Article

Impact of Nitrogen Fertilization on Tuber Yield, Sugar Composition and Nitrogen Uptake of Two Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) Genotypes

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Abstract: The tuberous root crop, yacon, is native to the Andean region and contains high amounts of fructooligosaccharides (FOS) with up to 70% of dry matter. Due to FOS, consumption of tubers may have health-promoting effects. However, regarding the overall cultivation system, no recommendations exist for farmers on nitrogen fertilization and nitrogen (N) uptake of yacon. Therefore, three different N fertilization levels (0, 40, and 80 kg N ha⁻¹) and two genotypes (brown-shelled (BG) and red-shelled (RG)) were examined in a two-year field experiment regarding their tuber yield, sugar composition, and nitrogen uptake. Tuber yields increased with increasing fertilization level and were highest for B80 and R80 (50 and 67 t FM ha⁻¹), while significant differences between the genotypes existed. Sugar and the amount of FOS slightly decreased with increasing N fertilization level, and ranged between 36% and 73% and 30% and 58% of dry matter, respectively. An overall decreasing amount of FOS led to a slight increase in the amount of FOS with a higher degree of polymerization. Regarding the N-use efficiency of tubers and the total plant, an N fertilization level of 40 kg N ha⁻¹ seems to favor tuber yield.

Keywords: fructooligosaccharide; yacon; nitrogen content

1. Introduction

The tuberous root plant, yacon (*Smallanthus sonchifolius*) ((Poepp. and Endl.) H. Robinson) is native to the Andean region and related to the family of *Asteraceae*. Apart from the Andean region, yacon has also been cultivated in Japan, Brazil, New Zealand, Czech Republic, Italy, and Germany [1,2]. Yacon is a perennial, herbaceous plant, which is not frost-tolerant. The plant grows to be 2–2.5 m, and the generally arrow-shaped leaves are dark green [3,4]. The average tuber yield of each plant amounts to 2–3 kg, or up to 5 kg [5–7]. Each tuber weighs between 200 and 500 g on average, but can reach a weight of up to 2000 g. The dry-matter content commonly ranges between 10–14% [3,8]. The color of the peel and flesh of the tubers can vary from white, creamy, yellow, and orange red, to purple [9].

In its tuberous roots, yacon mainly stores carbohydrates in the form of fructans, and particularly fructooligosaccharides (FOS) and inulin. FOS and inulin account for up to 70% of dry matter. The main components of FOS are kestose, nystose, and fructofuranosylnystose [5]. These polysaccharides may not be digested by the human intestinal tract, wherefore no increase in blood glucose level occurs after the consumption of fresh yacon [10,11]. Other carbohydrates stored in the tubers are sucrose, and the monosaccharides fructose and glucose. The composition of stored carbohydrates depends on various factors, such as the origin, growing conditions, and farming system [5].

As the interest in yacon has increased over the last 2–3 years, due to its potential as a natural sugar replacement, a proper crop management and cultivation system needs to be developed in order to cover the increasing demand. Up until now, only very limited information on the nitrogen (N) fertilization requirement of yacon and the influence of N on the sugar composition of yacon tubers has been available. Furthermore, the influence of N fertilization on tuber yield formation has not yet been reported.

Nitrogen is one of the most important nutrients in plant growth due to its direct effect on vegetative growth and related impacts on leaf area and light interception [12]. The expected N requirement of yacon reported in the literature ranges from 24 to 98 kg N ha^{−1} [6,13–15]. These values are below the N requirement of sweet potatoes and potatoes, which require up to 200 kg N ha^{−1} [12,16], while simultaneously having lower yield potentials per ha. In order to determine yacons' need for N fertilization, it is critically important to measure the N uptake of different plant parts, and thus to determine complete N removal.

Additionally, the impact of the N-level of tuber yield formation and sugar content has not been clearly defined. However, the impact of N-level on the root yield of sugar beet has been well-investigated. Increasing amounts of N fertilizer could lead to higher tuber yields, but decreasing sugar content [17–19]. Furthermore, N concentration in vegetative parts increases with increasing amounts of N application [20].

Besides the impact of N on plant growth and development, an adjusted fertilization strategy is also needed due to economic, as well as environmental reasons [21,22]. In the case where the applied amount of N exceeds the N uptake of the plant, the risk of N losses in ground water rises [23]. Due to a lack of knowledge about N fertilizer requirements of yacon, it is assumed that the amounts which are currently being applied do not meet the actual demand of the plant.

The aims of the present study are therefore to investigate the effect of different N fertilization levels on tuber yield formation and sugar composition, and also to evaluate the N uptake of different plant parts (above and below ground) of two different yacon genotypes. Based on the obtained results, the N fertilization requirement of yacon can be identified, and a specific fertilization strategy can be developed. The results of the current study can be used to considerably improve the environmental performance of yacon cultivation.

2. Materials and Methods

2.1. Field Site and Experimental Design

In 2016 and 2017, field experiments were carried out at the experimental station, Ihinger Hof of the University of Hohenheim (48° 44' N, 8° 55' E, and 475 a.s.l.) in Southwest Germany from May to October. The mean annual rainfall was 646.5 mm in 2016 and 653.9 mm in 2017. The average annual temperature was 9.1 °C in 2016 and 9.2 °C in 2017, respectively. During the cultivation period of yacon, the average temperature was 14.8 °C in 2016 and 13.5 °C in 2017. The rainfall amounted to 394.3 mm in 2016 and 464.7 mm in 2017 (Figure 1A,B).

The soil of both experimental years was a Vertic Cambisol [24]. In 2016, sugar beet was the preceding crop, while in 2017, winter wheat served as the preceding crop. The trials were set up as a randomized complete block design with three replicates. Each plot was 5 × 4 m. Yacon was planted in ridges (60 × 45 cm; 44 cm between the ridges; 114 cm between the center of ridges), which were formed using a common asparagus ridge-planting machine (Leofant, HMF-Hermeler Maschinenbau GmbH, Füchtorf, Germany). Each plot contained two ridges, which were directed from east to west, and 14 plants (0.7 × 1.14 m). Ridges were formed four weeks before planting. N_{min} in the soil was determined shortly before planting [25] and amounted to 28.8 kg NO₃ ha^{−1} in 2016 and 119 kg NO₃ ha^{−1} in 2017. To ensure that no water stress occurred, plots were irrigated by hand, twice in 2016 (27 May and 7 June) and four times in 2017 (26 and 30 May, 20 and 22 June) with 1.4 L per plant.

Weed control was done by hand once a week until the plant population was established and no weeds occurred anymore.

Figure 1. Temperature (●) and precipitation (bars) at the trial site, Ihinger Hof for the cultivation period of yacon in (a) 2016 and (b) 2017. Plotted are the average temperatures in degree Celsius, and the total rainfall in mm in each month. Temperature and precipitation in May and October (2016) and May and November (2017) only include the days, which were within the cultivation period.

2.2. Treatments

Two different genotypes with three different N fertilization levels were investigated over both experimental years. The two genotypes were a brown-shelled (B) and red-shelled (R) genotype. These two genotypes were chosen because of the known yield differences from other studies. Further, they are the most commonly distributed and available genotypes. As the genotypes differ also in their development and total duration of cultivation period, it was assumed that this will point out important differences in yield and the final N uptake. First, seedlings were received from a plant nursery (Helenion, Berlin, Germany), and then the rhizomes were stored after each harvest for the next year's cultivation period.

The different N fertilization levels were 0 (control), 40, and 80 kg N ha⁻¹. This resulted in six treatments (Table 1). In both years, all treatments were fertilized with ENTEC 26 (EuroChem Agro GmbH, Mannheim, Germany) prior to planting. ENTEC 26 is a stabilized fertilizer, and consists of 26% total N (18% ammonium nitrogen, 7.5% nitrate nitrogen) and 13% hydro-soluble sulphur with nitrification inhibitors 3,4-dimethyl-1H-Pyrazolophosphat (DMPP), with an effect duration of four to ten weeks, depending on the climate, weather, and soil. In 2016, divided seedlings after budding (DSAB) were generated from mother plants and pre-cultivated for six to eight weeks in the greenhouse (Doo et al., 2002). In 2017, the propagation method was adjusted and seedlings were obtained from rhizome pieces which were pre-cultivated for six to eight weeks in the greenhouse. Cultivation of mother plants began ten days later on 15 April 2016, and seedlings were produced over a period of seven days. Mother plants were cultivated in round pots (37 cm height, Ø 32 cm on ground, Ø 44 cm top, 0.035 m³) filled with 14 kg of standard soil (classic, expert substrate, Einheitserde Werkverband e.V., Sinntal-Altengronau, Germany) without further fertilization. To establish seedlings from the mother plant, the whole rhizomes were dug out every second day and rooted shoots were separated. Seedlings were transplanted into square planters (9 × 9 × 9.5 cm) filled with a standard soil (classic, expert substrate, Einheitserde Werkverband e.V., Sinntal-Altengronau, Germany) for further cultivation.

In 2017, cultivation of rhizome pieces started on 27 March. Whole rhizomes were sliced with a sterile knife into smaller segments (20–40 g), which were planted in square planters (9 × 9 × 9.5 cm) and filled with a standard soil (classic, expert substrate, Einheitserde Werkverband e.V., Sinntal-Altengronau,

Germany). In both years, seedlings were cultivated in the greenhouse for six to eight weeks at 21 °C during the day and 15 °C during the night, where the humidity was about 65%. No artificial light was used. Pots were irrigated every third day.

Seedlings were transplanted manually on 18 May 2016 and 11 May 2017. Plants were placed with a distance of 1.14 m between rows and 0.7 m between plants in rows. Consequentially, the plant density was 12,531 plants ha⁻¹. Immediately after planting 6 kg ha⁻¹, slug pellets (Arinex, ADAMA Deutschland GmbH, Cologne, Germany) were applied manually.

Table 1. List of two used genotypes (B = brown-shelled, R = red-shelled) and three different N fertilization levels (0, 40, and 80 kg N ha⁻¹). Given abbreviations for each treatment will be used throughout the text.

Abbreviation Treatment	Genotype	Fertilization (kg N ha ⁻¹)
B0	brown-shelled	0
B40	brown-shelled	40
B80	brown-shelled	80
R0	red-shelled	0
R40	red-shelled	40
R80	red-shelled	80

2.3. Field Measurements and Sample Preparation

The traits of plant height (from soil surface to youngest leaf, end of stem), number of leaves on the main stem, and number of ramifications on the main stem were determined non-destructively on six plants in the central part of each plot, every second week from transplanting until harvest. Four destructive measurements were conducted every four weeks from the beginning of tuberous root formation until harvest (Table 2). For each destructive measurement, one single plant per plot (in 2016) or two plants per plot (in 2017) were harvested manually with a sickle and a digging fork. Plants were washed to remove soil residues. Afterwards, plants were separated into four parts: leaf, stem, tuber, and rhizome. Fresh weight and dry weight was determined for every part and every plant. Additionally, the weight of every single tuber was determined. For determination of the dry weight of the leaf and stem, bulked samples of each plot were dried at 60 °C until they reached a constant weight. For determination of the dry weight of tubers and rhizomes, mixed samples of each plot were frozen with liquid N (−196 °C) in order to prevent any further enzymatic reactions, and were finally freeze-dried.

The final harvest took place on 28 October 2016 and 2 November 2017 (163 and 175 days after planting (DAP), respectively). The date of harvest was scheduled depending on the outside temperature, and took place immediately after the first frost. The six central plants in each plot, which were measured non-destructively during the cultivation period, were harvested to obtain final yield data as before plants were subdivided into parts and washed. Again, the fresh weight of each part and plant was recorded. Additionally, the single tuber weight was recorded. Subsequently, a bulked sample of each part and plot was frozen with liquid N and freeze-dried.

For further plant analysis, all samples were milled. Freeze-dried samples (tubers and rhizomes) were milled with GRINDOMIX GM 200 (Retsch GmbH, Haan, Germany). Leaf and stem samples were milled with Ultra-Zentrifugalmühle ZM 200 (Retsch GmbH, Haan, Germany).

The nitrogen concentration in each freeze-dried sample was analyzed with near-infrared spectroscopy analysis (NIRS) by using a Model 5000 NIRS spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). 3 mg of dried or freeze-dried milled samples of each plant part were placed in a cuvette. Double determination of each sample was also performed. For validation, 25% of the whole sample set was chosen randomly by the NIRS software, and the rest was assigned to the calibration set. Samples for validation were analyzed with a macro-elemental analyzer, Vario MAX CNS (Elementar, Hanau, Germany), according to the Dumas combustion method. The software WIN

ISI™ (Intrasoft Intl. S.A., Luxembourg) was used to perform validation and calibration. Throughout these procedures, aboveground (leaves and stems) and belowground (tubers and rhizomes) plant parts were handled separately.

The sugar composition of tubers was analyzed by using high-performance liquid chromatography (HPLC). Therefore, 0.5 g freeze-dried and milled samples were placed in Corning™ Falcon™ 50 mL (Fisher Scientific GmbH, Schwerte, Germany) and filled with a 25 mL mixture of HPLC-grade 70:30 Ethanol: Water (Chemsolute, Hamburg, Germany). Subsequently, mixtures were sonicated at 60 °C for 30 min, and then cooled at room temperature. Afterwards, the extract was filtered with an attached syringe filter holder (0.2 µm).

HPLC was performed using the Agilent 1290 Infinity (Agilent Technologies, Inc., Santa Clara, CA, USA). A Column A Phenomenex Rezex RSO (Phenomenex, Aschaffenburg, Germany) was used at a temperature of 85 °C. The eluent was 100% HPLC-grade water, and as the detector, a refractive index detector with a temperature of 40 °C was applied. The injection volume was 1 mL, and the flow rate was 0.4 mL min^{−1}. The content of fructofuranosylnystose (GF₄), nystose (GF₃), kestose (GF₂), fructose, glucose, and sucrose was determined using a standard curve drawn by an injection of standards (Merck KGaA, Darmstadt, Germany) of analyzed sugar.

In 2017, after each destructive measurement, soil samples in each plot were collected to determine N_{min} [25]. Therefore, the soil samples were immediately taken in each plot (besides the destructively measured plant) using an auger at a depth of 0–30 and 30–60 cm. Additionally, in both experimental years, soil samples after harvest were collected to determine N_{min} [25]. Therefore, two soil samples were taken from each plot at a depth of 0–30, 3–60, and 60–90 cm.

Table 2. Dates of transplanting, and the number of measurements and harvests in both experimental years, 2016 and 2017. Description includes the date and date of measurement (DAP) corresponding to all six tested treatments.

Year	Transplanting Date	Date of Measurement (DAP)				Harvest
		1.	2.	3.	4.	
2016	18.05	27.07. (70)	30.08. (104)	27.09. (132)	26.10. (161)	28.10. (163)
2017	11.05	27.07. (77)	24.08. (105)	21.09. (133)	23.10. (165)	02.11. (175)

2.4. Data Collection

For the calculation of N uptake, the dry matter of different plant fractions were multiplied with analyzed N concentration according to the following equation:

$$\text{N uptake (kg N ha}^{-1}\text{)} = \text{dry matter yield (kg ha}^{-1}\text{)} * (\% \text{ N}/100).$$

For the determination of tuber N utilization efficiency (TNUtE_D), tuber yields (kg ha^{−1} DM) were divided by tuber N uptake (kg ha^{−1}). For the tuber N utilization efficiency of sugar (TNUtE_S), the sugar yield (kg ha^{−1}) (data not shown) were divided by tuber N uptake (kg ha^{−1}). Total plant N utilization efficiency for sugar (PNUtE_S) was determined by dividing the sugar yield (kg ha^{−1}) by the N uptake of the total plant (kg ha^{−1}).

Total monosaccharides are given as a sum of glucose and fructose. Total FOS are given as a sum of GF₂, GF₃, and GF₄ [26]. Total sugar content is the sum of all analyzed sugar fractions (fructose, glucose, sucrose, and FOS).

2.5. Statistical Analysis

A mixed-model approach was used, and the following model was fitted to all traits:

$$y_{ijkl} = \mu + b_j + r_{jk} + a_i + \beta_l + (a\beta)_{il} + (ba)_{ij} + (b\beta)_{jl} + (ba\beta)_{ijl} + e_{ijkl},$$

where μ is the general effect, b_j is the fixed effect of the j^{th} year, r_{jk} is the fixed effect of the k^{th} replicate in the j^{th} year, a_i is the main effect of i^{th} genotype, β_l is main effect of l^{th} fertilizer, $(a\beta)_{il}$ is the interaction effect of the i^{th} genotype and l^{th} fertilizer, and e_{ijkl} is the error of y_{ijkl} with homogeneous or year-specific variances. $(ba)_{ij}$, $(b\beta)_{jl}$, and $(ba\beta)_{ijl}$ are random interaction effects between genotype and fertilizer, or their interactions and year, respectively. The Akaike information criterion (AIC) was used to select the error variance structure, and thus was used to select a model with homogeneous or year-specific error variance [27]. As the fertilizer is numeric (0, 40, and 80 kg N ha⁻¹), a linear regression for each genotype was fitted too, but was consequently not used as the lack of fit was significant (results not shown). Thus, the fertilizer trend is not linear. Residuals were checked graphically for normal distribution and homogeneous variance. After finding significant differences via the global F-test, significant differences were evaluated with a multiple t-test (Fisher's least significant difference test) at significance level of 5%. A letter display was used to present results of multiple comparisons [28]. Additionally, simple means were calculated for all traits for presentation purposes only.

Statistical analyses were performed using SAS Software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Figures were generated using SigmaPlot, version 13.0 (Systat Software Inc., CA, USA) and Excel 2013 (Microsoft Corporation, WA, USA).

3. Results

3.1. Tuber Yield

Fresh-matter (FM) tuber yield (t ha⁻¹) and dry-matter (DM) tuber yield (t ha⁻¹) were significantly affected by year-by-genotype and year-by-fertilizer interaction effects. Therefore, the two experimental years are presented separately (Table 3). For fresh-matter tuber yield, genotype means were significantly different from each other in 2017 only (Table A2). In 2016, 0 kg N ha⁻¹ yielded significantly higher tuber yield values (22.05 t ha⁻¹) compared to 80 kg N ha⁻¹ (16.44 t ha⁻¹) (Table A4). Both genotypes achieved lowest tuber yields in the highest fertilization level. In 2017, the average yield level increased with fertilization. Tuber yields reached from 42.81 t ha⁻¹ (B40) up to 67.32 t ha⁻¹ (R80). Both genotypes achieved highest tuber yields in the highest fertilization level of 80 kg N ha⁻¹.

Tuber dry matter showed significant fertilization-by-genotype interaction effects, but no interactions with year was found (Table 3, Table A5). Tuber DM ranged from 10.4 (B40) to 17.7% (R40) in 2016. Across both years, RG had significantly higher DM in all fertilization levels. Within the genotypes, R40 had significantly higher DM than R0 and R80. Within BG, no significant differences between the fertilization levels were determined.

Dry-matter (DM) tuber yield (t ha⁻¹) was significantly affected by year-by-genotype and year-by-fertilizer (Tables 3, A2 and A4). In 2016, DM tuber yield ranged from 1.95 (B80) to 3.90 (R40) t ha⁻¹. Significantly higher DM tuber yield were reached for both genotypes in 2017, and ranged from 4.51 (B40) to 11.40 (R80) t ha⁻¹. Furthermore, in both experimental years, RG reached significantly higher DM tuber yield than BG. With regard to the fertilization level, in 2016, 0 and 40 kg N ha⁻¹ reached significant higher tuber yields than 80 kg N ha⁻¹. In 2017, a reverse picture was shown, where the highest fertilization level reached significantly higher DM tuber yield than the other two fertilization levels.

Table 3. Tuber yield (t ha^{-1} FM), tuber dry matter (DM in %), and tuber yield (t ha^{-1} DM) for the two years (2016 and 2017) of the six treatments (B0, B40, B80, R0, R40, and R80) at harvest as mean value $\pm$ standard error.

Treatment		Tuber Yield		
		Fresh Matter [t ha ⁻¹ FM]	DM [%]	Dry Matter [t ha ⁻¹ DM]
2016				
B0		24.06 ± 2.45	11.83 ± 0.67	2.81 ± 0.3
B40		19.57 ± 2.45	10.36 ± 0.67	2.04 ± 0.3
B80		17.62 ± 2.45	11.28 ± 0.67	1.95 ± 0.3
R0		20.04 ± 2.45	15.03 ± 0.67	3.01 ± 0.3
R40		21.97 ± 2.45	17.68 ± 0.67	3.90 ± 0.3
R80		15.26 ± 2.45	16.25 ± 0.67	2.47 ± 0.3
2017				
B0		45.38 ± 3.55	10.58 ± 0.47	4.72 ± 0.5
B40		42.81 ± 3.67	10.45 ± 0.47	4.51 ± 0.5
B80		50.25 ± 3.55	11.31 ± 0.47	5.67 ± 0.5
R0		60.44 ± 3.66	17.04 ± 0.47	10.35 ± 0.5
R40		61.93 ± 3.55	17.00 ± 0.47	10.50 ± 0.5
R80		67.32 ± 3.55	17.00 ± 0.47	11.40 ± 0.5
Results of statistical analysis				
Factor	DF	<i>p</i> -Value		
Year × Rep	4	<0.0001	0.1060	0.0048
Year	1	<0.0001	0.6375	<0.0001
G	1	<0.0001	<0.0001	<0.0001
Year × G	1	<0.0001	0.1272	<0.0001
F	2	0.8731	0.6895	0.8622
Year × F	2	0.0137	0.6356	0.0057
G × F	2	0.4782	0.0486	0.2412
Year × G × F	2	0.9438	0.0698	0.5333

ANOVA table (factor (replication = REP; genotype = G; fertilizer = F), degree of freedom (DF) and *p*-value) carried out for tuber yield (t ha^{-1} FM), tuber DM (%) and tuber yield (kg ha^{-1} DM).

3.2. Sugar Composition

The fractions GF₄, GF₃, GF₂, sucrose, total FOS (GF₄, GF₃, and GF₂), and total sugar content (sum of all sugar fractions) were significantly affected by year-by-genotype-by-fertilizer interactions (Table 4). Fructose was significantly affected by year-by-genotype interactions. Only glucose was significantly affected by year and genotype, and therefore showed no interactions with the year. For this reason, the two experimental years, 2016 and 2017, were presented separately.

In 2016, all sugar components except GF₄ B40 showed the highest concentration. In 2017, B80 was best for all sugar components, except for monosaccharides (B0) and sucrose (B40). In general, concentrations in 2017 tended to be lower compared to 2016.

In 2016, GF₄ ranged from 10.5 to 18.6%. B0 (12.2%) and R0 (18.6%), as well as B80 (13.5%) and R80 (11.6%) were significantly different from each other. The other fertilization levels did not differ significantly between the genotypes. B80 reached the highest amounts of GF₄ (13.5%) and differed significantly from B0 and B40. Contrary results were shown for RG. The highest amounts of GF₄ were reached by R0 (18.6%), which were significantly different to R40 and R80. In 2017, GF₄ ranged from 9.9 to 14%. All fertilization levels differed significantly between the genotypes. Within the genotypes in each case with increasing fertilization, the amount of GF₄ increased. B80 (11.4%) differed significantly to B40 and B0. In addition, R40 and R0 differed significantly to R80 (14%).

The amount of GF₃ in 2016 ranged from 12 to 20.9%. Except for B0 (19.0%) and R0 (20.8%), all fertilization levels differed significantly between the genotypes. Estimates for fertilizer levels of BG

did not differ significantly between each other. Within RG, R0 (20.8%) reached significantly higher amounts of GF₃ than R40 and R80. In 2017, the amount of GF₃ ranged between 12.2 and 15.5%. Again, for all fertilizer levels, genotypes were significantly different from each other except B0 (13.5%) and R0 (12.2%). Within the genotypes, there were significant differences between fertilization levels similar to GF₄. In each case, the amount of GF₃ increased with increasing fertilization levels. An exception was R0 (20.8%) in 2016, which was significantly higher than R40 (12.0%) and R80 (12.4%). In 2017, B80 (15.5%) and R80 (13.7%) had significantly higher amounts of GF₃ than the other fertilization levels.

In 2016, the amount of GF₂ ranged between 7.4 and 25.1%. GF₂ values of the two genotypes differed for all fertilization levels, with BG showing higher values. Within BG, there were non-significant differences between the fertilization levels. Within RG, R0 achieved a significantly increased amount of GF₂ (14.6%) compared to R40 and R80. In 2017, the amount of GF₂ ranged from 6.0 (R0) to 15.1% (B80). In 2017, GF₂ values of genotypes differed for all fertilization levels, with BG again showing higher values. Within a genotype, no differences between fertilizer levels were found. However, an increase in fertilization was found to lead to an increase in the amount of GF₂.

In 2016, sucrose ranged from 1.8 (R40) to 7.1% (B40). In all fertilization levels, RG reached significantly lower amounts of sucrose than BG. R0 reached 4.2%, and therefore significantly higher amounts than R40 and R80. Within BG, there were no significant differences between the fertilization levels. In 2017, the sucrose amount ranged from 2.3 (R0) to 5.0% (B40). Again, for all fertilization levels, the estimates differed significantly between the genotypes. Within the genotypes, there were no significant differences between the fertilization levels, nor in the BG and in the RG.

The amount of fructose ranged from not detectable (coded as zero) (R0, R40, and R80) to 4.0% (B0). In both years, BG reached significantly higher amounts of fructose than RG. Furthermore, both genotypes reached significantly higher amounts of fructose in 2017 than in 2016.

The amount of glucose ranged from 1.7 (R40 in 2016) to 10.7% (B0 in 2017). For glucose, BG generally indicated significantly higher amounts than RG.

The total FOS amount in 2016 ranged from 30.8 (R40) to 58.2% (B80). Except B0 and R0, the other fertilization levels differed significantly between the genotypes. Within BG, there were no significant differences between the fertilization levels. R0 (54.0%) reached the highest amount and was significantly different to R40 and R80. In 2017, the content of total FOS ranged from 30.5 (R0) to 42.0% (B80). All fertilization levels were significantly different between the genotypes. Within the genotypes, there was a similar trend. B0 (36.2%) and R0 (30.5%) reached the lowest amounts of FOS and differed significantly to B80 (42.0%) and R80 (34.8%). B40 and R80 were not significantly different to the other two fertilization levels in the corresponding genotype.

The total sugar content was significantly affected by year. In 2016, the sugar content ranged from 34.3 (R40) to 73.7% (B40). Fertilization levels 40 and 80 kg N ha⁻¹ of BG reached significantly higher sugar contents than the same fertilizer levels in RG. Also within the genotypes, there were significant differences between the fertilization levels. BG had the highest sugar content in B40 (73.7%) and lowest in B0 (66.6%). B80 did not differ significantly to B0 or B40. Within the RG, R0 reached the highest sugar content of 60.7% and differed significantly to R40 (34.3%) and R80 (35.3%). B0, B40, B80, and R0 reached significantly higher sugar contents in 2016 than in 2017. Only R40 and R80 reached similar (R40) or higher sugar contents in 2017.

In 2017, the sugar content ranged from 36.2 (R0) to 58.8% DM (B40). In all fertilization levels, BG reached significantly higher sugar contents than RG. Within the genotypes, there were no significant differences between the fertilization levels for the sugar content of BG. Within RG, R80 had significantly higher sugar contents of 41.8% than R0 with 36.2% DM. R40 did not differ significantly to R0 or R80.

Table 4. Fructose, glucose, sucrose, GF₂, GF₃, GF₄, total fructooligosaccharides (FOS), and total sugar content in % of dry matter (DM) for the two years (2016 and 2017) of the six treatments (B0, B40, B80, R0, R40, and R80) at harvest, notated as the mean value $\pm$ standard error.

Treatment	Fructose	Glucose	Sucrose	GF ₂	GF ₃	GF ₄	Total FOS	Total Sugar	
2016									
B0	1.38 ± 0.6	4.67 ± 1.2	6.15 ^{aA} ± 0.4	23.16 ^{aA} ± 1.0	19.04 ^{aA} ± 0.6	12.22 ^{bAB} ± 0.5	54.42 ^{aA} ± 1.8	66.62 ^{aB} ± 2.16	
B40	2.63 ± 0.6	7.87 ± 1.2	7.09 ^{aA} ± 0.4	25.07 ^{aA} ± 1.0	19.30 ^{aA} ± 0.6	11.77 ^{aB} ± 0.5	56.15 ^{aA} ± 1.8	73.74 ^{aA} ± 2.16	
B80	1.60 ± 0.6	5.44 ± 1.2	6.58 ^{aA} ± 0.4	23.85 ^{aA} ± 1.0	20.85 ^{aA} ± 0.6	13.52 ^{aA} ± 0.5	58.22 ^{aA} ± 1.8	71.84 ^{aAB} ± 2.16	
R0	n.d.	2.48 ± 1.2	4.22 ^{bA} ± 0.4	14.59 ^{bA} ± 1.0	20.75 ^{aA} ± 0.6	18.64 ^{aA} ± 0.5	53.99 ^{aA} ± 1.8	60.69 ^{aA} ± 2.16	
R40	n.d.	1.68 ± 1.2	1.84 ^{bB} ± 0.4	8.20 ^{bB} ± 1.0	12.05 ^{bB} ± 0.6	10.50 ^{aB} ± 0.5	30.75 ^{bB} ± 1.8	34.27 ^{bB} ± 2.16	
R80	n.d.	2.01 ± 1.2	1.94 ^{bB} ± 0.4	7.38 ^{bB} ± 1.0	12.43 ^{bB} ± 0.6	11.55 ^{bB} ± 0.5	31.36 ^{bB} ± 1.8	35.31 ^{bB} ± 2.16	
2017									
B0	4.01 ± 0.4	10.66 ± 0.9	4.78 ^{aA} ± 0.2	12.89 ^{aA} ± 1.0	13.45 ^{aB} ± 0.6	9.86 ^{bB} ± 0.4	36.21 ^{aB} ± 1.2	55.65 ^{aA} ± 1.70	
B40	3.92 ± 0.4	10.50 ± 0.9	5.02 ^{aA} ± 0.2	14.22 ^{aA} ± 1.0	14.49 ^{aAB} ± 0.6	10.64 ^{bAB} ± 0.4	39.35 ^{aAB} ± 1.2	58.80 ^{aA} ± 1.70	
B80	3.18 ± 0.4	8.00 ± 0.9	4.37 ^{aA} ± 0.2	15.09 ^{aA} ± 1.0	15.54 ^{aA} ± 0.6	11.39 ^{bA} ± 0.4	42.02 ^{aA} ± 1.2	57.56 ^{aA} ± 1.70	
R0	0.08 ± 0.4	3.28 ± 0.9	2.32 ^{bA} ± 0.2	6.05 ^{bA} ± 1.0	12.23 ^{aB} ± 0.6	12.22 ^{aB} ± 0.4	30.50 ^{bB} ± 1.2	36.18 ^{bB} ± 1.70	
R40	0.17 ± 0.4	3.85 ± 0.9	2.68 ^{bA} ± 0.2	6.87 ^{bA} ± 1.0	13.12 ^{bAB} ± 0.6	13.24 ^{aAB} ± 0.4	33.23 ^{bAB} ± 1.2	39.92 ^{bAB} ± 1.70	
R80	0.15 ± 0.4	4.16 ± 0.9	2.63 ^{bA} ± 0.2	7.10 ^{bA} ± 1.0	13.72 ^{bA} ± 0.6	13.99 ^{aA} ± 0.4	34.81 ^{bA} ± 1.2	41.75 ^{bA} ± 1.70	
Results of statistical analysis									
Factor	DF	<i>p</i> -Value							
Year × Rep	4	0.2147	0.3612	0.0594	0.1646	0.1028	0.0843	0.1656	0.2596
Year	1	0.0064	0.0003	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001
G	1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Year × G	1	0.0156	0.1196	0.0003	<0.0001	<0.0001	0.0104	<0.0001	0.0006
F	2	0.5143	0.3753	0.1348	0.3982	0.0010	0.0001	0.0085	0.0523
Year × F	2	0.7009	0.7453	0.0834	0.0025	<0.0001	<0.0001	<0.0001	<0.0001
G × F	2	0.5364	0.2099	0.0100	0.0019	<0.0001	<0.0001	<0.0001	<0.0001
Year × G × F	2	0.6348	0.2230	0.0021	0.0095	<0.0001	<0.0001	<0.0001	<0.0001

ANOVA table (factor (replication = REP; genotype = G; fertilizer = F), degree of freedom (DF) and *p*-value) carried out for the sugar fractions GF₄, GF₃, GF₂, sucrose, fructose, glucose, total FOS and total sugar content in % of DM. Within the years, the same lowercase letter in one column indicates no significant differences between genotypes with the same fertilization level at *p* < 0.05. Within the years, the same capital letters in one column indicates no significant differences between fertilization levels of one genotype at *p* < 0.05.

3.2.1. Nitrogen Concentration and Nitrogen Uptake

Nitrogen concentration (% of DM) was significantly affected by year and genotype in the two plant parts, rhizome and tuber (Table 5). For rhizome, differences between fertilizer levels were found. In the case of aboveground biomass, there were significant effects by all two-way interactions of year, genotype, and fertilizer.

Table 5. Nitrogen concentration (%) in the aboveground biomass of rhizome and tuber at the last destructive measurement (161 and 165 DAP) immediately before harvest for two years (2016 and 2017) of the six treatments (B0, B40, B80, R0, R40, and R80), notated as the mean value $\pm$ standard error.

Treatment	% N in Different Plant Parts			
	Aboveground Biomass	Rhizome	Tuber	
2016				
B0	3.35 ± 0.11	1.46 ± 0.15	0.95 ± 0.07	
B40	3.06 ± 0.11	1.43 ± 0.15	0.96 ± 0.07	
B80	4.01 ± 0.11	1.75 ± 0.15	1.07 ±0.07	
R0	3.19 ± 0.11	1.25 ± 0.15	0.82 ± 0.07	
R40	3.31 ± 0.11	1.64 ± 0.15	0.73 ± 0.07	
R80	3.52 ± 0.11	1.47 ± 0.15	0.91 ± 0.07	
2017				
B0	3.21 ± 0.12	1.45 ± 0.05	0.91 ± 0.03	
B40	3.18 ± 0.12	1.52 ± 0.05	0.75 ± 0.03	
B80	3.19 ± 0.12	1.50 ± 0.05	0.78 ± 0.03	
R0	2.14 ± 0.12	0.98 ± 0.05	0.61 ± 0.03	
R40	2.31 ± 0.12	1.27 ± 0.05	0.77 ± 0.03	
R80	2.10 ± 0.12	1.34 ± 0.05	0.71 ± 0.03	
Results of statistical analysis				
Factor	DF	<i>p</i> -Value		
Year × Rep	4	0.0275	0.3944	0.2193
Year	1	<0.0001	0.0297	0.0002
G	1	<0.0001	0.0088	0.0018
Year × G	1	<0.0001	0.1362	0.2133
F	2	0.0097	0.0262	0.1710
Year × F	2	0.0010	0.9263	0.1677
G × F	2	0.0233	0.1500	0.7892
Year × G × F	2	0.2954	0.2032	0.1834

ANOVA table (factor (replication = REP; genotype = G; fertilizer = F), degree of freedom (DF) and *p*-value) for nitrogen concentration in different plant parts: aboveground biomass, rhizome and tuber.

In 2016, N concentration in aboveground biomass ranged from 3.1 (B40) to 4.0% (B80). Fertilization level 80 kg N ha⁻¹ differed significantly to the fertilization levels 0 and 40 kg N ha⁻¹ (Table A4). The two genotypes did differ significantly (Table A2). In 2017, the N concentration in the aboveground biomass ranged from 2.1 (R80) to 3.2% (B0) and did not differ significantly between the genotypes. There were also no significant differences between the fertilization levels.

The N concentration of the rhizomes ranged from 1.0 (R0) to 1.8% (B80). Across both experimental years, BG reached significantly higher N concentrations than RG. With regard to fertilization level, 40 and 80 kg N ha⁻¹ reached significantly higher N concentrations than 0 kg N ha⁻¹ (Table A3).

The N concentration of tubers ranged from 0.6 (R0 in 2017) to 1.0% (B80 in 2016) and, across both years, were significantly higher in BG than in RG (A1).

The N uptake (kg N ha⁻¹) of the aboveground and total plant were significantly affected by genotype and fertilizer (Table 6). The N uptake of the tuber was significantly different between genotypes-by-years combinations (Table 6, Table A1). For the N uptake of rhizomes, no significant effects were found. The RG had a significantly higher N uptake in aboveground biomass and total

plant compared to BG. The N uptake of aboveground biomass differed significantly between 0 and 40 kg N ha⁻¹, whereas 80 kg N ha⁻¹ did not differ significantly to both other fertilizer levels. Within the total plant, the N uptake of fertilization level 0 kg N ha⁻¹ was significantly lower than fertilization levels 40 and 80 kg N ha⁻¹.

The N uptake of aboveground biomass ranged from 88.6 (B0 in 2016) up to 226.0 kg ha⁻¹ (R80 in 2017). Across both years, RG reached significantly higher N uptakes than BG. Furthermore, the fertilization level of 40 kg N ha⁻¹ reached significantly higher N uptake than 0 kg N ha⁻¹. The fertilization level 80 kg N ha⁻¹ did not differ significantly to the other two fertilization levels.

The N uptake of rhizomes ranged from 9.3 (R0) to 29.3 kg ha⁻¹ (B80). No significant differences could be detected.

The N uptake in tubers ranged from 19.1 (B0) to 31.4 kg N ha⁻¹ (R0) and from 29.5 (B40) to 84.2 kg N ha⁻¹ (R80) in 2016 and 2017, respectively. In general, the N uptake of tubers increased with increasing fertilization.

The N uptake of the total plant ranged from 121.0 (B0 in 2016) 330.3 kg ha⁻¹ (R80 in 2017) and was significantly highest for RG and for fertilization levels 40 and 80 kg N ha⁻¹.

Table 6. Nitrogen uptake (kg N ha⁻¹) of different plant parts, and sum of total plant N uptake at the last destructive measurement (161 and 165 DAP, respectively) immediately before harvest for two years (2016 and 2017) of the six treatments (B0, B40, B80, R0, R40, and R80) notated as the mean value $\pm$ standard error.

Treatment	N Uptake (kg ha ⁻¹) of Different Plant Parts				
	Aboveground Biomass	Rhizome	Tuber	Total Plant	
2016					
B0	88.60 ± 28.6	13.31 ± 4.8	19.06 ± 6.7	120.98 ± 32.3	
B40	169.42 ± 28.6	22.35 ± 4.8	18.77 ± 6.7	210.56 ± 32.3	
B80	110.19 ± 28.6	12.20 ± 4.8	29.80 ± 6.7	152.18 ± 32.3	
R0	118.81 ± 28.6	10.84 ± 4.8	31.41 ± 6.7	161.07 ± 32.3	
R40	200.64 ± 28.6	20.46 ± 4.8	15.33 ± 6.7	236.42 ± 32.3	
R80	152.62 ± 28.6	17.03 ± 4.8	30.08 ± 6.7	199.73 ± 32.3	
2017					
B0	131.00 ± 18.3	12.74 ± 6.6	33.36 ± 8.7	177.10 ± 26.3	
B40	130.30 ± 18.3	22.66 ± 6.6	29.52 ± 8.7	182.48 ± 26.3	
B80	128.81 ± 18.3	29.32 ± 6.6	38.53 ± 8.7	196.66 ± 26.3	
R0	151.14 ± 18.3	9.30 ± 6.6	50.88 ± 8.7	211.31 ± 26.3	
R40	193.38 ± 18.3	15.07 ± 6.6	72.25 ± 8.7	280.71 ± 26.3	
R80	226.03 ± 18.3	20.03 ± 6.6	84.19 ± 8.7	330.25 ± 26.3	
Results of statistical analysis					
Factor	DF	<i>p</i> -Value			
Year × Rep	4	0.4321	0.4120	0.0149	0.1587
Year	1	0.1666	0.5236	<0.0001	0.0087
G	1	0.0033	0.3313	0.0004	0.0014
Year × G	1	0.3708	0.3095	0.0019	0.1509
F	2	0.0252	0.0856	0.0683	0.0191
Year × F	2	0.1153	0.2629	0.2708	0.1873
G × F	2	0.4400	0.9508	0.7648	0.4534
Year × G × F	2	0.6343	0.7186	0.1287	0.5043

ANOVA table (factor (replication = REP; genotype = G; fertilizer = F), degree of freedom (DF) and *p*-value) carried out for nitrogen uptake in different plant parts and total plant.

3.2.2. Indicators for Plant Efficiency

The three different indicators show the efficiency of different treatments related to N use and sugar production. All indicators were calculated as mentioned in 2.4. Therefore, in the following text, no units for indicators are given.

For $TNUtE_D$, no significant differences could be detected (Table 7). $TNUtE_D$ ranged from 35.3 (B80) to 185.0 (R40).

$PNUtE_S$ ranged from 6.1 (R80) to 13.4 (B0) and from 13.7 (B40) to 17.3 (R0) in 2016 and 2017, respectively. Across both experimental years and genotypes, efficiency of fertilization levels 0 kg N ha⁻¹ was significantly higher than of the other two fertilization levels (Table A3).

For $TNUtE_S$, no significant differences could be detected. $TNUtE_S$ ranged from 37.8 (R80) to 96.3 (B0). The general trend was decreasing efficiency with an increasing amount of N fertilization.

Table 7. Indicator for tuber N utilization efficiency for tuber dry-matter yield ($TNUtE_D$), total plant N utilization efficiency for sugar ($PNUtE_S$), and tuber N utilization efficiency for sugar ($TNUtE_S$) at the last destructive measurement (161 and 165 DAP) immediately before harvest for the two years (2016 and 2017) of the six treatments (B0, B40, B80, R0, R40, and R80), notated as the mean value $\pm$ standard error.

Treatment		$TNUtE_D$	$PNUtE_S$	$TNUtE_S$
2016				
B0		80.33 $\pm$ 40.3	13.38 $\pm$ 1.7	96.32 $\pm$ 20.9
B40		114.69 $\pm$ 40.3	5.68 $\pm$ 1.7	75.14 $\pm$ 20.9
B80		35.29 $\pm$ 40.3	6.74 $\pm$ 1.7	73.74 $\pm$ 20.9
R0		105.48 $\pm$ 40.3	9.56 $\pm$ 1.7	50.96 $\pm$ 20.9
R40		184.95 $\pm$ 40.3	7.25 $\pm$ 1.7	95.19 $\pm$ 20.9
R80		103.48 $\pm$ 40.3	6.11 $\pm$ 1.7	37.77 $\pm$ 20.9
2017				
B0		83.96 $\pm$ 7.0	14.88 $\pm$ 1.8	78.54 $\pm$ 3.6
B40		134.16 $\pm$ 7.0	13.70 $\pm$ 1.8	84.41 $\pm$ 3.6
B80		129.35 $\pm$ 7.0	15.71 $\pm$ 1.8	80.68 $\pm$ 3.6
R0		164.51 $\pm$ 7.0	17.26 $\pm$ 1.8	72.29 $\pm$ 3.6
R40		131.23 $\pm$ 7.0	14.41 $\pm$ 1.8	56.32 $\pm$ 3.6
R80		85.43 $\pm$ 7.0	15.17 $\pm$ 1.8	60.59 $\pm$ 3.6
Results of statistical analysis				
Factor	DF		<i>p</i> -Value	
Year $\times$ Rep	4	0.2726	0.9535	0.0789
Year	1	0.3445	<0.0001	0.9472
G	1	0.0912	0.9606	0.0605
Year $\times$ G	1	0.2456	0.3926	0.9026
F	2	0.1022	0.0281	0.4142
Year $\times$ F	2	0.4356	0.2328	0.4548
G $\times$ F	2	0.6241	0.7200	0.5396
Year $\times$ G $\times$ F	2	0.1571	0.3422	0.1979

ANOVA table (factor (replication = REP; genotype = G; fertilizer = F), degree of freedom (DF) and *p*-value) carried out for the indicators $TNUtE_D$, $PNUtE_S$ and $TNUtE_S$.

4. Discussion

4.1. Tuber Yield

Tuber yields were affected by year, and were significantly higher in 2017 than in 2016. The tuber yields were comparable to other studies. Under similar climatic conditions in the Czech Republic, tuber yield ranged from 21 to 29 t ha⁻¹ [29,30]. In 2016, tuber yields of this study (except B80) were in a similar range, and in 2017 were noticeably higher. However, plant density in the present study (12,531 plants ha⁻¹) was noticeably lower than the commonly used plant densities ranging

between 23,000 to 30,000 plants ha⁻¹ [31]. Therefore, the tuber yields reached were remarkably higher, considering the plant density. Yacon plants were conceivably able to compensate for the lower plant densities through a higher single-plant tuber yield [31]. Douglas et al. showed noticeable differences in tuber yields, which ranged from 11 to 81 t ha⁻¹, depending on location and, associated therewith, precipitation [31]. Also, Fernández et al. reported a very high impact of precipitation on tuber yield formation [29]. This is also true for the present study, where significantly higher tuber yields were reached in 2017, the year with higher precipitation (70 mm more than 2016) and a lower average temperature (1.5 °C lower than 2016). In particular, precipitation in 2017 was higher in July and August, the months where tuberous root formation started. Similarly, water stress is also one of the most important factors for tuber yield formation of chicory and Jerusalem artichoke [32,33]. In general, for yacon, 550 mm was sufficient for tuberous root production, even if higher precipitation could lead to higher yields [29].

With regard to the fertilization level, the two experimental years showed contrary results. In 2016, tuber yield decreased with increasing fertilization level, and the genotypes were not significantly different; whereas in 2017, a reverse picture was shown. In Jerusalem artichoke, the effect of N fertilization was more pronounced under non-water-limited conditions [34]. That could also explain the more pronounced effects of N fertilization in 2017 on yield, as the overall precipitation was higher. This would mean that the water requirement is perhaps different for optimum tuber yield formation and N utilization. The effects of fertilization level on tuber yield in 2017 were not significant. The tuber yield increased with increasing N fertilization, but no maximum could be achieved in both genotypes. Fernandes et al. reported that under high N rates, tuber yield by sweet potato were similar in all treatments. Losavio et al. also reported no significant effect of N fertilization on tuber yield by Jerusalem artichoke [16,35]. This is also in accordance with Maltas et al., who reported increasing tuber yields of potatoes with increasing N fertilization, but no significant differences in the higher N rates of about 120 kg N ha⁻¹ under comparable climatic conditions [21]. In general, the expected mineralization in the soil of the present study was quite high. Due to the formation of ridges, mineralization was stimulated because of oxygen supply and fast heating [36]. Furthermore, the long growing period leads to higher N mineralization [37]. As mineralization decreases with increasing N supply (N_{min} + additional fertilization) [37], no significant effects of higher N fertilization levels on tuber yield were obtained.

Significant differences between the genotypes may indicate that the RG had a higher yield potential in general, or better N-use efficiency, which is confirmed by the data shown in Table 7 where TNUtE_D showed higher values for RG. Also, several other studies reported significant differences between different yacon genotypes with regard to tuber yield [29,38]. As significant differences between the genotypes were only observed in 2017, it can be assumed that tuber yield formation did not reach its maximum in 2016. Tuber DM of BG was similar to DM reported in other studies and ranged from 10.3 to 14% [39–41]. The significantly higher DM of RG was similar to the findings of Campos et al., who reported DM up to 18.6% [42]. In accordance with the present study, Hermann et al. showed that tuber DM differed significantly between the genotypes [40,43].

4.2. Sugar Composition

Contrary to the obtained tuber yields, the sugar content, on average, was higher in 2016 than in 2017. That could be justified by the higher precipitation in 2017 than 2016. In the case of Jerusalem artichoke, sugar content increased in dry years, and vice versa [44]. With regard to the monosaccharides, fructose achieved rather low amounts, and could not be detected in RG in 2016. This is in contrast to the results of other studies where fructose represented the highest proportion of monosaccharides and carbohydrates in yacon tubers [7,40,45–47]. However, some of these investigations were carried out on stored tubers. Due to the reduction of FOS and sucrose, the amount of fructose was highest in tubers [45–47]. Contrary to that are the findings of Valentova et al., who also reported highest amounts of fructose, but without the storage of tubers [7]. Herman et al. showed both, depending

on the investigated genotype [40]. Similarly, Fukai et al. and Khajehei et al. detected the lowest amounts of fructose [48,49]. Therefore, the amount of fructose depends on genotype, but also on the post-harvest conditions of tubers. With increasing storage time, the amount of fructose increases [45]. In general, amounts of FOS decreased during storage, and sucrose, fructose, and glucose increased due to hydrolyzation of FOS [15,50]. Fructose amounts in Jerusalem artichoke decreased with later harvest dates, and therefore prolonged plant growth [51]. As RG started flowering, in contrast to BG, it might have been closer to physiological maturity than BG, leading to changes in sugar profiles.

In general, it is striking that the monosaccharides fructose and glucose were only affected by the year-by-genotype or genotype interactions, respectively. This indicates that the amount of monosaccharides were not influenced by N fertilization. Also, the fructose content in Jerusalem artichoke was not influenced by N fertilization, but by genotype [51,52]. In general, a reaction similar to that of Jerusalem artichoke or potato would have been expected, where higher amounts of N fertilizer delayed maturity [51,53]. This would lead to a decrease in DM content, as well as a decrease in carbohydrates [16,21]. Furthermore, a difference in sugar composition would be expected. In sugar-containing crops like sugar beet, or starch-containing crops like potatoes, sugar or starch content decreases with increasing fertilization, and the composition changes [54]. As no changes in yacon were observed, this might be related to either the date of application [51] or the N fertilizer used. In contrast to the studies on potatoes, all N was applied at planting. In addition, a slow-release fertilizer was used, which might have affected the N availability during the season not negatively affecting carbohydrate formation.

The amount of sucrose was significantly higher in BG in all fertilization levels and both years. It is reported in the literature that higher amounts of sucrose can lead to higher amounts of FOS [7,46,55]. This was confirmed in the present study, where the BG in all treatments reached significantly higher amounts of FOS than RG, except for R0 in 2016. This exception is due to significantly higher amounts of sucrose in R0 than in other treatments of RG, which led to significantly higher amounts of FOS. In addition, R0 (2016) was the only treatment which differed significantly to other fertilization levels within the genotype. All other treatments showed no significant influence of N fertilization.

The amount of total FOS ranged over both experimental years from 30.5% to 58.2% in DM. This is in the range of findings in other studies, which showed a wide range from 6.4 to 70% [8,40,48]. This wide range is due to a strong influence of year (climatic conditions) and genotype [5,45]. Furthermore, this wide range is due to different post-harvest conditions like those mentioned above. The FOS in yacon tubers are extremely sensitive to temperature and storage conditions [15,50]. Compared to the FOS content of Jerusalem artichoke, which ranged from 24.7 to 67%, it was quite similar [56–58]. Similar to yacon, tubers of Jerusalem artichoke were sensitive to different post-harvest conditions, which indicates that storage duration and storage temperature can have a high impact on FOS content.

If compared to other FOS-containing vegetables, such as artichoke and chicory (up to 2%), the amount in yacon tubers is really high [26]. In general, RG achieved lower amounts of FOS, which corresponded to the findings of Graefe et al. who also showed higher FOS amounts in a light-colored genotype, due to significant differences between genotypes [8]. Furthermore, it was reported that the total amount of FOS and degree of polymerization (DP) correlated. Tubers containing highest amounts of total FOS had higher amounts of FOS with lower DP. Tubers containing lower amounts of total FOS had higher amounts of FOS with higher DP [46–48]. This corresponds to findings in the present study, where BG had higher amounts of FOS and mainly the highest amount of GF₂. All treatments in 2017 had, on average, a higher DP than in 2016, which led to lower amounts of total FOS in nearly all treatments. Different climatic conditions, like those mentioned above and higher total N supply in both years could have led to delayed maturity [51]. Jerusalem artichoke and chicory, as the two other mayor crops containing FOS, generally had higher chain length of DP >10 [26]. A lower DP <10, as in yacon, is preferable because of higher bifidogenic effects [39,57].

Due to there being few or no significant differences between fertilization levels regarding FOS amounts in general, a higher influence of average DP on the total FOS amount when compared to the

N fertilization level is assumed. This hypothesis is supported by no significant differences between treatments across the years in spite of higher N availability in 2017.

The total sugar content of all treatments in the present study were below or equal to the normal range of 70–80% of DM [13,45]. Compared to the sugar content of Jerusalem artichoke (71% of DM), chicory (74% of DM), and artichoke (78–80% of DM), the sugar content in the present study were comparatively lower [33,35,59]. It has been reported that an increase of N leads to a decrease of sugar content for sugar beet and artichoke [19,35,60]. Besides the amount of applied N is, decisively, the date of application for sugar content by Jerusalem artichoke. Divided rates could lead to higher sugar yields, because of slow development of seedlings [19,51,61]. Furthermore, high N supply could have been the reason why the total sugar content was lower in 2017 [51]. Higher amounts of N supply lead to a delay in maturity [61], which would lead to a higher percentage of sugar in tubers without delayed maturity [51]. In general, sugar content was highly variable and differed significantly due to the impact of several parameters, such as origin, nutrient content, and cultivation conditions, wherefore it might be difficult to compare total sugar content in general. [5,6,41]. Overall, determination of the optimal N fertilization level for sugar production is not merely. It is necessary to maximize both tuber yields and sugar content. Certainly, the effect of climatic conditions and genotype were greater than the impact of N fertilization level.

4.3. Nitrogen Concentration and Uptake

N concentration of aboveground biomass was significantly influenced by the genotype-by-fertilizer interaction. This is similar to the findings of Sah et al., who reported a significant influence of N level on N concentration in the vegetative parts of Jerusalem artichoke and chicory, amongst others. N concentration ranged from 0.3 to 1.0 and 1.5 to 2.0%, respectively [20]. N concentrations in the present study were noticeably higher and comparable to those of fodder beet, which ranged from 1.7 to 3.3% [20]. Similar to these are findings of N concentration in leaves of sweet potatoes, which ranged from 2.9 to 4.2%, also influenced by fertilizer [16]. RG had significantly lower N concentrations in aboveground biomass, while overall N uptake was significantly higher. This was due to bigger plants of RG, and therefore more biomass in general (data not shown). Furthermore, Hermann et al. reported that yacon leaves had noticeably higher N concentrations than the stems [40]. Comparing the two tested genotypes, BG had a lower plant height and bigger leaves, whereas that of RG was higher and had smaller leaves. Both genotypes were comparable in their N uptake of aboveground biomass to sugar beet (up to 238 kg N ha⁻¹) [19].

The nitrogen concentration of tubers was significantly higher for BG than for RG, and higher than the N concentration reported by Lebeda et al., which ranged between 0.6 and 0.7% [55]. Compared to the N concentration of potato tubers, which ranged approximately between 0.9 to 1.2%, the N concentration of RG was slightly lower, and the N concentrations of BG were similar [62,63]. Bélanger et al. also showed significant differences in N concentration between two potato genotypes [63]. Similarly, in tubers of Jerusalem artichoke, N concentration differed significantly between genotypes and ranged from 1.2 to 2.1% of DM [64]. In general, the N concentration of storage parts of chicory ranged between 0.7 and 1.1%, which is similar to that in yacon [20]. Regarding the fertilization level, there were no significant effects on tuber N concentration. In the case of potatoes, there were similar findings. It is reported that the fertilization level had no effect, but that it did for the time of application and possible splitting. An increase in N concentration with increasing fertilization could also be detected [65]. The decrease in N concentration in 2017 was probably due to a dilution effect. The N uptake of tubers in 2017 was significantly higher in RG when compared to BG, resulting in significantly higher tuber yields, although the amount of N on a percentage basis was significantly higher in BG across the years. Hermann et al. reported that the N uptake of tubers ranged between 0.4 and 0.8 kg N per t FM [40]. Nitrogen uptake in the present study was slightly higher and ranged between 0.8 and 1.2 kg N per t FM. Zotarelli et al. reported an N uptake in potato tubers between 90 to 148 kg ha⁻¹ at different fertilization levels without any significant differences [59]. These results are

similar to findings in the present study, where the N uptake was lower, but no differences between the fertilization levels could be detected.

The N concentration of the rhizomes was significantly influenced by N fertilizer level. With increasing fertilization, N concentration in rhizomes increased, too. Therefore, N storage in rhizomes did not increase in all cases with increasing fertilization level. The highest amount of N was obtained in each case (except BG in 2016) in the treatment with the highest N uptake of the total plant. This shows an increase of translocation of N into the rhizomes with an increasing N uptake of the plant. Similar to findings of Kleijn et al. regarding *V. album*, the amount of N in the whole plant was lowest in rhizomes. The amount of N was in decreasing order, being the highest in aboveground biomass, tubers, and lastly in rhizomes [66].

Total plant N uptake is composed of the N content of the three fractions—aboveground biomass, rhizome, and tuber. Similar to the N uptake of aboveground biomass, the genotype and fertilizer had a significant influence. Compared to the N uptake of sugar beet, which ranged from 58 to 285 kg N ha⁻¹, findings in the present study are in a comparable range [67,68]. The N uptake of fodder beet (150–370 kg N ha⁻¹) and Jerusalem artichoke (80–300 kg N ha⁻¹) confirmed that findings in the present study are in a normal range [20].

The significantly higher N uptake of RG can be explained by a significantly higher N uptake of aboveground biomass and tubers.

Overall, N uptakes exceeded N supply, which is due to a high mineralization rate that is common in soils high in organic matter [18]. Laufer et al. reported N uptakes of sugar beet roughly 130–180 kg above the given soil N_{min} content without any further fertilization, and attributed this to the preceding crops—winter wheat, or winter barley [67,69]. In addition, the effect of the previous crop in 2016 is decisive. The leaves of sugar beet have a N content of 20 to 30 mg N g⁻¹, and can be easily decomposed. The N content in soil is slightly higher with crop residues of sugar beet. The long-term effect is particularly remarkable [70].

4.4. Indicators for Plant Efficiency

The chosen indicators described relationships between tuber yield DM, N uptake, and sugar content for the different treatments. They are useful indicators for comparing the treatments and genotypes with each other, and with other appropriate crops as well.

The indicators TNUtE_D and TNUtE_S performed quite similarly. With increasing fertilization level, the efficiency of tubers for accumulating DM or sugar decreased in general. This is similar to the findings of Fernandes et al. who reported a decreasing N uptake efficiency under high N levels above 120 kg N ha⁻¹ for sweet potatoes [16]. Also, Maltas et al. reported decreasing N utilization and uptake efficiency with increasing N fertilizer (above 120 kg N ha⁻¹) in potatoes [21]. This is explainable by a stronger sink effect of aboveground biomass [20]. Higher N fertilization mostly affects the vegetative growth of the plant [19,22]. TNUtE_D showed differences between the genotypes. Both in 2016 and 2017, the highest BG efficiency was obtained at 40 kg N ha⁻¹. In 2016, RG efficiency was higher when the level of fertilization was 40 kg N ha⁻¹, and in 2017 when there was no fertilization. TNUtE_S was higher under low N fertilization conditions (B0 and R40) only in 2016. This is similar to the findings in 4.2, which showed lower sugar content under a higher N supply.

PNUtE_S decreased with increasing fertilization level (except B80 in 2017) and is an indicator for significant differences between the fertilization levels. PNUtE_S was highest for the unfertilized treatment, 0 kg N ha⁻¹. This is similar to several studies, which showed a decreasing amount of carbohydrates in potatoes, sugar beet, and Jerusalem artichoke [21,51,54]. Hence, the aboveground biomass seems to be particularly decisive for plant efficiency, because TNUtE_D and TNUtE_S did not show any significant differences.

Compared to the PNUtE_S of sugar beet and fodder beet under comparable climatic conditions in Germany and the Netherlands which ranged from 48 to 110, the determined efficiency of yacon can be seen to be quite low [69]. Possible reasons for this can be the high amounts of aboveground biomass

up to 54 t ha^{-1} , and thus the high N uptakes of yacon [3]. With increasing amounts of aboveground biomass, the overall efficiency of a plant decreases. This is similar to findings of Sah et al., who reported higher efficiency of chicory than for Jerusalem artichoke, due to noticeably higher amounts of aboveground biomass by Jerusalem artichoke [20].

Considering all three indicators, higher amounts of N did not inescapably lead to higher efficiency. Even though tuber yields were significantly higher in 2017 and lower fertilization amounts in 2017 were favorable for almost all indicators, the requirement of N is apparently covered by high $N_{\min}$ values, and no additional fertilization is needed. The difference between the genotypes is also noticeable—for RG, lower amounts of N fertilization were suitable for higher efficiency than for BG, due to different growth and development patterns, especially of aboveground biomass. Ojala et al. reported potatoes' optimum maturity when the DM content was highest and carbohydrate content was lowest [53]. Present findings would suggest that RG is closer to physiological maturity while BG still shows vegetative growth. Considering the desired aim of the farmer, to produce sugar contents as high as possible, BG might be a better option. However, overall tuber yields have to be considered.

5. Conclusions

This study tested the influence of N fertilization on the tuber yield, sugar composition, and N uptake of yacon in a two-year field study. In general, differences of determined traits between years and the two tested genotypes were considerably higher than differences between the N fertilization levels.

In general, tuber yield increased with increasing N fertilization in 2017, while the reverse effect was obtained in 2016. With increasing yields, sugar and FOS content slightly decreased. A decreasing amount of FOS led to a slight increase in the amount of FOS, with a higher degree of polymerization.

Overall, for an optimum efficient tuber yield production, an N fertilization level of 40 kg N ha^{-1} is recommended, depending on the $N_{\min}$ value. This optimum N amount might be different if the precipitation is significantly higher. As mentioned above, the amount of precipitation is one of the key factors for tuber yield formation of yacon, even though it proved to be sufficient in the present study. Nevertheless, it is conceivable that under inexhaustible water supplies, the N uptake can change. At this point, it is necessary to conduct further investigations about the N uptake of yacon under various water conditions. For the climatic conditions in Central Europe, the amount of precipitation in the present study reflected the average quantity. In general, the two tested genotypes differed in their N uptake due to differences in aboveground biomass and tuber yield.

In summary, yacon can cope with lower N fertilizer rates than other tuberous crops, such as potatoes and sugar beet, leading to high tuber and sugar yields overall on a hectare basis.

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Appendix A

Table A1. Results of a multiple t-test at significance level of 5% after finding significant differences via global F-test for the factor genotype (brown and RG). Same lowercase letters indicate no significant differences between the genotypes.

Genotype	Glucose [% DM]	Total Monosaccharides [% DM]	Total Sugar Content [% DM]	N Uptake Aboveground Biomass	N Uptake Total Plant [kg ha ⁻¹]	% N Rhizome	% N Tuber
B	7.86 ^a ± 0.4	16.31 ^a ± 0.7	64.03 ^a ± 0.8	126.39 ^b ± 9.8	173.33 ^b ± 12.0	1.52 ^a ± 0.04	0.89 ^a ± 0.02
R	2.91 ^b ± 0.4	5.38 ^b ± 0.7	41.35 ^b ± 0.8	173.77 ^a ± 9.8	236.58 ^a ± 12.0	1.32 ^b ± 0.04	0.76 ^b ± 0.02

Table A2. Results of a multiple t-test at significance level of 5% after finding significant differences via global F-test for the factor year × genotype. Same lowercase letters indicate no significant differences between the years and same genotype. Same capital letters indicate no significant differences between genotypes within one year.

Year × Genotype	Tuber Yield [t ha ⁻¹ FM]	Tuber Yield [t ha ⁻¹ DM]	Fructose [% DM]	N Uptake Tuber [kg ha ⁻¹]	% N Aboveground Biomass
2016 × B	20.42 ^{bA} ± 1.4	2.26 ^{bB} ± 0.2	1.87 ^{bA} ± 0.4	22.55 ^{aA} ± 3.9	3.47 ^{aA} ± 0.06
2016 × R	19.08 ^{bA} ± 1.4	3.19 ^{bA} ± 0.2	n.d.	33.80 ^{bA} ± 5.0	3.19 ^{aB} ± 0.07
2017 × B	46.15 ^{aB} ± 2.0	4.93 ^{aB} ± 0.3	3.70 ^{aA} ± 0.3	25.61 ^{aB} ± 3.9	3.34 ^{aA} ± 0.06
2017 × R	63.23 ^{aA} ± 2.0	10.75 ^{aA} ± 0.3	0.13 ^{aB} ± 0.3	69.11 ^{aA} ± 5.0	2.19 ^{aA} ± 0.07

Table A3. Results of a multiple t-test at significance level of 5% after finding significant differences via global F-test for the factor fertilizer (0, 40 and 80 kg N ha⁻¹). Same lowercase letters indicate no significant differences between the fertilization levels.

Fertilizer	N Uptake Aboveground Biomass [kg ha ⁻¹]	N Uptake Total Plant [kg ha ⁻¹]	% N Rhizome	PNUtEs
0	122.39 ^b ± 12.0	167.62 ^b ± 14.7	1.29 ^b ± 0.05	13.77 ^a ± 0.9
40	173.44 ^a ± 12.0	227.54 ^a ± 14.7	1.47 ^a ± 0.05	10.26 ^b ± 0.9
80	154.41 ^{ab} ± 12.0	219.71 ^a ± 14.7	1.52 ^a ± 0.05	10.93 ^b ± 0.9

Table A4. Results of a multiple t-test at significance level of 5% after finding significant differences via global F-test for the factor year × fertilizer. Same lowercase letters indicate no significant differences between the years and same fertilization level. Same capital letters indicate no significant differences between the fertilization levels within one year.

Year × Fertilizer	Tuber Yield [t ha ⁻¹ FM]	Tuber Yield [t ha ⁻¹ DM]	Total Sugar Content [% DM]	% N Aboveground Biomass
2016*0	22.05 ^{bA} ± 1.7	2.89 ^{bA} ± 0.2	63.65 ^{aA} ± 1.5	3.27 ^{aB} ± 0.07
2016*40	20.77 ^{bAB} ± 1.8	3.08 ^{bA} ± 0.2	54.00 ^{aB} ± 1.5	3.19 ^{aB} ± 0.07
2016*80	16.44 ^{bB} ± 1.7	2.21 ^{bB} ± 0.2	53.57 ^{aB} ± 1.5	3.77 ^{aA} ± 0.07
2017*0	52.91 ^{aA} ± 2.6	7.48 ^{aB} ± 0.3	45.91 ^{bA} ± 1.2	2.68 ^{bA} ± 0.08
2017*40	52.37 ^{aA} ± 2.6	7.50 ^{aB} ± 0.3	49.36 ^{bA} ± 1.2	2.74 ^{bA} ± 0.08
2017*80	58.80 ^{aA} ± 2.5	8.54 ^{aA} ± 0.3	49.66 ^{aA} ± 1.2	2.65 ^{bA} ± 0.08

Table A5. Results of a multiple t-test at significance level of 5% after finding significant differences via global F-test for the factor genotype × fertilizer. Same lowercase letters indicate no significant differences between the genotypes and same fertilization level. Same capital letters indicate no significant differences between the fertilization levels within one genotype.

Genotype × Fertilizer	Tuber DM	% N Aboveground Biomass
B0	11.21 ^{bA} ± 0.41	3.28 ^{aB} ± 0.08
B40	10.41 ^{bA} ± 0.41	3.12 ^{aB} ± 0.08
B80	11.30 ^{bA} ± 0.41	3.59 ^{aA} ± 0.08
R0	16.04 ^{aB} ± 0.41	2.67 ^{bA} ± 0.08
R40	17.34 ^{aA} ± 0.41	2.81 ^{bA} ± 0.08
R80	16.63 ^{aAB} ± 0.41	2.81 ^{bA} ± 0.08

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