The effects of harvest date and frequency on the yield, nutritional value and mineral content of the paludiculture crop cattail (*Typha latifolia* L.) in the first year after planting

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SUMMARY

The use of drained peatlands as dairy grasslands leads to long-term organic matter losses, CO_2 emissions and soil subsidence. It also yields grass with increased N and P contents compared to grass grown on mineral soils due to peat mineralisation, which often leads to greater farm surpluses of these elements. Growing *Typha latifolia* as a forage crop on rewetted peatlands (paludiculture) could reduce these issues. Therefore, the effects of harvest date and frequency on yield and nutritional value were studied in three experiments during the first growing season after establishment of two different *T. latifolia* plantations. *T. latifolia* produced 40–68 shoots m^{-2} and maximum dry matter (DM) yields of 9.81–10.89 Mg ha^{-1}. Harvesting before flowering resulted in the highest nutritional value per kg DM, of 563–575 g *in vitro* digestible organic matter (IVDOM), 120–128 g crude protein (CP), 287–300 g crude fibre (CF) and 1.5 g P. Surprisingly, harvesting at intervals of three or six weeks resulted in similar cumulative DM yields ($p = 0.190$). Also, average nutritional values per kg DM, especially of biomass harvested at 3-week intervals, remained similar to a May yield of 466–591 g IVDOM, 103–134 g CP and 286–303 g CF. Growing *T. latifolia* fodder for inclusion in grass-based diets could reduce the environmental impacts of dairy farming on peat.

KEY WORDS: harvesting procedure, rewetted peatland, ruminant feed

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INTRODUCTION

Peatlands cover about 3 % of the planet's surface (Urák *et al.* 2017) and nearly 10 % of the surface of Europe if shallow peatlands (< 30 cm peat) are also taken into account (Tanneberger *et al.* 2017). Oxic conditions of peat due to drainage lead to constant degradation and long-term losses of organic matter and CO_2 emissions (van den Akker *et al.* 2008). In The Netherlands about 223,000 ha of the total 290,000 ha of peat soils are used as intensively managed dairy grassland and, therefore, have to be permanently drained (Schothorst 1977, van den Akker *et al.* 2008). In Dutch peatland areas used for dairy farming, the intensive management of grassland is leading to discharge of surplus N and P to surface water (van Beek 2007) and estimated soil subsidence of 1–2 cm $year^{-1}$ (Schothorst 1977, van den Akker *et al.* 2007). Moreover, the constant organic matter degradation in dairy grassland on peat leads to a higher grass mineral uptake from the soil than on mineral soils, yielding more N and P dense forage (De Visser *et al.* 2001, van der Meer *et al.* 2004). When the higher nutrient density of the forage is not balanced with components low in N and P such as maize in the cows' diets, the cows will receive a dietary surplus of N and P which, in turn, will lead to a higher farm N and P surplus than for a dairy farming system of similar intensity on mineral soil (De Visser *et al.* 2001). Since the cultivation of maize on peat is undesirable because it requires more intensive tillage than perennial grass cultivation (and thus leads to increased peat degradation), this is not an option for most farms (De Visser *et al.* 2001).

Alternatively, increasing groundwater levels or completely rehydrating peat soils reduces peat degradation and reduces or completely stops soil subsidence (van den Akker *et al.* 2007, FAO 2014, Wilson *et a*l. 2016). Cultivating crops in (re)hydrated conditions on peat (i.e. paludiculture) may be a viable alternative, maintaining economic activities in peatland areas (Wichtmann *et al.* 2016, Dragoni *et al.* 2017), and could be part of a more 'nature-inclusive' farming system (Erisman *et al.* 2016). In The Netherlands little effort has been devoted to growing flood resistant grains (e.g. *Oryza*, *Zizania*), submerged starch crops (e.g. *Sagitaria*) and flood tolerant grasses (*Phalaris*, *Glyceria*), even though large areas of Dutch agricultural land have subsided below sea level. *Typha latifolia* (broadleaf cattail) can be successfully grown and harvested in wet peatlands, is potential livestock fodder (González *et al.* 2000, do Nascimento *et al*. 2014), can produce considerable biomass, and can take up large amounts of nutrients from e.g. farm runoff and soil accumulations that have resulted from intensive farming (Maddison *et al*. 2005, Geurts *et al*. 2017).

Yields of *T. latifolia* seem to depend on time since crop establishment and harvest date, and are known to vary spatially and between years (Pfadenhauer & Wild 2001, Maddison *et al*. 2005, Maddison *et al*. 2009, Heinz 2012, Günther *et al*. 2015). However, to our knowledge, relatively little is known about the biomass production and nutritional value in relation to harvest date and frequency of *T. latifolia* grown as a paludiculture crop. In a dairy farming system, *T. latifolia* could possibly be grazed or harvested repeatedly during the season in order to utilise younger and nutritionally superior biomass than would be available from a single seasonal harvest. Only a few studies have been performed on the stability of *Typha* spp. regrowth after harvesting, mostly aiming to reduce the abundance of *Typha* spp. in wetlands (Hellsten *et al.* 1999). The objectives of the current work were to assess the production, nutritional value and mineral content of *T. latifolia* green biomass harvested at different dates and frequencies during a growing season. We hypothesised that:

1) *T. latifolia* biomass yields would peak between the middle and end of the growing season;
2) the nutritional value of *T. latifolia* would decrease with increasing crop age, with a sharp decrease before onset of flowering, as in (other) grasses; and
3) increased harvesting frequency (as in a situation with grazing) would result in a maintained nutritional value compared to harvesting before onset of flowering but would drastically reduce productivity and shoot density during a growing season.

METHODS

Study sites

Three experiments were performed on two stands of *T. latifolia* planted on transformed dairy grassland at the Knowledge Transfer Centre Zegveld in The Netherlands (location used for Experiment I: 52° 08' 04.8" N, 4° 50' 10.4" E; location for Experiments II and III: 52° 08' 20.0" N, 4° 50' 19.6" E). The experimental sites were established by planting young *T. latifolia* plants which had been reared in a greenhouse using seeds obtained from natural stands (Aquaflora, The Netherlands). The seeds were spread in 1 × 1 m soil beds for germination, after which individual seedlings were planted in pots (4.8 × 4.5 × 11.0 cm) filled with potting soil. Before planting out, the experimental fields were prepared by mowing the grass to a stubble height of 2–3 cm then removing the topsoil layer including the grass sod (± 10 cm). The removed soil was used to make ridges around the field, in order to permanently maintain a water level of 20–30 cm above the soil surface. Water levels were maintained using a solar-powered water pump equipped with a water level sensor. At planting (June 2015 for Experiment I, July 2016 for Experiments II and III), plants were 30–60 cm high, and were planted by hand (Experiment I) or semi-mechanically (Experiments II and III). Planting densities were 15 plants m^{-2} for Experiment I and 3.5 plants m^{-2} for Experiments II and III. Immediately after planting the experimental fields were inundated by 10–20 cm to reduce desiccation damage and repress the growth of competitive plants. In Experiment I and in the first year of Experiments II and III (2016) no additional nutrients were applied. During winter, all plants were mowed at 5–10 cm above water level using a brush cutter and cut biomass was either removed (Experiment I) or left in place (Experiments II and III). In May 2017, Experiments II and III received applications of 150 kg ha^{-1} N in the form of coated urea and 150 kg ha^{-1} K as coated potassium nitrate (Ekompany, the Netherlands). The N and K loads were based on observed N:P and N:K ratios in growing *T. latifolia* biomass (Geurts *et al*. 2017, Vroom *et al*. 2018) to simulate nutrient supply from farm runoff and to stimulate plant P uptake.

Experimental setup and sample collection

Experiment I consisted of a field of 60 m^2, in which ten different 0.5 × 0.5 m plots were randomly assigned to five different harvesting dates (31 May, 07 July, 03 August, 15 September and 28 October 2016) (Figure 1). At each harvest, two plots were harvested and a subsample of fresh biomass per plot was taken for further analyses.

Experiments II and III were performed on a field of 61.4 m^2 which was split into 24 equal plots of 1.6 × 1.6 m. For Experiment II, 22 plots in total were used to determine the effect of harvest date on biomass yield and nutritional value (Figure 2; plots A–I). Experiment II comprised eight different harvest dates (19 May, 02 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017) which were randomly assigned to the 22 plots. At 19 May, eight plots (combined use with Experiment III) were used; and at the subsequent seven other harvest dates, two plots per harvest date were used.

For Experiment III, in total eight plots were used to determine the effect of a 3-week or 6-week harvest interval (Figure 2; plots A and B, four plots per harvest interval). Harvest dates were 19 May, 02 June (only 3-week harvest interval), 30 June, 21 July (only 3-week harvest interval), 11 August, 01 September (only 3-week harvest interval) and 22 September 2017. Treatments were randomly assigned to the plots. At each harvest, the number of shoots and flowers were counted. Thereafter, a bamboo stick was placed in the middle of each plot and plants were harvested from a circle of radius 50 cm centred on the stick, resulting in a harvested surface of 0.79 m^2. After the harvest, fresh biomass was weighed and subsamples of all plots were taken for dry matter (DM) analyses. In Experiment II all subsamples and in Experiment III two randomly selected subsamples per harvest date and scheme (n = 7 and n = 4 for the 3-week and 6-week harvest interval schemes, respectively) were taken for further nutrient and

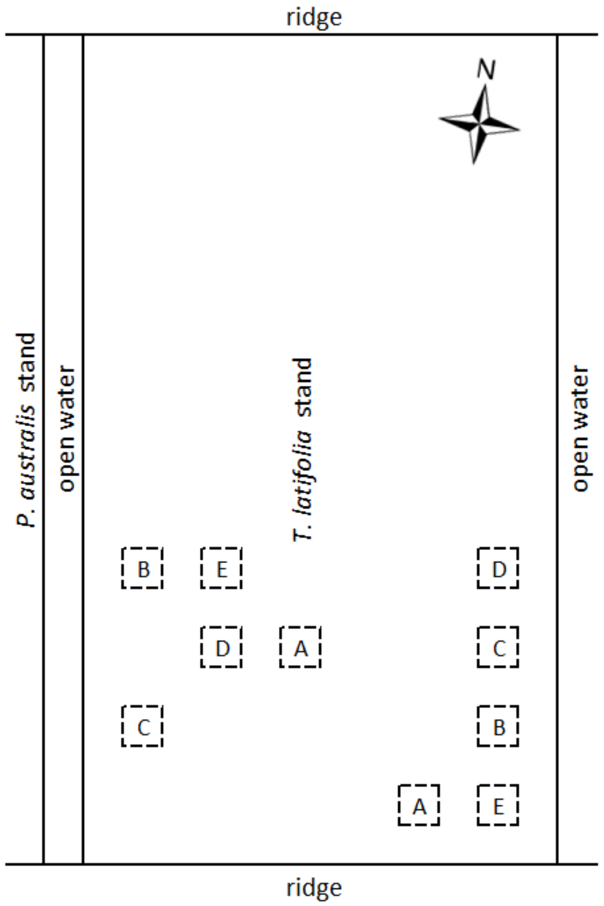

Figure 1. Experiment I. Schematic overview of the experimental field (6 × 10 m) used for harvesting *T. latifolia* biomass. The field was surrounded by soil ridges at north and south sides and by open water (≥ 0.75 m) at west and east sides. Harvested plot surfaces were 0.25 m^2, and harvest dates were 31 May (A), 07 July (B), 03 August (C), 15 September (D) and 28 October 2016 (E)

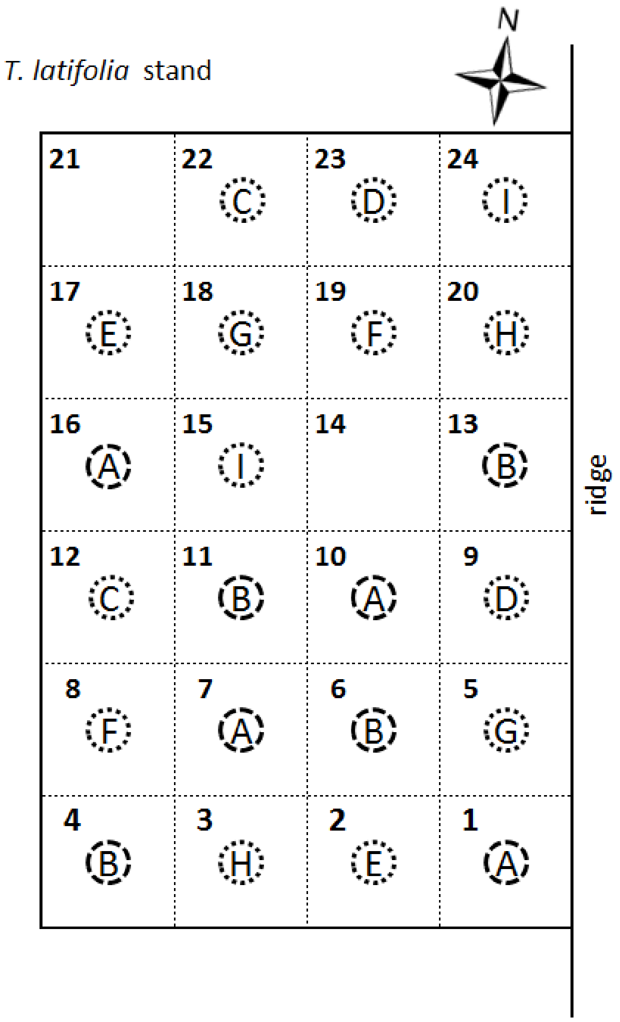

Figure 2. Experiment II and III. Schematic overview of experimental field (6.4 × 9.6 m) divided into 24 equal plots (1.6 × 1.6 m) used for harvesting *T. latifolia* biomass. The experimental field was a subfield (~ 0.35 ha) of a planted *T. latifolia* stand. *T. latifolia* plants surrounded the experimental field on the north, west and south sides. On the east side of the field there was a soil ridge. Harvested surfaces were circles of 0.79 m^2 per plot (0.5 m radius). Plots with letters A and B (dashed circles) were used for Experiments II and III. Plots with letters C–I were only used for Experiment II (dotted circles). Plants outside the harvested surfaces (circles) were not harvested during 2017. Two subplots (nos. 14 and 21) were not used for the experiments. Harvest dates were 19 May (A, B), 02 June (C), 09 June (A), 16 June (D), 30 June (A, B, E), 21 July (A, F), 11 August (A, B, G), 01 September (A, H) and 22 (A, B, I) September 2017.

mineral analyses. In all experiments, plants were harvested at 5–10 cm above the water level using secateurs.

In Experiment I, soil samples were taken in May 2015, June 2017 and September 2017. Ditch water samples were taken in July and November 2015, and four times in 2016 with a monthly interval from July 2016 onwards. In Experiments II and III, soil samples were taken in July 2017 at four sub-locations distributed over the field and were analysed separately. Ditch water samples were taken four times at monthly intervals from May 2017 onwards. Average results of soil and ditch water analyses are shown in Table 1. During the years of sample collection, 2016 (Experiment I) and 2017 (Experiments II and III), average temperatures from March to the end of October were equal to (Experiment I) or 0.5 °C higher than (Experiments II and III) the 20-year average. Total precipitation from March to the end of October were 37 mm (Experiment I) and 11 mm (Experiments II and III) lower than the 20-year average (de Bilt, KNMI, Table 1).

Analytical methods used for evaluation of nutritional values

Nutritional value was evaluated following widely used methods and component analyses for nutritional evaluation of forage and determination of ruminant dietary requirements. These were:

1) the proximate analyses which include moisture, crude ash (ash), crude protein (CP), crude fibre (CF), crude fat, starch and sugars (CVB 2016, NRC 2001, Sauvant *et al*. 2004);

2) the fractions neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (Van Soest *et al*. 1991, CVB 2016, NRC 2001, Sauvant *et al*. 2004);

3) an *in vitro* organic matter digestibility (IVOMD) assay for forages (Tilley & Terry 1963); and

4) macro and micro mineral analyses of relevant minerals (CVB 2016).

Biomass samples from all experiments were analysed at Eurofins Agro (the Netherlands) for

Table 1. Soil, ditch water and weather averages (± standard deviations) determined at the sites used for Experiments I and II + III.

	Experiment I	Experiments II and III
Soil		
Soil organic matter (%)	47 ± 0.1	75 ± 1.2
Total C (g kg^{-1})	240 ± 0.2	397 ± 0.6
Total N (g kg^{-1})	20 ± 0.4	24 ± 0.4
Total P (g kg^{-1})	1.8 ± 0.24	0.5 ± 0.15
Total K (g kg^{-1})	3.2 ± 1.94	1.8 ± 0.35
pH-NaCl	5.3 ± 0.0	4.1 ± 0.2
Ditch water		
NO_3-N (mg L^{-1})	0.20 ± 0.31	0.07 ± 0.07
NH_4-N (mg L^{-1})	0.27 ± 0.13	2.10 ± 2.00
P (mg L^{-1})	0.28 ± 0.21	0.09 ± 0.01
K (mg L^{-1})	7.6 ± 2.4	13.3 ± 1.7
Weather		
Mean temperature March–October °C, year of measurement	13.5	14.0
Precipitation March–October mm, year of measurement	545	571
Mean temperature March–October °C, 1998–2017	13.5 ± 0.5	
Precipitation March–October mm, 1998–2017	582 ± 116	

moisture content (oven drying at 70 °C for 24 h), ash (NEN-ISO 5984), CP (determined as total N × 6.25; NEN-ISO 5983-2, Kjeldahl method), CF (NEN-EN-ISO 6865) and IVOMD. Dry matter (DM) was calculated by subtracting the moisture content from the total sample weight, organic matter (OM) was calculated by subtracting ash from DM, and consequently the quantity of *in vitro* digested organic matter (IVDOM) was calculated using the IVOMD. Samples from Experiment I were also analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) (Van Soest *et al*. 1991) and samples of the first two harvest dates were analysed for crude fat (NEN-ISO 6492), starch (NEN-EN-ISO 15914) and sugars (NEN-ISO 3571).

Samples from Experiments II and III were analysed for P, Na, K, S, Ca, Mg, Mn, Cu, Zn, Fe, I, Mo (Experiment II only), Co (Experiment II only) and Se at Radboud University Nijmegen (Netherlands). Several grams of oven-dried biomass (24 h at 70 °C) were incinerated at 550 °C for four hours in a muffle furnace (Nabertherm GmbH, Lilientahl, Germany) to obtain the crude ash fraction. This remaining fraction of the oven-dried biomass was ground in a ball mill for 4 min at 400 RPM (Fritsch Pulverisette Ball Mill, Fritsch GmbH, Idar-Oberstein, Germany). Total phosphorous and total potassium contents were determined by digesting 200 mg soil in 4 mL HNO_3 (65 %) and 1 mL H_2O_2 (35 %) in Teflon vessels, heated in an Ethos D microwave (Milestone, Sorisole Lombardy, Italy). Subsequently, inductively coupled plasma emission spectrometry (ICP-OES) was used to measure P, Na, K, S, Ca, Mg, Mn and Fe (IRIS Intrepid II, Thermo Electron corporation, Franklin, MA, USA). Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure Co, Cu, Zn, Se, Mo and I (X-series I, Thermo Electron, Bremen, Germany).

Analysis of data

In Experiment III cumulative (c) yields (cDM, cOM, cCP, etc.) were calculated by accumulating yields of subsequent harvests. Development of biomass yields, contents (Experiments I and II) and cumulative biomass yields (Experiment III) over time were approximated by fitting a linear, quadratic or logistic function with harvest day as dependent variable, using stepwise regression. Model selection was based on lowest Akaike Information Criterion (AIC). When a quadratic and a logistic term were both included in the model according to the lowest AIC, a maximum model with either a quadratic or a logistic term was chosen based on the highest adjusted r^2. Model response variates were biomass content or yield parameters and harvest day in which 31 May 2016 for Experiment I, and 19 May 2017 for Experiments II and III were considered as day 1. For Experiment III, effect of harvest frequency was included as a fixed factor in the model with two levels (3- or 6-weekly harvest interval), and model coefficients were analysed for statistical differences between harvest frequency using ANOVA. All analyses were performed in R (version 3.4.0, R Core Team 2017) using the functions ‘lm’, ‘step’ and ‘anova’ (using package stats version 3.4.0).

RESULTS

Experiments I and II: effect of harvest date on biomass yields and nutritional values

Average shoot densities were 68 ± 1.9 m^{-2} and 40 ± 1.9 shoots m^{-2} in Experiments I and II, respectively, and on average 16 % and 43 % of the shoots produced an inflorescence in Experiments I and II, respectively. In both experiments, lowest observed biomass DM yields were observed at the first harvest (5.20 Mg ha^{-1} for Experiment I at 31 May and 2.89 Mg ha^{-1} for Experiment II at 19 May). Peak DM yields were observed at 15 September (9.81 Mg ha^{-1}, Experiment I) and 30 June (10.89 Mg ha^{-1}, Experiment II), while later in the season observed DM yields were lower (7.39 Mg ha^{-1} for Experiment I at 28 October and 7.30 Mg ha^{-1} for Experiment II at 22 September) (Figure 3, Table 2).

In both experiments harvested biomass showed an increase in DM, OM and CF, and a decrease in IVDOM and CP, over time; with highest IVDOM and CP, and lowest CF, at the first harvest and the greatest nutritional value changes between the first and the second harvests (Figure 3, Table 2). In both experiments, the DM increase was approximated by a logistic function (r^2 adj. 0.26 and 0.99 for Experiments I and II, respectively), with the difference that in Experiment I average DM contents at 31 May and 07 July were similar while in Experiment II the DM content of wet biomass increased during that part of the season (86 g kg^{-1} increase between 02 June and 21 July). Dry biomass OM content increases (r^2 adj. 0.71 and 0.85 for Experiments I and II, respectively) and CP decreases (r^2 adj. 0.74 and 0.96 for Experiments I and II, respectively) over time were approximated by logistic functions because of the relatively strong OM increase and strong CP decrease between the first two harvest dates. In both experiments, CP decreased on average from 125 to 75 g kg^{-1} between the first two harvest dates. The IVDOM decrease and CF increase over time were approximated by linear curves for Experiment I (r^2 adj. 0.86 in both cases) and logistic

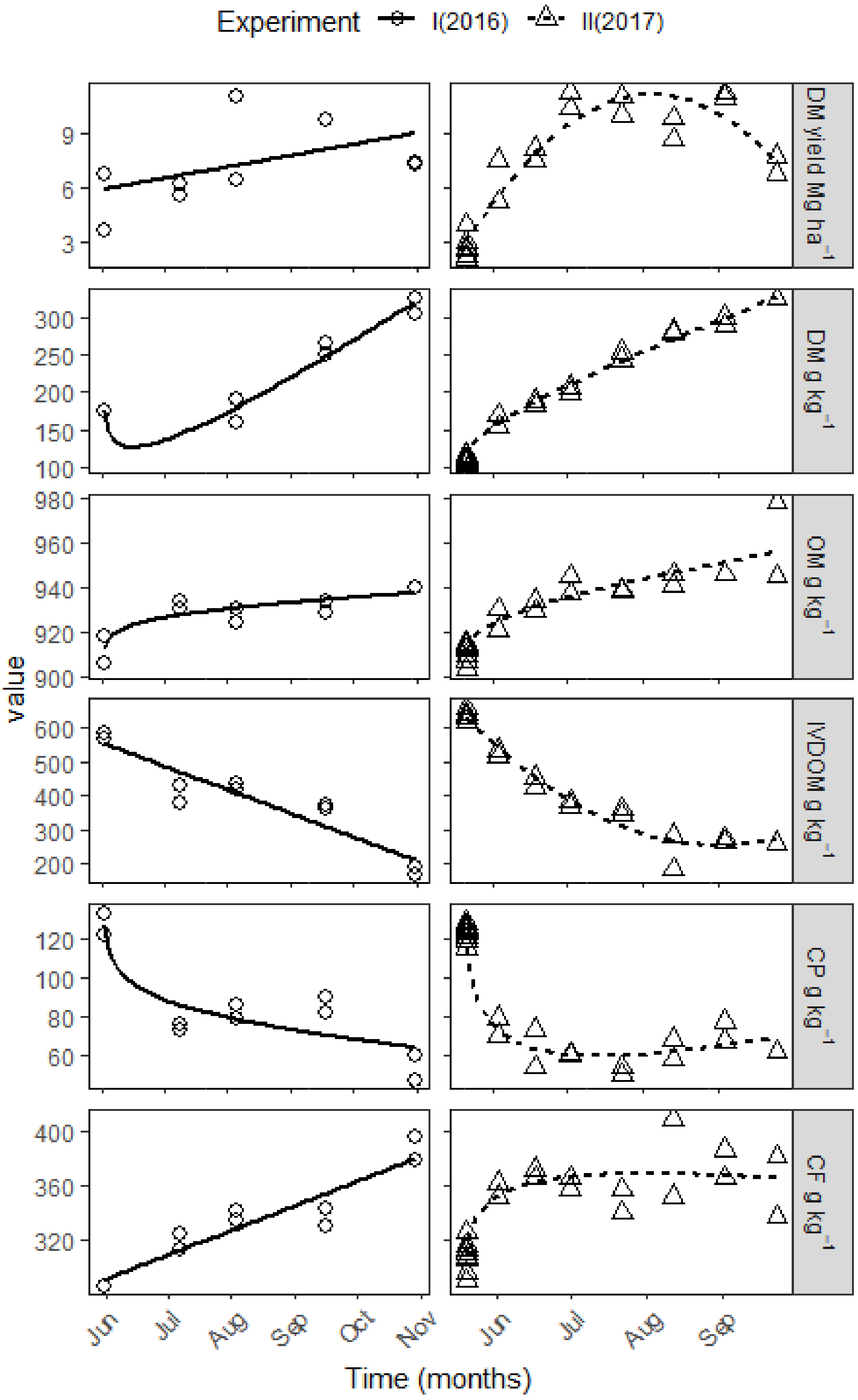

Figure 3. Experiments I and II: Effect of harvest day on dry matter (DM) yield and biomass DM, and on the dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) in *T. latifolia* biomass one year after planting. Harvest dates were 31 May, 07 July, 03 August, 15 September and 28 October 2016 for Experiment I and 19 May, 09 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017 for Experiment II. Each set of points per harvest date ($n \geq 2$) represents the nutrient concentrations of biomass harvested for a first time in the growing season. In Experiment I DM contents of the 07 July harvest were not determined. Fit parameters of the curves are shown in Table 2.

Table 2. Experiments I and II: Effect of harvest day on dry matter (DM) yield and DM, and on the dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) in *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) function $y = intercept + harvest\ date + harvest\ date^2 + log(harvest\ date) + error$, in which *y* represents DM yield or biomass content variables and *harvest date* the number of days after the first harvest date (31 May for Experiment I, 19 May for Experiment II). SE = standard error.

	Experiment	Intercept		Harvest date		Harvest date^2		log(Harvest date)		Adj. r^2
		Value	SE	Value	SE	Value	SE	Value	SE	
DM yield (Mg ha^{-1})	I	5.90	1.11	0.021	0.012					0.26
	II	2.87	0.38	0.219	0.019	-1.44×10^{-3}	1.61×10^{-4}			0.90
DM (g kg^{-1})	I	172.9	11.2	1.88	0.24			-27.35	6.60	0.96
	II	108.4	2.4	1.21	0.07			13.43	1.57	0.99
OM (g kg^{-1})	I	912.4	3.9	0.04	0.06			3.72	1.82	0.71
	II	910.6	2.5	0.18	0.07			4.68	1.60	0.85
IVDOM (g kg^{-1})	I	554.6	27.3	-2.28	0.30					0.86
	II	634.9	9.7	-7.35	0.52	3.54×10^{-2}	4.61×10^{-3}			0.97
CP (g kg^{-1})	I	126.3	9.2	-0.06	0.15			-10.59	4.28	0.74
	II	122.4	2.2	0.38	0.07			-20.94	1.49	0.96
CF (g kg^{-1})	I	290.8	6.9	0.59	0.08					0.86
	II	309.1	5.9	-0.24	0.17			18.00	3.78	0.72

curves for Experiment II (r^2 adj. 0.97 and 0.72 for IVDOM and CF, respectively).

The IVOMD decreased from 63 % to 19 % in Experiment I between 31 May and 28 October (Table 3) and from 69 % to 28 % in Experiment II between 19 May and 22 September. In Experiment I, at the first two harvests (31 May and 07 July), the crude fat content was on average 20 g kg^{-1}, the starch content was on average 21 g kg^{-1} and the sugar content was on average 64 g kg^{-1} (dry mass basis in all cases). Moreover, in Experiment I, the dry mass NDF, ADF and ADL contents increased between the first and last harvests. NDF, ADF and ADL averages ranged from 653 to 742, 344 to 512 and 52 to 93 g kg^{-1}, respectively (Table 3).

In Experiment II P, K and S contents versus harvest date were approximated linearly (r^2 adj. 0.20, 0.67 and 0.27, respectively) with the highest concentrations at the first harvest date (Figure 4, Table 4). Ca, Mg, Mn, I and Se contents versus harvest date were approximated by a logistic function (r^2 adj. 0.39, 0.37, 0.09, 0.32 and 0.25, respectively), all with the highest approximated content in early June, around the time the inflorescences appeared. Fe content was lowest around the fourth harvest (30 June), and Fe content versus harvest date was approximated with a quadratic curve (r^2 adj. 0.23). For Na, Cu, Zn, Mo and Co contents versus harvest date no specific relationship seemed to be present.

Experiment III: effect of harvest frequency on *T. latifolia* stand development, biomass yields and nutritional values

Frequent cutting apparently prevented the plants from producing inflorescences in Experiment III. On average, the observed number of shoots decreased during the experiment from 29 to 15 shoots m^{-2} at the 3-week harvest interval and 35 to 26 shoots m^{-2} at the 6-week harvest interval. However, the developments of the number of shoots (Figure 5 and Table 5) appeared difficult to approximate by linear functions (r^2 adj. 0.29). According to the linear models, the number of shoots differed significantly at the first harvest date (19 May) ($p = 0.003$) but the approximated linear decrease did not differ significantly between the 3-week and 6-week harvest intervals ($p = 0.711$). Average cumulative DM yields did not differ significantly ($p = 0.190$, $n = 4$ per harvest interval) and were 5.16 ± 0.57 Mg ha^{-1} (3-week harvest interval) and 6.51 ± 0.71 Mg ha^{-1} (6-week harvest interval). DM yields per cut and harvest interval decreased between 19 May and 22 September from 2.97 Mg ha^{-1} to 0.09 Mg ha^{-1} at the 3-week harvest interval and from 2.80 Mg ha^{-1} to 0.43 Mg ha^{-1} at the 6-week harvest interval.

The 6-weekly harvesting frequency resulted in a faster increase of cCF yields ($p = 0.015$ and $p = 0.032$ for the linear and quadratic effects, respectively) and a tendency towards a faster increase of cOM yield

Table 3. Experiment I: Effect of harvest day on *in vitro* organic matter digestibility (IVOMD), and the dry matter contents of crude fat (Cfat), sugars, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in *T. latifolia* biomass one year after planting. Each figure represents measurements from two different subplots, harvested for the first time in the growing season (2016). DM = dry matter; ND = not determined.

Harvest date	IVOMD (%)	Cfat (g kg^{-1})	Sugars (g kg^{-1})	Starch (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})	ADL (g kg^{-1})
31 May	63.1	21	57	10	653	344	52
07 July	43.3	18	71	32	655	396	58
03 August	45.7	ND	ND	ND	663	422	69
15 September	39.3	ND	ND	ND	676	438	67
28 October	19.0	ND	ND	ND	742	512	93

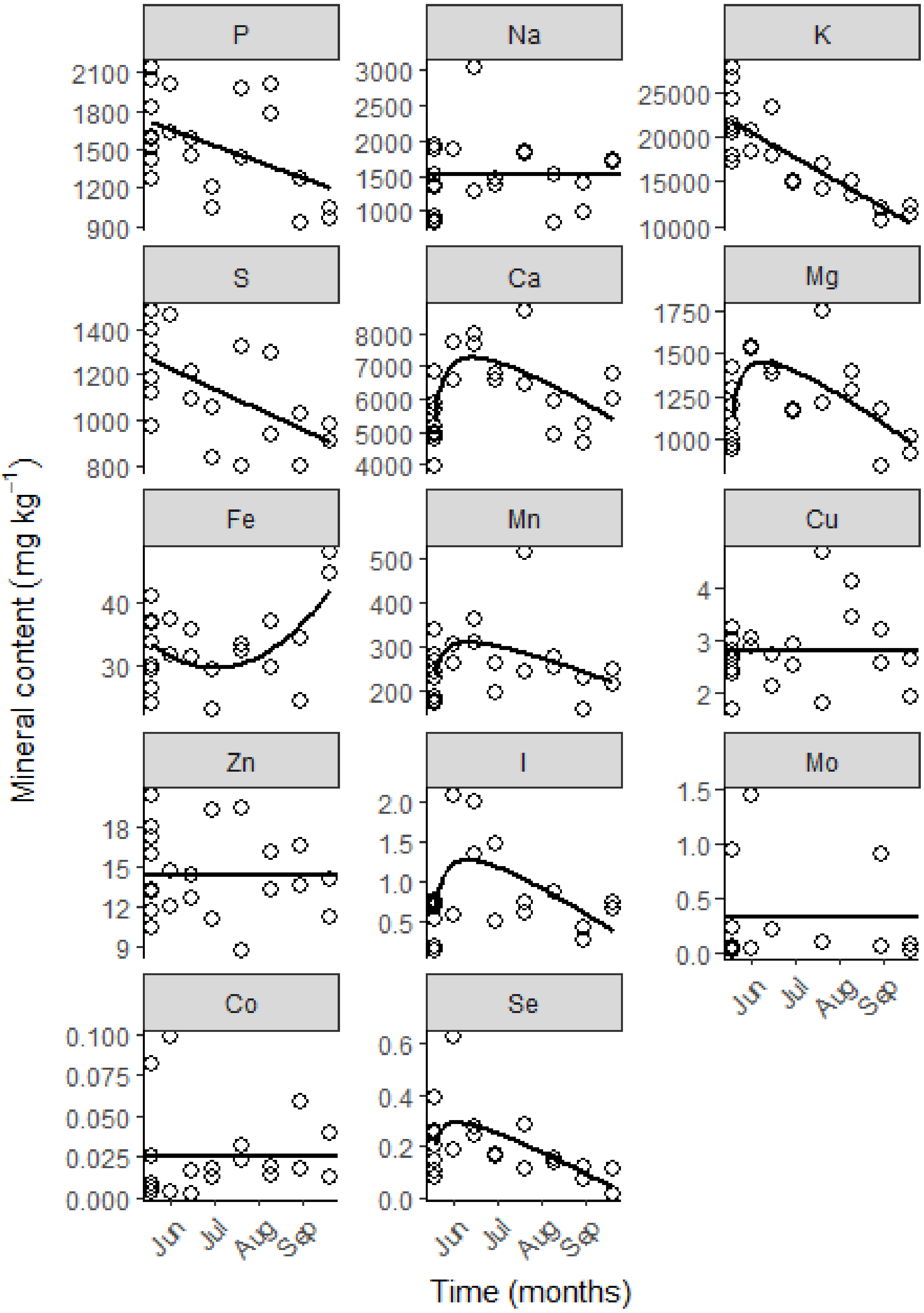

Figure 4. Experiment II: Effect of harvest day on the dry matter contents of minerals in *T. latifolia* biomass one year after planting. Harvest dates were 19 May, 02 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Each set of points per harvest date (n ≥ 2) represents biomass mineral concentrations from two different subplots, harvested for the first time in the growing season. Fit parameters of the curves are shown in Table 4.

Table 4. Experiment II: Effect of harvest day on dry matter mineral contents of *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) models $y = intercept + harvest\ day + harvest\ day^2 + error$ or $y = intercept + harvest\ day + log(harvest\ day) + error$, in which y represents biomass concentration and *harvest day* the harvest date where the 19 May 2017 was set as day 1. SE = standard error.

	Intercept		Harvest day		Harvest day ^{2}		log(Harvest day)		r^2 adj.
	Value	SE	Value	SE	Value	SE	Value	SE	
P (mg kg^{-1})	1705	99	-4.00	1.61					0.20
Na (mg kg^{-1})	1517	106							
K (mg kg^{-1})	21693	832	-89.70	13.53					0.67
S (mg kg^{-1})	1262	59	-2.84	0.96					0.27
Ca (mg kg^{-1})	5330	335	-31.78	9.78			842.23	215.09	0.39
Mg (mg kg^{-1})	1132	65	-7.05	1.91			152.34	42.05	0.37
Fe (mg kg^{-1})	33.3	1.9	-0.171	0.097	1.89×10^{-3}	8.18×10^{-4}			0.23
Mn (mg kg^{-1})	237.8	25.7	-1.419	0.752			33.36	16.53	0.09
Cu (mg kg^{-1})	2.8	0.1							
Zn (mg kg^{-1})	14.4	0.7							
I (mg kg^{-1})	0.6	0.1	-0.014	0.004			0.318	0.094	0.32
Mo (mg kg^{-1})	3.22×10^{-1}	1.29×10^{-1}							
Co (mg kg^{-1})	2.60×10^{-2}	6.06×10^{-3}							
Se (mg kg^{-1})	2.09×10^{-1}	3.80×10^{-2}	-3.12×10^{-3}	1.11×10^{-3}			4.78×10^{-2}	2.44×10^{-2}	0.25

($p = 0.072$ for the linear slope coefficient of cOM) compared to the 3-weekly harvesting frequency. Harvesting frequency did not significantly affect the development of cDM, cOM, cIVDOM and cCP yields, since there were no significant differences between the slope coefficients of the different harvesting frequencies. When comparing the average IVDOM, CP and CF contents of biomass harvested after May to biomass harvested in May, the nutritional value of biomass harvested at 3-week intervals remained similar to the nutritional quality of spring biomass harvested in May while biomass harvested at 6-week intervals had lower IVDOM ($p < 0.001$) contents (Table 6). DM contents of biomass harvested after May was higher at both harvest frequencies compared to the harvest at 19 May ($p < 0.001$).

Harvesting frequency did not significantly affect the development of cumulative mineral yields over time. However, for cSe a tendency towards a faster yield increase for the 6-weekly compared to the 3-weekly harvesting frequency was observed (Figure 6 and Table 7). Harvesting frequency had a significant effect on the intercept of the approximated yield developments for cNa ($p = 0.002$), cMg ($p = 0.039$), cCu ($p = 0.015$), cI ($p = 0.037$) and cSe ($p = 0.001$).

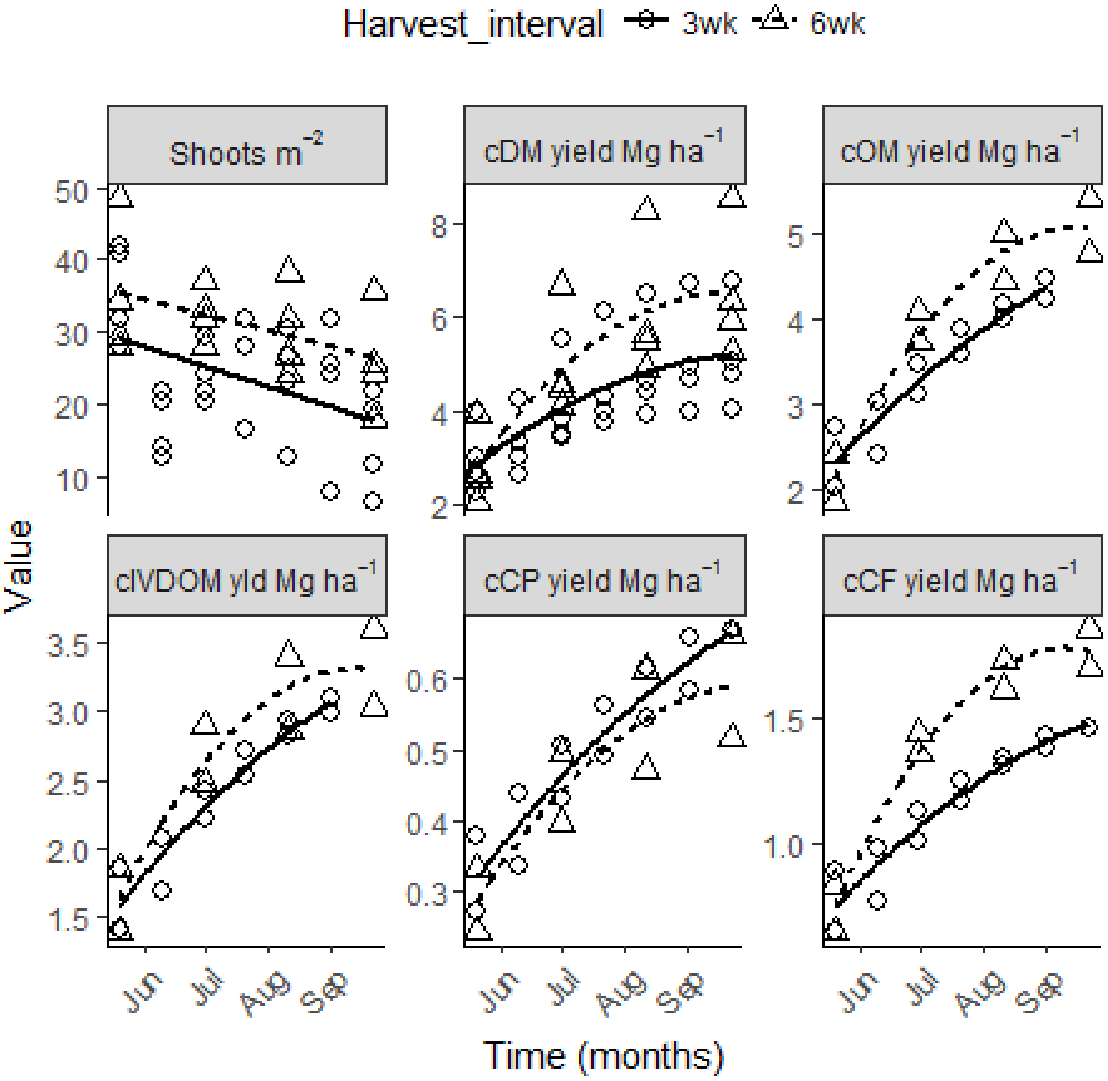

Figure 5. Experiment III: Effect of harvesting frequency (3-week or 6-week intervals) on shoot density and cumulative dry matter (DM), organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) yields of *T. latifolia* biomass contents one year after planting. Harvest dates were 19 May, 02 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Fit parameters of the curves are shown in Table 5.

Table 5. Experiment III: Effect of harvesting frequency (3-weekly or 6-weekly) on shoot density and cumulative (c) dry matter (DM), organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) yields of *T. latifolia* biomass contents one year after planting. Fitted parameters to the (maximum) model $y = \mathit{intercept} \times \mathit{harvest\ interval} + \mathit{harvest\ day} \times \mathit{harvest\ interval} + \mathit{harvest\ day}^2 \times \mathit{harvest\ interval} + \mathit{error}$, in which y represents the biomass yield, *harvest interval* is 3 weeks or 6 weeks and *harvest day* is number of days after 19 May 2017 (set as day 1). SE = standard error.

	Intercept					Harvest day					Harvest day^2					
Harvest interval	3 weeks		6 weeks			3 weeks		6 weeks		*p*-value	3 weeks		6 weeks		*p*-value	r^2 adj.
	Value	SE	Value	SE	*p*-value	Value	SE	Value	SE		Value	SE	Value	SE		
Shoot density (m^{-2})	29.1	2.6	8.9	4.1	0.003	-0.091	0.034	0.054	0.053	0.711						0.29
cDM (Mg ha^{-1})	2.702	0.414	0.613	0.556	0.016	4.06×10^{-2}	1.30×10^{-2}	2.36×10^{-2}	7.09×10^{-3}	0.141	-1.73×10^{-4}	9.48×10^{-5}	-1.73×10^{-4}	9.48×10^{-5}		0.54
cOM (Mg ha^{-1})	2.315	0.195	2.117	0.285	0.001	2.49×10^{-2}	8.58×10^{-3}	4.86×10^{-2}	1.16×10^{-2}	0.072	-5.11×10^{-5}	7.71×10^{-5}	-2.00×10^{-4}	9.70×10^{-5}	0.147	0.91
cIVDOM (Mg ha^{-1})	1.492	0.133	1.666	0.183	0.014	2.42×10^{-2}	4.35×10^{-3}	2.52×10^{-2}	2.66×10^{-3}	0.696	-9.46×10^{-5}	3.68×10^{-5}				0.87
cCP (Mg ha^{-1})	0.310	0.033	0.292	0.044	0.223	4.18×10^{-3}	1.03×10^{-3}	3.86×10^{-3}	5.84×10^{-4}	0.587	-1.16×10^{-5}	7.90×10^{-6}	-1.16×10^{-5}	7.90×10^{-6}		0.80
cCF (Mg ha^{-1})	0.739	0.055	0.733	0.081	< 0.001	8.63×10^{-3}	2.09×10^{-3}	1.81×10^{-2}	3.09×10^{-3}	0.015	-2.21×10^{-5}	1.66×10^{-5}	-7.84×10^{-5}	2.38×10^{-5}	0.032	0.94

Table 6. Experiment III: Dry matter (DM) yield, and dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) for the May harvest (Experiment II) and average contents of harvested biomass from the 3-weekly (6 cuts) and 6-weekly (3 cuts) subsequent harvests. SEM = standard error of the mean.

	19 May harvest (first cut)	3-week harvest intervals, 08 June to 22 September	6-week harvest intervals, 30 June to 22 September	SEM	*p*-value
DM (g kg^{-1})	112^{c}	153^{b}	195^{a}	7.9	<0.001
OM (g kg^{-1})	911^{ab}	901^{a}	917^{b}	2.6	0.020
IVDOM (g kg^{-1})	624^{a}	591^{a}	466^{b}	16.3	<0.001
CP (g kg^{-1})	123^{ab}	134^{a}	103^{b}	5.1	0.023
CF (g kg^{-1})	304	286	303	5.7	0.328

^{abc} Values with an unequal superscript differ significantly ($P \leq 0.05$)

DISCUSSION

Effect of harvest date on yields and nutritional values

We hypothesised that biomass yields would peak between the middle and end of the growing season and that, as in (other) grasses, the nutritional value of *T. latifolia* would decrease with increasing crop age, with a sharp decrease before onset of flowering. These hypotheses were confirmed in Experiments I and II. The IVOMD of *T. latifolia* was, especially from June onwards, low (on average < 50 %). Thus, for optimal forage quality *T. latifolia* biomass should be harvested in May, before the appearance of inflorescences and the onset of nutrient translocation to below-ground biomass as known for other perennial grasses such as *Lolium perenne* (Parsons 1988). From June onwards we observed a decrease of 42 and 50 % of CP content in Experiments I and II, respectively. Similar results were reported by Grosshans (2014) who observed that due to translocation the content of nutrients in the shoot tissue can decrease by more than 50 % in *Typha glauca*, and by Maddison *et al.* (2009) who found that N and P were translocated from shoots to reserve organs (mainly rhizomes but also inflorescences) after the flowering stage. Maddison *et al.* (2009) measured, at a water N load of 138 kg ha^{-1} year^{-1}, dry matter CP contents between 79 and 151 g kg^{-1} during the growing season, which corresponds well to our results from Experiments I and II (CP content of 75 to 125 g kg^{-1}). In our Experiments I and II we observed similar CP content decreases despite a fertiliser N amendment and higher soil and ditch water N contents at the site of Experiment II compared to Experiment I. It is possible that plant N uptake or growth was limited by other factors since we observed very similar CP levels in both experiments.

Apparently, 0.5 °C lower average temperatures during the growing season, lower N concentrations in soil and ditch water (Table 1), no N and K amendments and biomass removal in the year of planting in Experiment I compared to Experiment II had limited effects on the biomass yield and contents in the consequent year, since we observed similar peak DM yields (9.81–10.89 Mg ha^{-1}) and developments of OM, IVDOM, CP and CF in Experiments I and II, although peak yields were in September for Experiment I and in June for Experiment II. Biomass nutrient removal from the site could have significantly affected nutrient cycling (Jordan *et al*. 1990). Possibly, the higher average plant density (and thus lower yield per shoot) in Experiment I (68 ± 1.9 shoots m^{-2}) compared to Experiment II (40 ± 1.9 shoots m^{-2}) compensated the yield per ha.

DM yields in our study could have been limited because the stand was not fully developed, despite the observed great increases in shoot densities (increase from 15 to 68 in Experiment I and from 3.5 to 40 shoots m^{-2} between the first year of planting and the second year of measurements). Possibly, plants

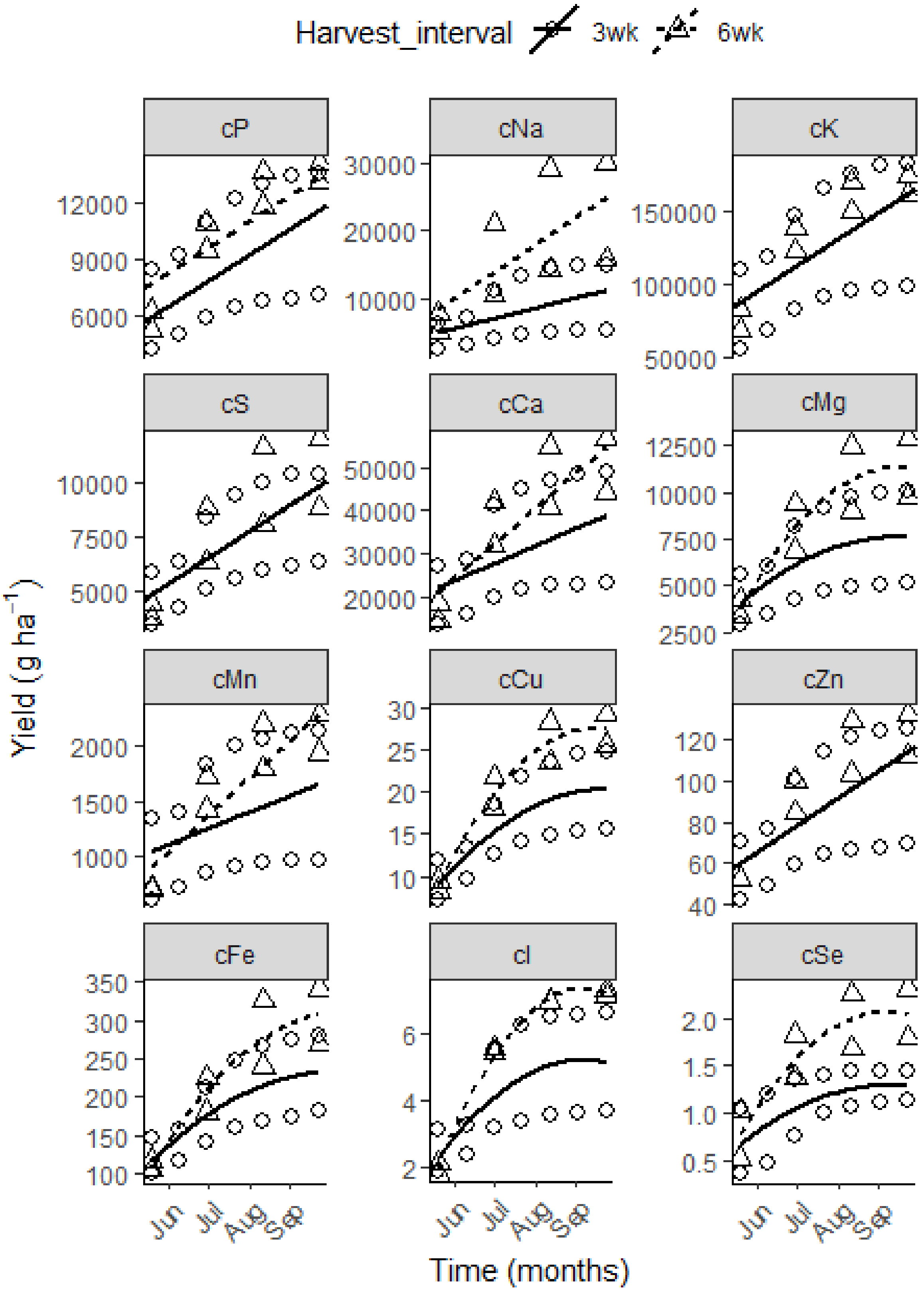

Figure 6. Experiment III: Effect of harvest interval (3-week or 6-week intervals) on cumulative (c) mineral yields of *T. latifolia* biomass one year after planting. Harvest dates were 19 May, 02 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Each set of points per harvest date (n = 2) represents biomass nutrient concentrations from two different subplots. Fit parameters of the curves are shown in Table 7. Cumulative yields of K, S and Zn were approximated by (nearly) the same curve for both harvest intervals, therefore only the fitted curve for the 3-week interval is visible for these minerals.

Table 7. Experiment III: Effect of harvest interval (3 weeks or 6 weeks) on cumulative (c) mineral yields of *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) model $y = intercept \times harvest\ interval + harvest\ day \times harvest\ interval + harvest\ day^2 \times harvest\ interval + error$ or $y = intercept \times harvest\ interval + harvest\ day \times harvest\ interval + \log(harvest\ day) \times harvest\ interval + error$, in which *y* represents biomass yield variables, *harvest interval* the harvesting interval (3-week or 6-week interval) and *harvest day* the number of days after the first harvest on 19 May 2017. SE = standard error.

	Intercept					Harvest day					Harvest day^2		
Harvest interval	3 weeks		6 weeks			3 weeks		6 weeks			3 and 6 weeks		
	Value	SE	Value	SE	*p*-value	Value	SE	Value	SE	*p*-value	Value	SE	r^2 adj.
cP (g ha^{-1})	5918	1069	7698	1156	0.140	45.4	12.7						0.39
cNa (g ha^{-1})	4689	2659	8113	4209	0.002	50.5	34.7	133.0	53.8	0.142			0.52
cK (g ha^{-1})	86694	12596				592.4	162.3						0.37
cS (g ha^{-1})	4855	724				39.3	9.3						0.44
cCa (g ha^{-1})	17847	6046	17506	8116	0.127	363.8	189.3	498.3	103.5	0.211			0.43
cMg (g ha^{-1})	3725	1230	3959	1652	0.039	75.2	38.5	107.1	21.1	0.148	-0.37	0.28	0.50
cMn (g ha^{-1})	1040.4	231.1	894.5	365.7	0.257	4.79	3.02	10.92	4.68	0.207			0.33
cCu (g ha^{-1})	8.2	2.1	9.3	2.8	0.015	0.22	0.06	0.27	0.04	0.124	-1.00×10^{-3}	4.74×10^{-4}	0.71
cZn (g ha^{-1})	59.6	8.6	60.0	0.1	0.195	0.43	0.11						0.42
cFe (g ha^{-1})	110.2	24.9	109.8	33.4	0.061	1.91	0.78	1.90	0.01	0.140	0.659	0.426	0.64
cI (g ha^{-1})	2.0	0.7	2.1	0.9	0.037	0.07	0.02	0.08	0.01	0.122	-3.42×10^{-4}	1.52×10^{-4}	0.64
cSe (g ha^{-1})	0.603	0.173	0.814	0.232	0.001	1.42×10^{-2}	5.41×10^{-3}	1.94×10^{-2}	2.96×10^{-3}	0.097	-7.27×10^{-5}	3.96×10^{-5}	0.66

put most of their resources into offspring when competition is limited. Fiala (1978) observed that each *T. latifolia* plant can produce up to 46 new shoots in the first year after planting when there was no competition. Heinz (2012) reported summer dry matter (DM) yields of up to 12 Mg ha^{-1} in the second year after establishment and Pfadenhauer & Wild (2001), who planted *T. latifolia* at a density of 0.5–2 plants m^{-2} in 1998, observed average increases to 75 and 102 shoots m^{-2} in the growing seasons of 1999 and 2000, respectively, with corresponding DM yield increases to about 8.9 Mg ha^{-1} in 1999 and 20.1 Mg ha^{-1} in 2000. Furthermore, observed yields might have been affected by (unknown) inter-annual variations. Maddison *et al*. (2009), Heinz (2012) and Günther *et al*. (2015) reported inter-annual variations of 2–8 Mg ha^{-1} DM yield in different *T. latifolia* stands, which only in some of the cases could be related to events such as late winter or spring harvests before the growing season, storms and spatial variations in stand densities. Thus, to determine the effects of increased stand ages and inter-annual variations on the sustainability of biomass production potential and biomass contents of *T. latifolia* in any particular situation, the current work should be considered as tentative and long-term effects need further investigation.

Effect of harvest frequency on yields and nutritional values

We hypothesised that an increased harvesting frequency (as in a situation with grazing) may maintain nutritional value but reduce productivity and shoot density during a growing season. The reduction of productivity is not confirmed by the results of Experiment III because the results showed that *T. latifolia* can be harvested repeatedly and intensively (at intervals of either 3 or 6 weeks) during a growing season and still produce biomass after each harvest. However, frequent harvesting seemed to have adverse effects on shoot density, which decreased over time, and this may influence productivity in the years to come. Also, DM yields decreased during the season and cumulative DM yields in Experiment III were lower than the yield from a single harvest per growing season when harvested in mid-June or later in the season (Experiments I and II), which may have been related to the decreasing shoot density. Therefore, the effects of frequent harvesting and grazing on biomass production in subsequent years need further investigation as our observations are limited to one growing season. Possibly, harvesting only two or three times instead of four or seven times during a growing season could result in an improved balance between yields, fodder quality, plant regrowth and stand development. Our observation that *T. latifolia* regrowth persisted during the most intensive harvesting scheme is in line with the findings of Hellsten *et al*. (1999) and Lishawa *et al*. (2017), who studied effects of mowing *Typha spp.* underwater with the objective of plant removal and concluded that above-water biomass removal is not an efficient strategy to completely remove natural stands.

The average fodder quality of cumulatively harvested biomass, when defined as highest IVDOM and CP and lowest CF contents, was very similar to that of a single harvest in May, especially for biomass harvested at 3-week intervals. This confirmed our hypothesis that intensive harvesting may lead to a rather stable nutritional value. Three-week harvest intervals led to a slightly better nutritional value compared to 6-weekly harvest intervals, which is similar to grasses such as *L. perenne* where more frequent harvesting also leads to improved fodder value (Parsons 1988).

General findings

We concluded that a harvest in July and August was optimal for yielding the greatest quantity of *T. latifolia* fodder. For the highest nutritional value, the optimal harvest was around May, before the appearance of inflorescences and at relatively low standing crop. The nutritional value of *T. latifolia* was inferior to fresh biomass from grassland dominated by *L. perenne* (Eurofins Agro 2017) when using dairy cow requirements (CVB 2016) as a quality standard, even when harvested before the appearance of inflorescences.

Furthermore, we found that the regrown biomass harvested at either 3-week or 6-week intervals after a first harvest in May can be nutritionally similar to biomass harvested in May. Thus, *T. latifolia* could potentially be grazed by adapted cattle (e.g. *Bubalus arnee*). However, cumulative DM yields were lower than a single yield per growing season when harvested in mid-June or later in the season. For the interpretation of our results, it is important to point out that the experiments described here were done at a relatively low nutrient availability and at a young stand age, which probably resulted in lower seasonal biomass DM yields than could be obtained from older or further developed *T. latifolia* stands with higher nutrient availabilities (Maddison *et al*. 2009, Pfadenhauer & Wild 2001, Heinz 2012).

Based on our results, we suggest that *T. latifolia* biomass harvested later in the season could be used as fibrous roughage at low dietary inclusion rates, whereas the biomass harvested before the appearance of inflorescences could be used at higher inclusion rates in grass based dairy rations. This introduces a promising prospect for lowering dietary N and P contents, which would allow the reduction of N and P losses to the environment from dairy farms on peat soils. Also, *T. latifolia* biomass appeared to have higher contents of Se, which is interesting because biomass from grassland dominated by *L. perenne* is typically deficient in Se in terms of dairy cow requirements (Table 8; CVB 2016, Eurofins Agro 2017).

To further unlock the potential of *T. latifolia* as a (complementary) forage in combination with (intensive) dairy farming on peat, further research is needed on aspects such as breeding, establishing and maintaining crops, managing optimal nutrient supplies, and optimising harvesting and conservation techniques. Furthermore, effects of dietary inclusion on animal performance and possible nutritional constraints should be investigated, as well as the impact on the economy and ecology of farms and surrounding regions.

Table 8. Average dry matter mineral contents in *T. latifolia* biomass Experiment II) and fresh grass from *L. perenne* dominated dairy grasslands in The Netherlands (Eurofins Agro 2017).

	T. latifolia biomass	*L. perenne* dominated fresh grass biomass
P (g kg^{-1})	1.7	4.2
Na (g kg^{-1})	1.3	2.3
K (g kg^{-1})	22.1	34.1
S (g kg^{-1})	1.2	3.5
Ca (g kg^{-1})	5.3	5.3
Mg (g kg^{-1})	1.1	2.4
Fe (mg kg^{-1})	32.2	169.0
Mn (mg kg^{-1})	237.8	73.0
Cu (mg kg^{-1})	2.6	8.4
Zn (mg kg^{-1})	15.0	40.0
I (mg kg^{-1})	0.6	0.2
Mo (µg kg^{-1})	263	1900
Co (µg kg^{-1})	25	67
Se (µg kg^{-1})	199	56

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