.70) | 0.80 (±0.02) | 1.40 (±0.23) | 0.41 (±0.05) | 0.10 (±0.01) | 4.24 (±0.50) | 0.42 (±0.03) | 0.63 (±0.13) | 0.06 (±0.01) | 2.44 (±0.26) |
| | | 42 | 3.18 (±0.37) | 0.75 (±0.10) | 1.14 (±0.13) | 0.49 (±0.06) | 0.20 (±0.03) | 1.89 (±0.19) | 0.22 (±0.02) | 0.58 (±0.08) | 0.11 (±0.02) | 3.74 (±0.08) |
| | 4 | 20 | 1.95 (±0.37) | 0.38 (±0.07) | 0.60 (±0.1) | 0.34 (±0.06) | 0.11 (±0.02) | 1.26 (±0.24) | 0.17 (±0.03) | 0.28 (±0.06) | 0.03 (±0.01) | 3.17 (±0.08) |
| | | 24 | 3.98 (±0.65) | 1.06 (±0.15) | 0.92 (±0.15) | 0.46 (±0.06) | 0.22 (±0.02) | 2.92 (±0.50) | 0.36 (±0.08) | 0.38 (±0.07) | 0.07 (±0.01) | 4.34 (±0.22) |
| | | 36 | 8.81 (±2.22) | 1.45 (±0.28) | 2.17 (±0.99) | 1.05 (±0.25) | 0.29 (±0.06) | 6.10 (±1.53) | 0.53 (±0.11) | 1.28 (±0.36) | 0.17 (±0.04) | 2.80 (±0.29) |
| | | 42 | 1.27 (±NA) | 0.34 (±NA) | 0.49 (±NA) | 0.27 (±NA) | 0.11 (±NA) | 0.79 (±NA) | 0.11 (±NA) | 0.17 (±NA) | 0.04 (±NA) | 4.26 (±NA) |
| | 5 | 20 | 1.67 (±0.29) | 0.31 (±0.05) | 0.56 (±0.1) | 0.30 (±0.05) | 0.09 (±0.02) | 1.08 (±0.18) | 0.15 (±0.02) | 0.22 (±0.05) | 0.02 (±0.00) | 2.99 (±0.05) |
| | | 24 | 3.88 (±0.50) | 1.07 (±0.08) | 1.08 (±0.12) | 0.42 (±0.04) | 0.19 (±0.02) | 2.76 (±0.38) | 0.41 (±0.02) | 0.34 (±0.08) | 0.06 (±0.01) | 4.52 (±0.37) |
| | | 32 | 6.53 (±0.67) | 1.03 (±0.09) | 1.68 (±0.2) | 0.44 (±0.04) | 0.12 (±0.02) | 4.98 (±0.63) | 0.51 (±0.04) | 0.74 (±0.04) | 0.08 (±0.01) | 2.54 (±0.16) |
| | | 36 | 2.38 (±1.07) | 0.59 (±0.27) | 0.82 (±0.32) | 0.36 (±0.14) | 0.16 (±0.06) | 1.46 (±0.69) | 0.21 (±0.09) | 0.34 (±0.13) | 0.05 (±0.03) | 3.87 (±0.11) |
| 2020 | 1 | 32 | 7.06 (±0.37) | 0.88 (±0.04) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | 1.99 (±0.09) |
| | 2 | 24 | 8.53 (±1.37) | 1.21 (±0.19) | 1.97 (±0.27) | 0.84 (±0.12) | 0.30 (±0.05) | 5.77 (±1.07) | 0.47 (±0.08) | 1.16 (±0.16) | 0.17 (±0.04) | 2.28 (±0.09) |
| | | 36 | 4.16 (±0.40) | 0.69 (±0.08) | 0.97 (±0.06) | 0.51 (±0.07) | 0.18 (±0.03) | 3.30 (±0.46) | 0.33 (±0.05) | 0.60 (±0.03) | 0.09 (±0.01) | 2.64 (±0.07) |
| | 3 | 20 | 2.08 (±0.48) | 0.44 (±0.10) | 0.65 (±0.16) | 0.37 (±0.09) | 0.13 (±0.03) | 1.28 (±0.34) | 0.20 (±0.05) | 0.26 (±0.07) | 0.05 (±0.01) | 3.43 (±0.14) |
| | | 32 | 8.82 (±1.87) | 0.82 (±0.20) | 1.71 (±0.27) | 0.67 (±0.20) | 0.09 (±0.02) | 6.31 (±1.12) | 0.54 (±0.10) | 1.09 (±0.16) | 0.10 (±0.03) | 1.75 (±0.04) |
| | | 42 | 2.99 (±0.60) | 0.62 (±0.13) | 0.86 (±0.12) | 0.49 (±0.08) | 0.21 (±0.03) | 1.83 (±0.40) | 0.20 (±0.04) | 0.45 (±0.08) | 0.09 (±0.02) | 3.32 (±0.10) |
| | 4 | 20 | 2.29 (±0.08) | 0.41 (±0.01) | 0.73 (±0.02) | 0.39 (±0.02) | 0.11 (±0.01) | 1.63 (±0.15) | 0.23 (±0.02) | 0.31 (±0.01) | 0.04 (±0.00) | 2.85 (±0.12) |
| | | 24 | 2.83 (±0.19) | 0.51 (±0.05) | 0.93 (±0.03) | 0.46 (±0.01) | 0.18 (±0.01) | 1.72 (±0.16) | 0.20 (±0.02) | 0.46 (±0.03) | 0.06 (±0.01) | 2.88 (±0.14) |
| | | 36 | 5.10 (±0.36) | 0.73 (±0.06) | 1.19 (±0.18) | 0.49 (±0.05) | 0.15 (±0.01) | 3.46 (±0.19) | 0.34 (±0.01) | 0.74 (±0.10) | 0.10 (±0.01) | 2.30 (±0.05) |
| | | 42 | 1.88 (±0.69) | 0.42 (±0.13) | 0.58 (±0.14) | 0.33 (±0.08) | 0.14 (±0.03) | 1.17 (±0.54) | 0.15 (±0.05) | 0.26 (±0.06) | 0.05 (±0.01) | 3.84 (±0.21) |
| | 5 | 20 | 1.95 (±0.23) | 0.32 (±0.04) | 0.62 (±0.06) | 0.33 (±0.03) | 0.08 (±0.01) | 1.45 (±0.19) | 0.19 (±0.03) | 0.28 (±0.03) | 0.03 (±0.00) | 2.65 (±0.04) |
| | | 24 | 2.95 (±0.15) | 0.53 (±0.03) | 0.88 (±0.05) | 0.43 (±0.01) | 0.17 (±0.01) | 1.80 (±0.11) | 0.22 (±0.01) | 0.45 (±0.02) | 0.06 (±0.01) | 2.89 (±0.05) |
| | | 32 | 6.44 (±1.32) | 0.76 (±0.2) | 1.35 (±0.2) | 0.49 (±0.06) | 0.13 (±0.01) | 3.43 (±0.59) | 0.36 (±0.06) | 0.87 (±0.16) | 0.08 (±0.02) | 2.22 (±0.30) |
| | | 36 | 0.87 (±0.34) | 0.21 (±0.08) | 0.27 (±0.09) | 0.14 (±0.04) | 0.06 (±0.02) | 0.52 (±0.22) | 0.08 (±0.02) | 0.14 (±0.05) | 0.02 (±0.01) | 3.87 (±0.21) |
| | | 42 | 1.54 (±0.23) | 0.39 (±0.06) | 0.58 (±0.08) | 0.34 (±0.04) | 0.14 (±0.02) | 0.83 (±0.13) | 0.14 (±0.02) | 0.27 (±0.04) | 0.05 (±0.01) | 4.10 (±0.08) |

Table A2. Average yields for biomass and all fractions resulting from green biorefinery, including the respective crude protein (CP) yields, for *Festuca arundinacea* (RCG) under harvest frequencies of one to five annual cuts, for each harvest occurrence as indicated by calendar week and for both assessed years. Standard error is given in brackets. All values are expressed in t $ha^{-1}$ $yr^{-1}$ (DM or CP) except for nitrogen (N) (%). Missing data (NA = not available) could not be determined due to technical difficulties. Values for 2019 are taken from Nielsen *et al*. (2021).

| Year | Cuts | Week | Biomass (DM) | CP in biomass | Juice (DM) | Protein paste (DM) | CP in protein | Pulp (DM) | CP in pulp | Brown juice (DM) | CP in brown juice | Biomass N (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2019 | 1 | 32 | 6.10 (±0.95) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) |
| | 2 | 24 | 7.86 (±0.93) | 1.81 (±0.27) | 1.49 (±0.17) | 0.73 (±0.09) | 0.28 (±0.04) | 6.02 (±0.73) | 0.72 (±0.06) | 0.78 (±0.14) | 0.14 (±0.03) | 3.69 (±0.36) |
| | | 36 | 5.46 (±1.43) | 1.05 (±0.19) | 2.01 (±0.55) | 0.54 (±0.15) | 0.19 (±0.04) | 3.52 (±0.9) | 0.37 (±0.07) | 0.99 (±0.29) | 0.13 (±0.01) | 3.35 (±0.38) |
| | 3 | 20 | 1.29 (±0.38) | 0.26 (±0.08) | 0.43 (±0.13) | 0.23 (±0.07) | 0.08 (±0.02) | 0.85 (±0.25) | 0.13 (±0.03) | 0.17 (±0.05) | 0.02 (±0.01) | 3.20 (±0.09) |
| | | 32 | 5.13 (±1.34) | 0.80 (±0.17) | 1.89 (±0.72) | 0.40 (±0.09) | 0.10 (±0.02) | 3.76 (±0.93) | 0.41 (±0.10) | 0.65 (±0.21) | 0.07 (±0.02) | 2.61 (±0.20) |
| | | 42 | 2.57 (±0.59) | 0.68 (±0.12) | 0.92 (±0.23) | 0.44 (±0.13) | 0.18 (±0.05) | 1.46 (±0.33) | 0.21 (±0.04) | 0.49 (±0.11) | 0.12 (±0.01) | 4.34 (±0.23) |
| | 4 | 20 | 0.98 (±0.32) | 0.19 (±0.06) | 0.34 (±0.12) | 0.19 (±0.07) | 0.06 (±0.02) | 0.64 (±0.21) | 0.09 (±0.03) | 0.12 (±0.04) | 0.01 (±0.00) | 3.20 (±0.14) |
| | | 24 | 2.69 (±0.78) | 0.72 (±0.18) | 0.69 (±0.19) | 0.33 (±0.10) | 0.15 (±0.05) | 1.89 (±0.54) | 0.30 (±0.09) | 0.25 (±0.09) | 0.05 (±0.01) | 4.41 (±0.28) |
| | | 36 | 5.47 (±0.83) | 1.13 (±0.08) | 1.72 (±0.30) | 0.56 (±0.10) | 0.19 (±0.04) | 3.69 (±0.59) | 0.40 (±0.04) | 0.85 (±0.19) | 0.15 (±0.02) | 3.42 (±0.28) |
| | | 42 | 0.96 (±NA) | 0.33 (±NA) | 0.35 (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | 5.53 (±NA) |
| | 5 | 20 | 0.87 (±0.25) | 0.16 (±0.05) | 0.29 (±0.10) | 0.15 (±0.04) | 0.05 (±0.01) | 0.58 (±0.16) | 0.08 (±0.02) | 0.11 (±0.04) | 0.01 (±0.00) | 2.94 (±0.05) |
| | | 24 | 2.39 (±0.88) | 0.61 (±0.21) | 0.55 (±0.18) | 0.30 (±0.10) | 0.13 (±0.04) | 1.69 (±0.62) | 0.26 (±0.09) | 0.26 (±0.10) | 0.04 (±0.01) | 4.22 (±0.25) |
| | | 32 | 4.69 (±0.62) | 0.83 (±0.10) | 1.49 (±0.38) | 0.41 (±0.07) | 0.12 (±0.02) | 3.46 (±0.36) | 0.42 (±0.04) | 0.61 (±0.14) | 0.07 (±0.01) | 2.87 (±0.17) |
| | | 36 | 1.07 (±0.06) | 0.30 (±0.01) | 0.46 (±0.02) | 0.14 (±0.03) | 0.06 (±0.02) | 0.63 (±0.04) | 0.12 (±0.01) | 0.19 (±0.01) | 0.04 (±0.01) | 4.53 (±0.14) |
| | | 42 | 1.08 (±0.07) | 0.32 (±0.03) | 0.44 (±0.01) | 0.25 (±0.00) | 0.12 (±0.00) | 0.61 (±0.04) | 0.12 (±0.01) | 0.17 (±0.01) | 0.04 (±0.00) | 4.71 (±0.08) |
| 2020 | 1 | 32 | 5.03 (±0.59) | 0.58 (±0.07) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | NA (±NA) | 1.84 (±0.08) |
| | 2 | 24 | 3.85 (±0.53) | 0.59 (±0.11) | 0.92 (±0.11) | 0.39 (±0.05) | 0.15 (±0.03) | 2.44 (±0.33) | 0.22 (±0.04) | 0.51 (±0.05) | 0.08 (±0.02) | 2.39 (±0.14) |
| | | 36 | 5.05 (±0.23) | 0.84 (±0.05) | 1.28 (±0.06) | 0.54 (±0.03) | 0.20 (±0.02) | 3.33 (±0.13) | 0.35 (±0.02) | 0.72 (±0.03) | 0.14 (±0.03) | 2.67 (±0.11) |
| | 3 | 20 | 0.14 (±0.06) | 0.04 (±0.01) | 0.06 (±0.02) | 0.03 (±0.01) | 0.01 (±0.00) | 0.12 (±0.05) | 0.02 (±0.01) | 0.02 (±0.01) | NA (±NA) | 3.32 (±0.20) |
| | | 32 | 5.35 (±0.61) | 0.82 (±0.04) | 1.05 (±0.14) | 0.50 (±0.05) | 0.15 (±0.03) | 4.15 (±0.54) | 0.40 (±0.04) | 0.55 (±0.09) | 0.10 (±0.01) | 2.51 (±0.23) |
| | | 42 | 2.18 (±0.25) | 0.46 (±0.05) | 0.71 (±0.07) | 0.40 (±0.04) | 0.15 (±0.01) | 1.31 (±0.15) | 0.17 (±0.02) | 0.33 (±0.04) | 0.06 (±0.01) | 3.41 (±0.08) |
| | 4 | 20 | 0.41 (±0.14) | 0.07 (±0.02) | 0.13 (±0.05) | 0.07 (±0.03) | 0.02 (±0.01) | 0.31 (±0.1) | 0.05 (±0.02) | 0.06 (±0.02) | 0.01 (±0.00) | 2.96 (±0.08) |
| | | 24 | 2.89 (±0.38) | 0.53 (±0.06) | 0.78 (±0.14) | 0.39 (±0.06) | 0.15 (±0.02) | 1.80 (±0.25) | 0.19 (±0.02) | 0.42 (±0.08) | 0.07 (±0.01) | 2.97 (±0.11) |
| | | 36 | 3.20 (±0.60) | 0.53 (±0.08) | 0.75 (±0.12) | 0.32 (±0.04) | 0.10 (±0.01) | 2.19 (±0.41) | 0.26 (±0.04) | 0.44 (±0.09) | 0.06 (±0.01) | 2.74 (±0.19) |
| | | 42 | 1.24 (±0.38) | 0.28 (±0.09) | 0.37 (±0.11) | 0.23 (±0.07) | 0.09 (±0.03) | 0.73 (±0.23) | 0.11 (±0.03) | 0.15 (±0.04) | 0.03 (±0.01) | 3.61 (±0.06) |
| | 5 | 20 | 0.61 (±0.22) | 0.11 (±0.04) | 0.18 (±0.06) | 0.10 (±0.03) | 0.03 (±0.01) | 0.48 (±0.19) | 0.07 (±0.03) | 0.08 (±0.02) | 0.01 (±0.00) | 2.92 (±0.13) |
| | | 24 | 2.59 (±0.59) | 0.46 (±0.09) | 0.69 (±0.15) | 0.31 (±0.06) | 0.12 (±0.02) | 1.66 (±0.39) | 0.17 (±0.04) | 0.37 (±0.09) | 0.05 (±0.01) | 2.86 (±0.10) |
| | | 32 | 6.92 (±2.58) | 0.71 (±0.10) | 1.09 (±0.27) | 0.44 (±0.09) | 0.14 (±0.03) | 3.19 (±0.37) | 0.38 (±0.02) | 0.67 (±0.18) | 0.09 (±0.02) | 2.65 (±0.26) |
| | | 36 | 0.83 (±0.12) | 0.18 (±0.03) | 0.24 (±0.03) | 0.13 (±0.02) | 0.05 (±0.01) | 0.56 (±0.10) | 0.09 (±0.01) | 0.12 (±0.02) | 0.02 (±0.01) | 3.53 (±0.16) |
| | | 42 | 0.83 (±0.12) | 0.18 (±0.03) | 0.24 (±0.03) | 0.13 (±0.02) | 0.05 (±0.01) | 0.56 (±0.10) | 0.09 (±0.01) | 0.12 (±0.02) | 0.02 (±0.01) | 3.53 (±0.16) |

Table A3. Linear mixed effects model outcomes for the effects of years (Year), harvest timing (Week) and the different harvest intensities (Cuts) for crude protein (CP) yield in biomass and in the protein precipitate. Significance codes: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

| | Dependent variable | |
|---|---|---|
| | CP yield biomass | CP yield protein |
| Year2019 | 0.167 | 0.168*** |
| Year2020 | 0.133 | 0.151*** |
| Week24 | 0.778*** | 0.101*** |
| Week32 | 0.628*** | 0.033 |
| Week36 | 0.556*** | 0.093*** |
| Week42 | 0.292** | 0.080*** |
| Cuts3 | 0.628*** | -0.077*** |
| Cuts4 | 0.141 | -0.074*** |
| Cuts5 | 0.173 | -0.104*** |
| Year2020:Week24 | 0.002 | -0.013 |
| Year2020:Week32 | -0.541*** | 0.037 |
| Year2020:Week36 | -0.034 | -0.062* |
| Year2020:Week42 | -0.426*** | -0.006 |
| Observations | 216 | 210 |
| Log Likelihood | -66.551 | 250.029 |
| Akaike Inf. Crit. | 165.101 | -470.059 |
| Bayesian Inf. Crit. | 219.106 | -419.852 |

Table A4. Performance of the generalised additive model used for predictions of crude protein content in protein precipitate. Significance codes: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

**Parametric coefficients:**

| | Estimate | Std. Error | t value | Pr(>\|t\|) | |
|---|---|---|---|---|---|
| (Intercept) | 0 | 0 | NaN | NaN | |
| 1) Species TF | -0.04169 | 0.010607 | -3.931 | 0.000169 | *** |
| 2) Number of days without precipitation over last 30 days | 0.010999 | 0.000527 | 20.884 | < 2e-16 | *** |

**Smooth terms:**

| | edf | Ref.df | F | p-value | |
|---|---|---|---|---|---|
| 1) RVI | 1 | 1 | 11.51 | 0.00104 | ** |
| 2) Avg. 10 day temp. * No. of days since last cut | 2.961 | 2.998 | 16.652 | < 2e-16 | *** |
| 3) No. of days above 10°C over last 30days | 1 | 1 | 5.409 | 0.02236 | * |
| 4) Avg 10 day precipitation | 1 | 1 | 41.053 | < 2e-16 | *** |

| | |
|---|---|
| | n = 95 |
| R-sq.(adj): 0.637 | Deviance explained: 65.5 % |
| -REML: -125.14 | Scale est.: 0.002168 |