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The Influence of Three Years of Supplemental Nitrogen on Above- and Belowground Biomass Partitioning in a Decade-Old *Miscanthus* × *giganteus* in the Lower Silesian Voivodeship (Poland)

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Abstract: Because of the different opinions regarding nitrogen (N) requirements for *Miscanthus* × *giganteus* biomass production, we conducted an experiment with a set dose of nitrogen. The objective of this study was to examine the effects of nitrogen fertilization on the biomass yield, water content, and morphological features of rhizomes and aboveground plant parts in various terms during a growing season over the course of three years (2014–2016) in Lower Silesia (Wroclaw, Poland). The nitrogen fertilization (dose 60 kg/ha and control) significantly affected the number of shoots ($p = 0.0018$), the water concentration of rhizomes ($p = 0.0004$) and stems ($p = 0.0218$), the dry matter yield of leaves ($p = 0.0000$), and the nitrogen uptake ($p = 0.0000$). Nitrogen fertilization significantly affected the nitrogen uptake in all plant parts ($p = 0.0000$). Although low levels of nitrogen appeared to be important in maintaining the maximum growth potentials of mature *Miscanthus* × *giganteus*, the small reductions in the above- and belowground biomass production are unlikely to outweigh the environmental costs of applying nitrogen. More studies should use the protocols for the above- and belowground yield determination described in this paper in order to create site- and year-specific fertilizer regimes that are optimized for quality and yield for autumn (green) and spring (delayed) harvests.

Keywords: *Miscanthus*; nitrogen fertilization; rhizomes; stem; leaves

1. Introduction

New technologies, excessive fossil fuel combustion, and future fossil fuel depletion will contribute to permanent changes in the natural environment. One of the most pivotal environmental problems is climate change, which is caused by the anthropogenic heating of the atmosphere as a result of rising greenhouse gas concentrations [1–5]. To overcome this difficulty, we must increase the use of renewable energy sources. Renewable energy sources play an increasingly essential role in the energy policy of European countries [6]. Among all renewable energy sources, plant biomass deserves special attention. Fast growing bioenergy crops are characterized by a great potential to provide raw material for renewable energy. *Miscanthus* has been proposed as a biomass energy crop in Europe [7,8], and its use could increase in the near future, as it is one of the most productive plants among bioenergy crops [9–13]. Additionally, biomass combustion is regarded to be more beneficial for the environment than fossil fuel combustion [14–16].

The success of this bioenergy crop is also determined by its low environmental requirements—for instance, its low nitrogen and water requirements, the mechanization of its planting and harvesting, and the resistance of the plants to diseases and pests [13,14,16,17]. Because of its low nutrient requirements, *Miscanthus* can be successfully cultivated on sandy and high organic matter soils with a wide pH range. Additionally, it is being successfully grown in unused marginal areas and has a tolerance to various abiotic stresses, including excessive salinity, low humidity, or the presence of heavy metals [7,18,19]. According to Galatsidas et al. (2018) [20], the total area of marginal land that is appropriate for *Miscanthus* cultivation in Europe is thought to be as high as 11.11 million ha.

For the successful development of *Miscanthus* production, it is necessary to consider the end specific uses and precise information on the effective management of nitrogen fertilization for different soil types under various climatic and growth conditions [14,21]. Although nitrogen is the main element that determines the efficiency of biomass production, it can have negative environmental effects such as water eutrophication and increased carbon dioxide emissions [22,23].

The literature varies regarding the nitrogen requirements for *Miscanthus* × *giganteus* biomass production [14,24–27], because the nitrogen applications of *Miscanthus* × *giganteus* are characterized by variable productivity results. The N requirements of *Miscanthus* × *giganteus* are low compared to those of other bioenergy crops [16,28,29]. According to Cadoux et al. (2011, 2012) [30,31], these low nutrient requirements are caused by various factors, including a high nutrient use efficiency and the nutrient recycling accumulated in the rhizomes. However, there is a serious debate about the exact need for N fertilizer in a given crop and whether N fertilizer should be required at all. The translocation of nitrogen to rhizomes during the late vegetation period is a major factor in the high efficiency of nitrogen utilization [17]. There are divergent results regarding the requirements of Mischanthus ×*giganteus* for N fertilization. The findings are divided on this matter; some studies have shown that the yield increases after the application of N fertilizer [14,25–27,32], while some state the contrary [13,33–38].

There are many European studies that provide estimates of the belowground biomass for *M. giganteus* at a single point in time [29,36–39]; however, there have been few previous studies that determined the dynamics of the rhizome yield which were not based on regular sampling through the growing season [40,41].

The organ of wintering in the *Mischanthus* is the rhizome, an underground part that grows horizontally that is important for nutrient storage and accumulation. Most research on the yield and biometric traits of *Mischanthus* is concentrated on the aboveground parts of the plants [9,14,29,42]. The main aspects of experimental research are mainly focused on the environmental impact of *Mischanthus*, the different terms of harvesting, the different genotypes of *Mischanthus,* and its chemical composition during multiannual study periods. Thus far, the elemental composition and resistance to frost and salinity have been examined in the rhizomes; however, there is a lack of information on the water content in the rhizomes during the whole growing season [43–46]. A new aspect of our research is the determination of the changes in the rhizome water content during the entire vegetation period (May–December) on a 10-year-old plant.

The objective of this study was to examine the effects of nitrogen fertilization on the number, height, and diameter of leaves on a shoot, as well as the water concentration, dry mass yield, and nitrogen uptake of *Miscanthus* × *giganteus*. The growth rate of the aboveground and belowground biomass of *Miscanthus* × *giganteus* (Greef et Deu) was evaluated in the conditions of southwest Poland, with and without nitrogen fertilization. Additionally, research was undertaken to determine the influence of nitrogen fertilization on the dynamics of the water content changes in the rhizomes during the whole vegetation period.

2. Materials and Methods

2.1. Study Site and Fertilization Treatments of Miscanthus × Giganteus

An investigation of *Miscanthus × giganteus* and nitrogen fertilization was conducted after 10 years of establishing crops (2014–2016) at the Experimental Station of Wroclaw University of Environmental and Life Sciences, Pawlowice (geographical location 17°7′ E and 51°08′ N in the Lower Silesian Voivodship, Wrocław, Poland). Pawlowice is characterized by a vegetation period (March–November) that lasts 223–230 days, with an average temperature during the growing season of 14.5 °C and an annual rainfall ranging between 500 and 600 mm (around 350 mm during the growing season). The soil conditions were defined as alluvial soil, very light on loose sand, and sandy gravel (V grade) (soil classification used in Poland). These soils are weak with a low humus level, and are poor in organic matter. The fifth class of soil quality (6 classes of soil quality: I class—the best arable land; VI—the weakest arable soil) comprises weak arable soils [47].

Plowing was carried out in 2003 at the depth of 20–25 cm, followed by rotary harrowing before planting. Miscanthus rhizomes (10 cm long with 3–6 nodes) were planted in a row spaced 75 cm apart and another row spaced 48 cm apart (on 1 ha–27,777 rhizomes). *Miscanthus × giganteus* was planted in 2004. Plantation was fertilized annually from the year 2004 to 2013 at the beginning of the growing season using the following doses: 40 kg ha^{-1} of N ammonium nitrate 32%, 17.5 kg ha^{-1} of P 40% enriched superphosphate, and 50 kg ha^{-1} of K potassium salt. The plots were separated by a distance of 1.0 m, and all measurements (non-destructive and destructive) were taken at least 0.2 m from the edge of the plot in the years 2014–2016. The dimension of the plot was around 20 m^2. Nitrogen treatments of 0 and 60 kg/ha were applied in March/April during each of the 3 years (17/3/2014, 18/3/2015, 17/4/2016) after pulling out the bedding. Fertilization was annually (from 2014 to 2016) applied during the field experiment, where the following doses were used: 17.5 kg ha^{-1} of P 40% enriched superphosphate, 50 kg ha^{-1} of K potassium salt. After fertilization, the mulch was placed in its original position.

Fertilization was applied via a hand broadcast at the beginning of the vegetation period.

No significant pests and weeds were found in the *Miscanthus* cultivation during the experiment, so the use of herbicides was not necessary.

2.2. Plant Growth Measurement

Miscanthus sampling started from the 30th day of the vegetation period and every 30 days until the end of vegetation period (June, July, August, September, October, November, and December) in the years 2014–2016. At each date of sampling, a plant sample of the aboveground part of the plant and rhizomes was sampled from an area of 0.25 m^2. The fresh mass of the rhizomes and the aboveground part was determined. Additionally, 10 randomly selected shoots were sampled from each replication to perform measurements on plant material—the height of the upper leaf, the diameter measured 10 cm from the soil surface, and the number of leaves per one stem. All the measurements (except the number of shoots) were made on 10 shoots per plot. The number of shoots was counted from a unit of 0.25 m^2 from each replication. Both white and yellow rhizomes were sampled.

Terminal (from outer rows) plants from the external rows were not included in the analysis because of the so-called edge effect. After the end of the vegetation period, *Miscanthus* was harvested at 10–15 cm using a circular saw. Harvested crops were weighed and the percentage of dry matter was determined. The dry biomass weight was determined by drying samples (specific weight, 500 g) to 60 °C for up to 48 h, then drying them at 105 °C for 4 h. Further, the harvested crops were weighed and the fresh mass yield was determined. The dry biomass weight was determined by drying samples (specific weight, 500 g) to 60 °C for up to 48 h, then drying them at 105 °C for 4 h. On this basis, the dry biomass yield per 1 m^2 in a given year was calculated.

Water concentration was calculated according to the Formula (1):

$$\text{Water concentration (\%)} = (100 \times (\text{FM} - \text{DM}))/\text{FM}. \tag{1}$$

FM—fresh mass.
DM—dry mass.

2.3. Soil and Weather Conditions

Tables 1 and 2 summarize the soil conditions for the *Miscanthus* plantation in this trial. Soil samples were twice taken (April, July) during the vegetation period and after its end (November) each year. These dates were presented as annual mean values. Soil samples were taken from the experimental field at a 0–20 cm soil depth and were thoroughly mixed to make a representative composite soil sample. The analysis was comprised of pH, humus, C, N, P, K, S, and micronutrients. Analyses were performed according to the following methods: the soil reaction (pH/KCl (potassium chloride)) was found using the potentiometric method; the total organic carbon was found using Tiurin's method [48]; the total nitrogen (classical distillation) content was found using the Kjehdal method both in soil and plant material [48]; the available forms of potassium and phosphorus were found using the Egner–Rhiem method; magnesium was found using the Schachtschabel method [49]; the total carbon content (TOC) was found via oxidimetric titration [50]; sulfur in the extract was found using the Johnson–Nishita procedure [51]; humic substances (HS) were found using the short fractionation method [52]; and the total contents of Fe, Mn, Zn, and Cu were found using an atomic absorption spectrophotometer (ASA) after mineralization with a concentrated mixture of acids using atomic-absorbent flame spectrophotometry Varian spectra AA 200 [52].

Table 1. The content of organic matter and soil abundance in macronutrients for a depth of 0–20 cm in 2014–2016.

Year	pH 1 N KCl	C g kg^{-1}	Humus g kg^{-1}	N g kg^{-1}	C:N	P mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	S mg kg^{-1}
2014	5.0	5.82	10.00	0.58	10.53	119.6	114.0	24.3	188.0
2015	5.0	5.86	10.05	0.60	10.60	119.6	115.3	27.3	192.6
2016	4.8	5.86	10.05	0.59	10.63	119.7	112.6	26.0	190.0

Table 2. Soil abundance in the micronutrients at the depth of 0–20 cm in 2014–2016.

Year	Fe mg kg^{-1}	Mn mg kg^{-1}	Zn mg kg^{-1}	Cu mg kg^{-1}
2014	428	93.4	82.3	1.82
2015	461	97.1	79.4	1.69
2016	463	95.2	78.5	1.78

The soil's carbon stock was typical for light alluvial soils, and the C: N ratio was on average 10.6:1, which indicates the appropriate process of the organic decomposition (Table 1). In the experimental years, the soil reaction ranged from 4.8 to 5.0 (acidic), which was favorable for *Miscanthus* cultivation, and the arable layer's richness in nutrients was as follows: P—very high; K—medium; Mg—low; S—medium; Fe—low; Mn—medium; Zn—high; and Cu—low (Tables 1 and 2). The assessment of the soil's nutrient content was determined by limit numbers to assess the content of elements developed by the Polish Institute of Soil and Plant Cultivation in Puławy [47].

Monthly data on the temperature and precipitation in the years 2014–2016 are presented in Table 3. The temperatures in the years 2014–2016 oscillated between ±9 °C in IV through to an average of ±17 °C from V to VIII. During the experimental years, the thermal conditions were favorable for the development of *Miscanthus*, with mild winters characterized by positive temperatures. The highest temperatures were recorded in 2015, while the lowest were in 2016 (Table 3).

The optimal amount of rainfall for *Miscanthus* × *giganteus* depends on many factors, including the air temperature, soil type, and groundwater level; however, 600 mm was sufficient for the development of *Miscanthus* [14,26]. The year with the lowest rainfall was 2015. Despite the lack of rainfall, there were no reduction in the yield. The highest rainfall during the growing season was recorded in 2016 (Table 3).

Table 3. Weather conditions during 2014–2016 with a 30-year average for Wroclaw, Lower Silesia (Poland).

Month	Temperature [°C]				Precipitation [mm]			
	2014	2015	2016	Average 1981–2010	2014	2015	2016	Average 1981–2010
I	0.0	2.3	−1.2	−0.8	35.8	46.0	33.4	31.9
II	3.7	1.5	3.8	0.3	1.2	15.6	56.2	26.7
III	7.0	5.4	4.3	3.8	40.1	39.5	55.9	31.7
IV	10.6	8.9	8.7	8.9	55.2	15.8	46.4	30.5
V	13.3	13.5	15.3	14.4	101.4	21.0	5.3	51.3
VI	16.6	16.6	18.6	17.1	40.2	73.3	44.6	59.5
VII	21.2	20.3	19.5	19.3	52.9	55.6	114.3	78.9
VIII	17.3	22.7	17.9	18.3	75.0	5.6	27.1	61.7
IX	15.5	15.1	16.4	13.6	72.2	23.2	44.7	45.3
X	10.7	8.4	8.5	9.1	59.4	20.0	83.8	32.3
XI	6.6	6.2	3.4	3.9	15.5	52.4	36.3	36.6
XII	2.3	5.4	1.2	0.2	17.5	24.0	36.1	37.4
Average annual air temperature and total precipitation	10.4	10.5	9.7	9.0	566.4	392.0	584.1	523.8

2.4. Statistical Analysis

The experiment was conducted with a randomized block design in four replications to test the effects of N fertilization on the morphological traits and yield of *Mischanthus*. The analysis of variance (ANOVA) and the mixed model with repeated measurements was used. The doses of nitrogen fertilizers were assumed to be a fixed factor, while the years were random. The results of the biometric measurements of the *Mischanthus* were analyzed via ANOVA in the Statistica program (13.1 StatSoft, Kraków, Poland).

3. Results

3.1. Effect of Nitrogen Fertilization on Morphological Features of *Miscanthus* × *Giganteus*

Nitrogen fertilization had a significant influence on the number of leaves on the shoot ($p = 0.0018$) during the field experiment (Table 4). Both the number of shoots and the height of the plants increased significantly until the end of vegetation period (Figures 1 and 2). Without N fertilization, the shoots reached 3.34 m in height, whereas the height of plants after an application of 60 kg ha^{-1} N was 3.31 m. The highest increases in height of shoots on unfertilized plots were found between June and July, while in fertilized plants they was found between July and August. The greatest increase in shoot diameter was found at the beginning of the vegetation period (Figure 3). A fast increase in the number of leaves on the shoot was observed in September. Between September and November, the differences were insignificant (Figure 4). The number of leaves on both fertilized and unfertilized shoots increased until November. After this period, it decreased.

Table 4. Morphological features of *Miscanthus* × *giganteus* (average for years 2014–2016).

Dose kg ha^{-1} N	Number of Days Starting from Beginning of Vegetation Period	Number of Shoots per 1 m^2	Height of Plants (m)	Diameter of Shoots (mm)	Number of Leaves on Shoot
0	June	52	0.18	8.5	3.1
	July	59	1.23	10.3	5.9
	August	64	2.14	9.7	8.7
	September	64	2.33	10.5	10.7
	October	66	2.98	9.6	11.3
	November	74	3.07	10.0	11.7
	December	72	3.34	10.8	8.9
60	June	53	0.21	9.1	3.8
	July	64	1.04	9.9	5.9
	August	66	2.24	10.1	8.8
	September	76	2.71	10.8	11.3
	October	78	3.09	10.2	12.1
	November	72	3.13	10.5	12.4
	December	78	3.31	10.8	11.2
	p value	0.2884	0.0001	0.4553	0.0322
Averages for Factors and Years					
0	-	64	2.18	9.9	8.6
60	-	70	2.25	10.2	9.3
	p value	0.0018	0.7020	0.1004	0.1484
2014	-	67	2.28	10.0	8.5
2015		66	2.20	10.0	8.6
2016		68	2.16	10.2	9.9
	p value	0.4112	0.8354	0.4200	0.4040

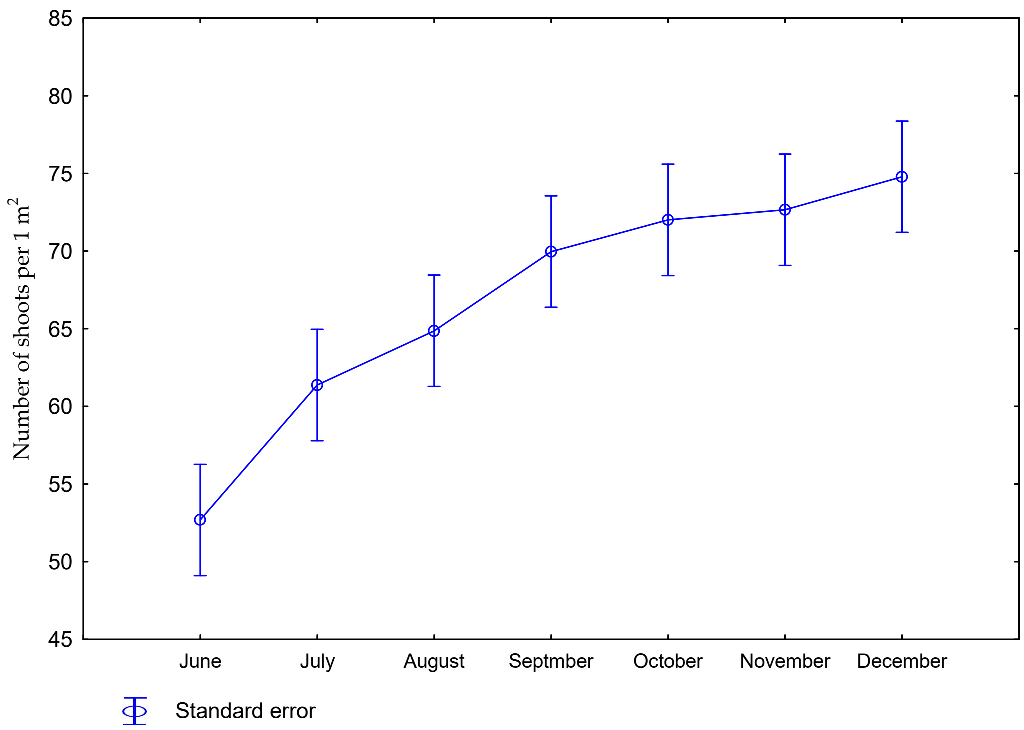

Figure 1. The number of shoots during the vegetation period in the years 2014–2016 (average for years).

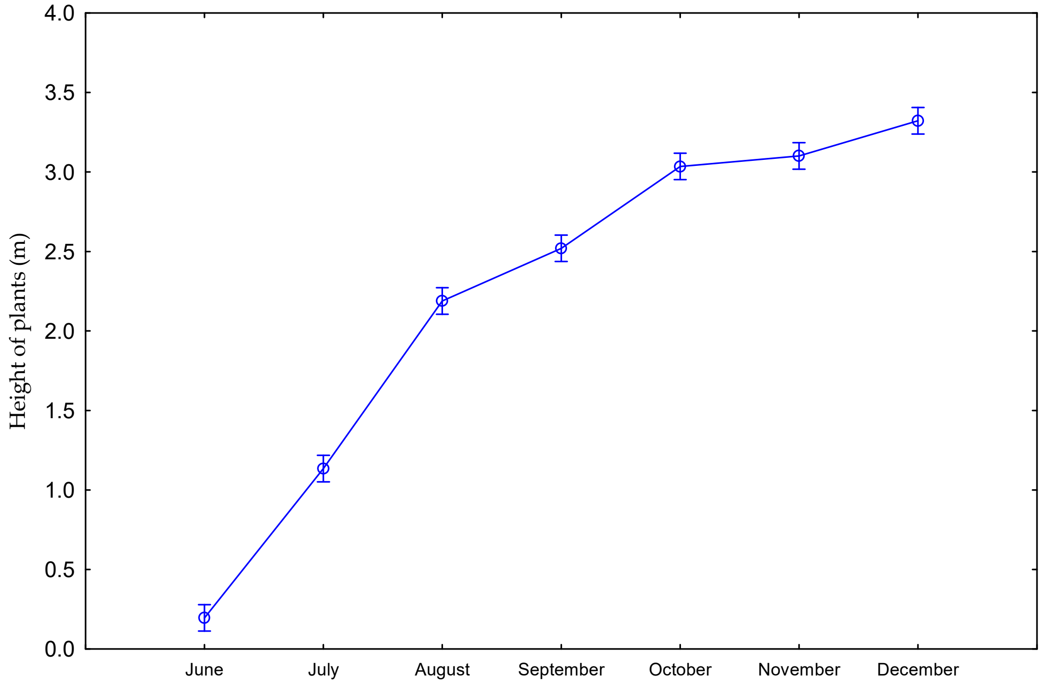

Figure 2. The height of plants during the vegetation period in the years 2014–2016 (average for years).

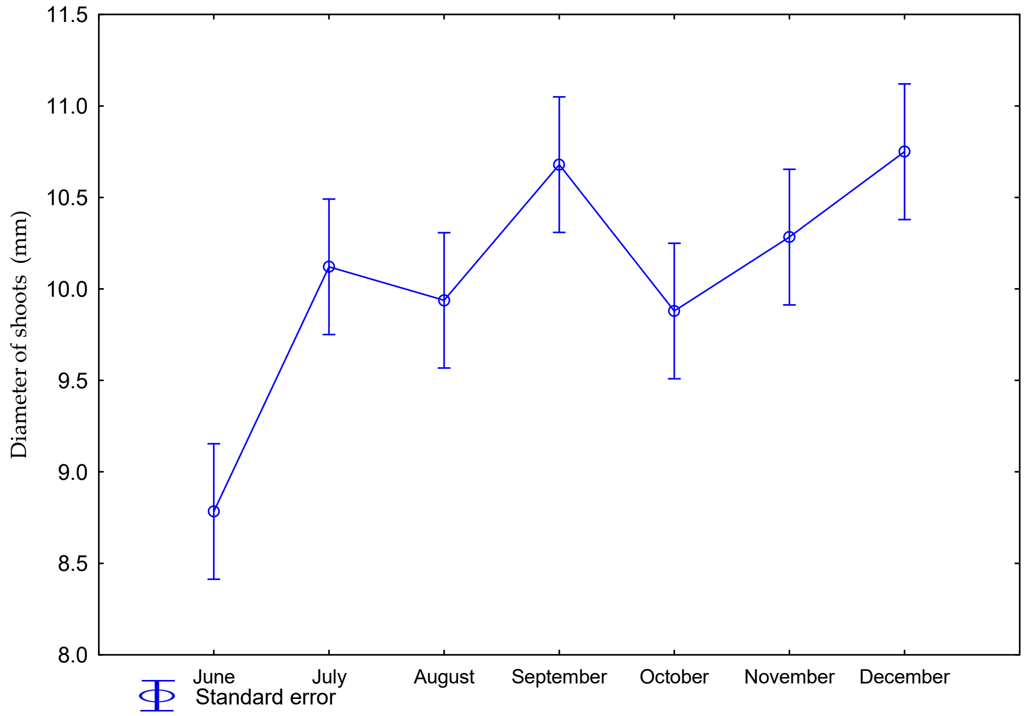

Figure 3. Diameter of shoots during the vegetation period in the years 2014–2016 (average for years).

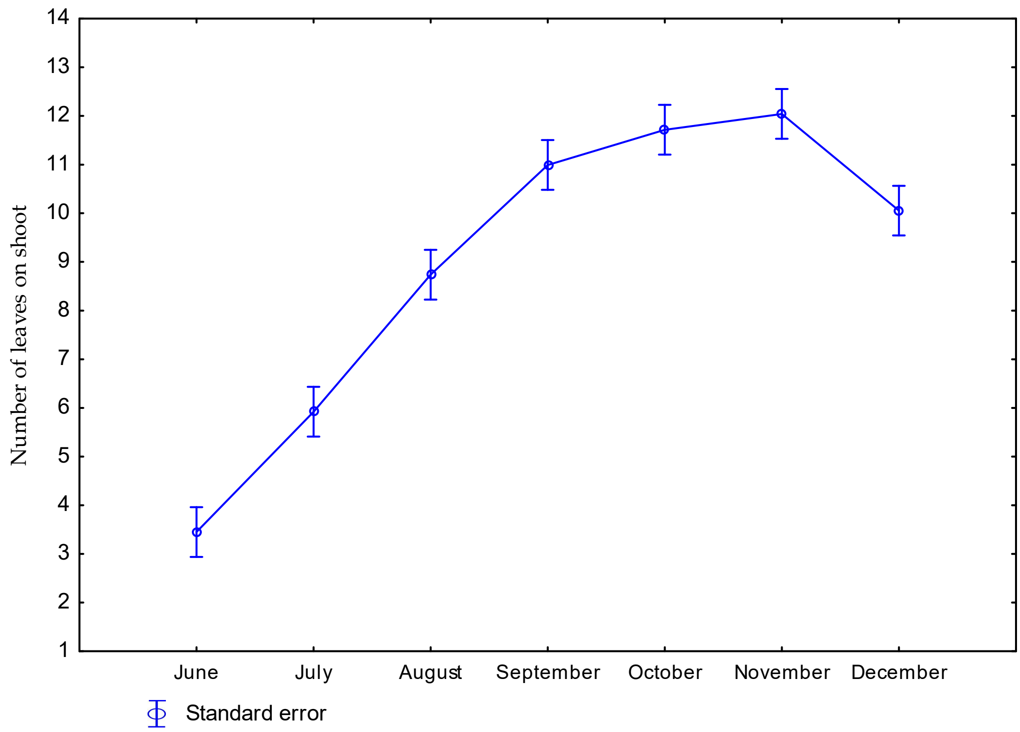

Figure 4. Number of leaves on the shoots during the vegetation period in the years 2014–2016 (average for years).

3.2. Effect of Nitrogen Fertilization on Water Concentration of *Miscanthus* × *Giganteus*

The water concentration was characterized with differences between the examined parts of plants. The rhizomes, stems, and leaves were characterized by a higher water concentration at the beginning of the growing season (Table 5, Figures 5 and 6). On fertilized and unfertilized plots, the water content in the leaves (p = 0.0260) and stems (p = 0.0015) decreased until the end of the vegetation period. For rhizomes, the water content decreased until October and then increased at about 7 g in the unfertilized plot and 31 g in the fertilized plot. There was a significantly higher water concentration found in the rhizomes (p = 0.004) and stems (p = 0.0218) fertilized with nitrogen. The water concentration was significantly different during the experimental years. The highest content of water was observed in the rhizomes (p = 0.0000), stems (p = 0.0022), leaves (p = 0.0000), and whole aboveground parts of plants (p = 0.0025) in the third year of the study (Table 5). A greater water content in the aboveground part of plants was observed until November (Figure 6).

Table 5. Water concentration in the fresh mass of *Miscanthus* × *giganteus* (g kg^{-1}) (average for years 2014–2016).

Dose kg ha^{-1} N	Number of Days Starting from Beginning of Vegetation Period	Rhizomes	Stems	Leaves	Aboveground Part of Plant
	June	722	-	-	882
	July	689	870	879	875
	August	709	772	777	775
0	September	684	697	715	702
	October	663	691	702	694
	November	663	662	698	672
	December	670	622	679	635

Table 5. *Cont.*

Dose kg ha^{-1} N	Number of Days Starting from Beginning of Vegetation Period	Rhizomes	Stems	Leaves	Aboveground Part of Plant
60	June	744	-	-	883
	July	707	867	853	862
	August	712	827	810	820
	September	713	764	781	769
	October	673	720	740	726
	November	683	676	707	685
	December	704	661	701	672
	p value	0.4958	0.0015	0.0260	0.00120
Average for Factors and Years					
0		686	719	742	748
60		705	752	765	774
	p value	0.0004	0.0218	0.0669	0.0693
2014		673	714	722	738
2015		693	722	735	750
2016		721	771	803	795
	p value	0.0000	0.0022	0.0000	0.0025

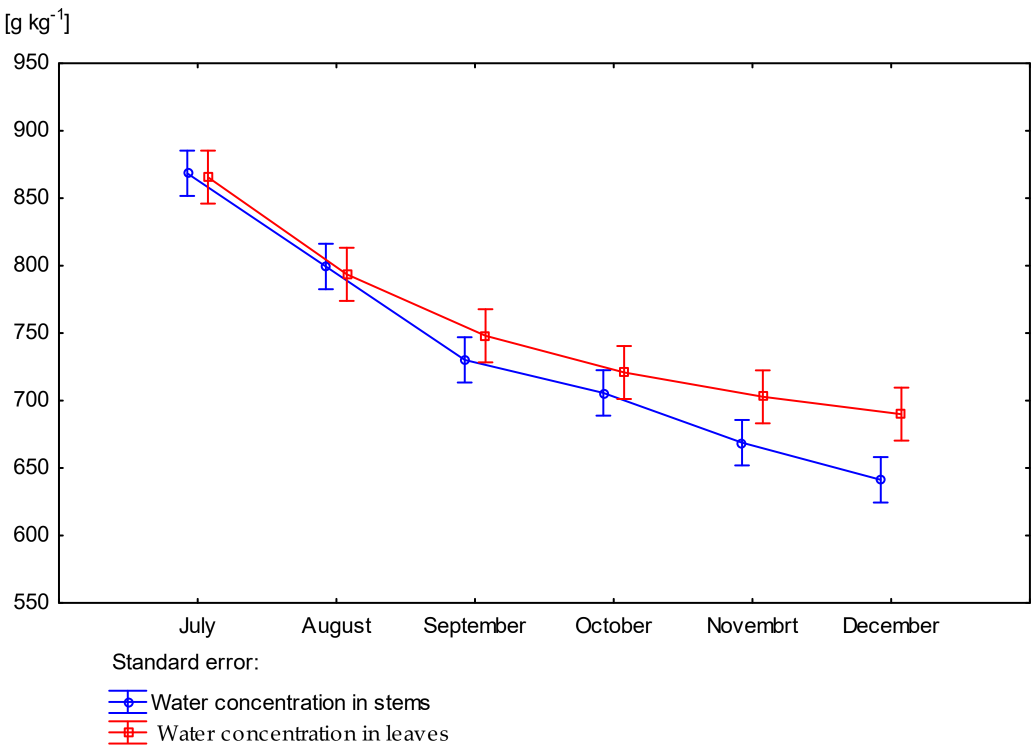

Figure 5. Water concentration in leaves and stems during the vegetation period of the years 2014–2016 (average for years).

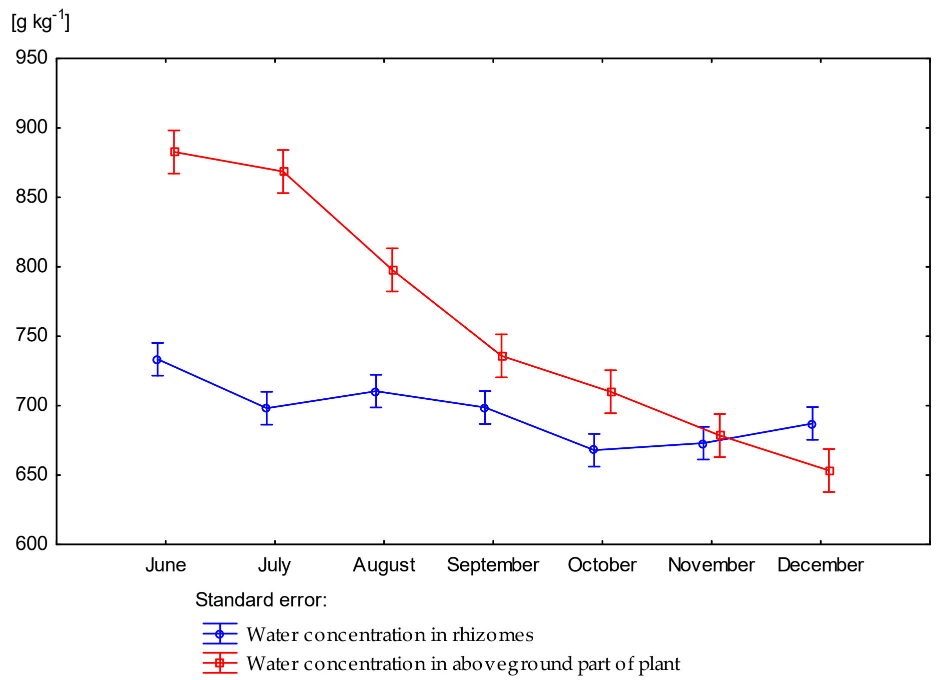

Figure 6. Water concentration in the rhizomes and aboveground part of plants during the vegetation period of the years 2014–2016 (average for years).

3.3. Effect of Nitrogen Fertilization on Dry Matter Yield of Miscanthus × Giganteus

Nitrogen fertilization significantly contributed to an increase in the dry matter yield of leaves (p = 0.0000). The nitrogen fertilization and lack of fertilization of biomass sampling was characterized by an increasing tendency in the dry mass of rhizomes and aboveground parts of plants. The dry mass of the stems grew faster than that of the leaves over the whole vegetation period (Figure 7). The highest yield growth dynamics of the whole plant was observed between August and September (Table 6, Figure 8).

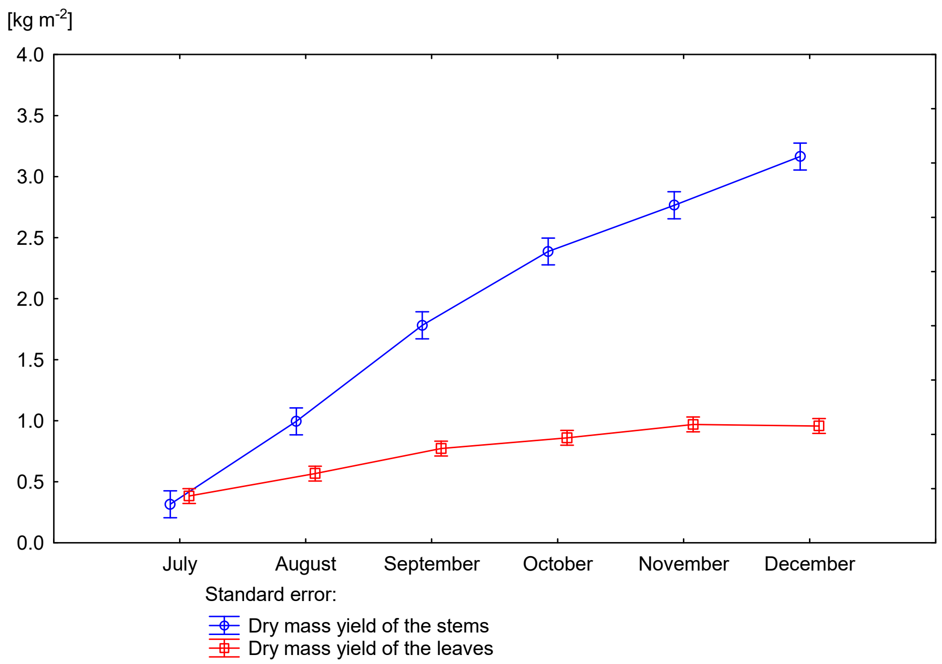

Figure 7. Dry mass yield of the leaves and stems during the vegetation period in the years 2014–2016 (average for years).

Table 6. The yield of the dry mass of *Miscanthus* × *giganteus* (kg m^{-2}) (average for years 2014–2016).

Dose kg ha^{-1} N	Number of Days Starting from Beginning of Vegetation Period	Rhizomes	Aboveground Part			Rhizomes and Aboveground Part
			Stems	Leaves	All Together	
0	June	0.52	-	-	0.34	0.86
	July	0.65	0.28	0.32	0.60	1.25
	August	0.77	0.94	0.45	1.39	2.16
	September	1.15	1.67	0.63	2.30	3.45
	October	1.34	2.22	0.78	3.00	4.34
	November	1.67	2.81	0.89	3.70	5.37
	December	1.73	3.08	0.84	3.92	5.65
60	June	0.73	-	-	0.44	1.17
	July	0.77	0.35	0.45	0.80	1.57
	August	0.97	1.06	0.68	1.74	2.71
	September	1.22	1.90	0.92	2.82	4.04
	October	1.64	2.55	0.94	3.49	5.13
	November	1.64	2.72	1.06	3.78	5.42
	December	1.83	3.25	1.07	4.32	6.15
	p value	0.0125	0.1223	0.1393	0.0153	0.0056
Average for Factors and Years						
0		1.12	1.83	0.65	2.18	3.29
60		1.26	1.97	0.85	2.48	3.74
	p value	0.0524	0.4310	0.0000	0.1586	0.1181
2014		1.20	2.00	0.75	2.41	3.61
2015		1.15	1.91	0.71	2.30	3.45
2016		1.21	1.79	0.79	2.27	3.48
	p value	0.7318	0.6005	0.3165	0.8522	0.8881

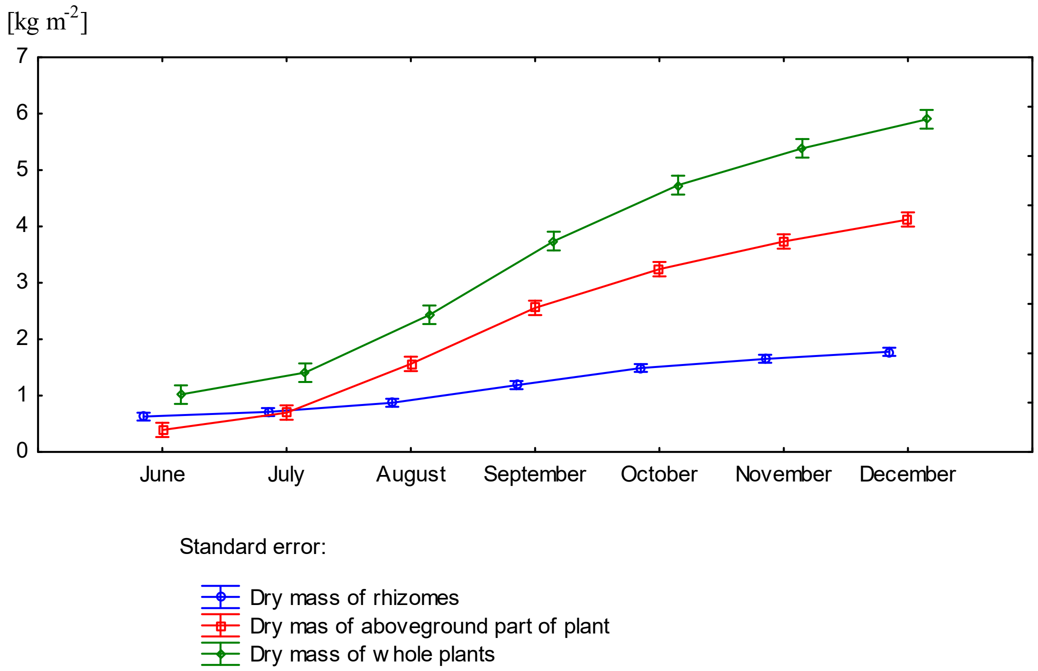

Figure 8. Dry mass yield of the rhizomes and the aboveground part of the plant during the vegetation period of the years 2014–2016 (average for years).

The dry mass of aboveground parts of plants (p = 0.0153) and rhizomes (p = 0.0125) in 30-day intervals significantly differentiated from June to November, in which we obtained the highest values in December (Table 6, Figure 8). In July, the dry matter of leaves was slightly greater than that of the stems, and from this month the increase in the dry matter of stems was greater than that of the leaves. The period between June and July and the November and December vegetation days, constituted 29%

of the entire vegetation period. During this time, a more than 18% increase in the dry weight of the rhizomes and aboveground parts was observed.

3.4. Nitrogen Uptake by Miscanthus × Giganteus

Nitrogen fertilization caused a significant increase in the nitrogen uptake in all the examined parts of plants ($p = 0.0000$). For the control object, the nitrogen uptake by rhizomes decreased until July, whereas in fertilized plots it decreased until August ($p = 0.0118$) (Table 7). The highest uptake of nitrogen in rhizomes was found in December, while in whole plants it was found in November. Therefore, it can be presumed that rhizomes can be a nitrogen reserve for shoots. In the initial vegetation period, the nitrogen uptake in leaves was higher than that in stems. The accumulation of nitrogen in stems was found to be higher than in leaves starting in August (Figure 9). The highest nitrogen uptake was found in the case of whole plants, with an increasing tendency from July to September, where the differences became insignificant (Figure 10). The fastest increase in the N uptake by rhizomes was observed from October to November (Figure 10). In the case of the aboveground parts of plants, the nitrogen uptake increased from June to September and then decreased (Figure 10).

Table 7. Nitrogen uptake of *Miscanthus × giganteus* (kg m^{-2}) (average for years 2014–2016).

Dose kg ha^{-1} N	Number of Days from the Start of the Growing Season	Rhizomes	Aboveground Part of Plants			Rhizomes and Aboveground Part of Plants
			Stems	Leaves	Together	
0	June	5.35	-	-	4.75	10.10
	July	3.25	3.16	4.34	7.50	10.75
	August	3.65	8.24	4.79	13.03	16.68
	September	5.29	10.24	5.99	16.24	21.53
	October	6.62	8.50	6.04	14.54	21.16
	November	10.19	8.61	6.66	15.27	25.46
	December	10.68	7.97	3.97	11.95	22.63
60	June	9.24	-	-	7.60	16.84
	July	5.79	5.08	6.98	12.05	17.84
	August	5.40	8.63	9.52	18.15	23.55
	September	6.91	14.36	11.01	25.37	32.28
	October	7.72	15.71	8.78	24.51	32.23
	November	14.07	12.82	7.19	20.01	34.08
	December	14.94	12.40	5.74	18.15	33.09
	p value	0.0118	0.0000	0.000	0.0000	0.0000
Means for Factors and Years						
0		6.43	7.79	5.30	11.90	18.33
60		9.15	11.50	8.20	17.98	27.13
	p value	0.0000	0.0000	0.0000	0.0000	0.0000
	2014	8.18	10.07	6.53	15.06	23.24
	2015	7.48	10.10	7.05	15.55	23.03
	2016	7.71	8.77	6.67	14.19	21.89
	p value	0.6315	0.1925	0.5895	0.5205	0.6493

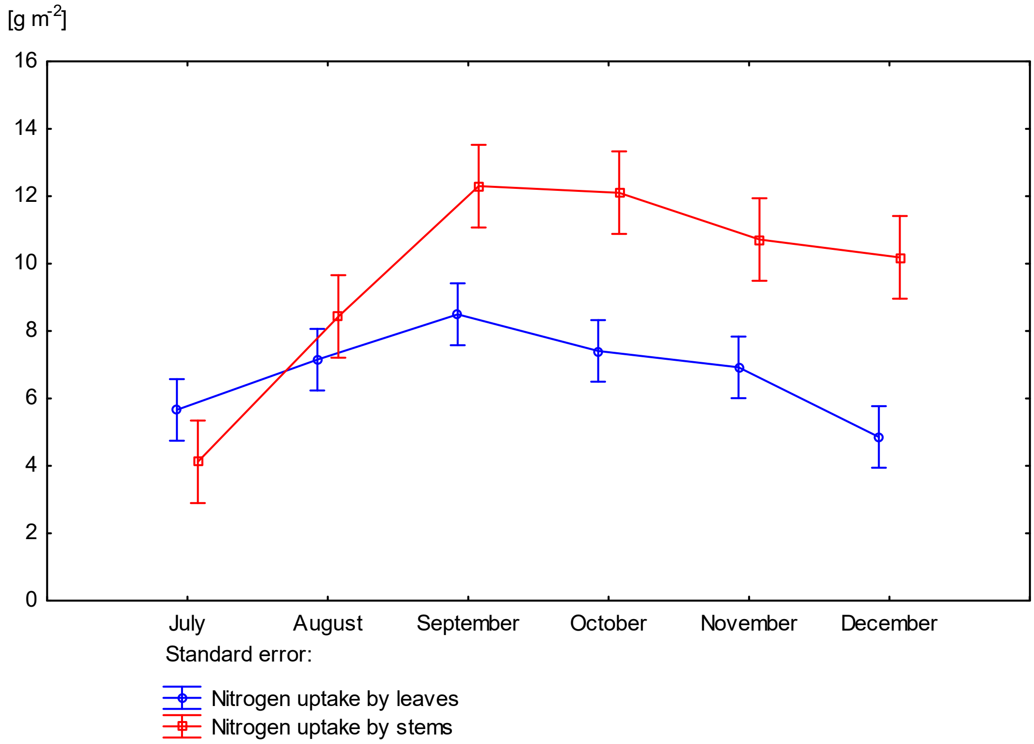

Figure 9. Nitrogen uptake by leaves and stems during the vegetation period in the years 2014–2016 (average for years).

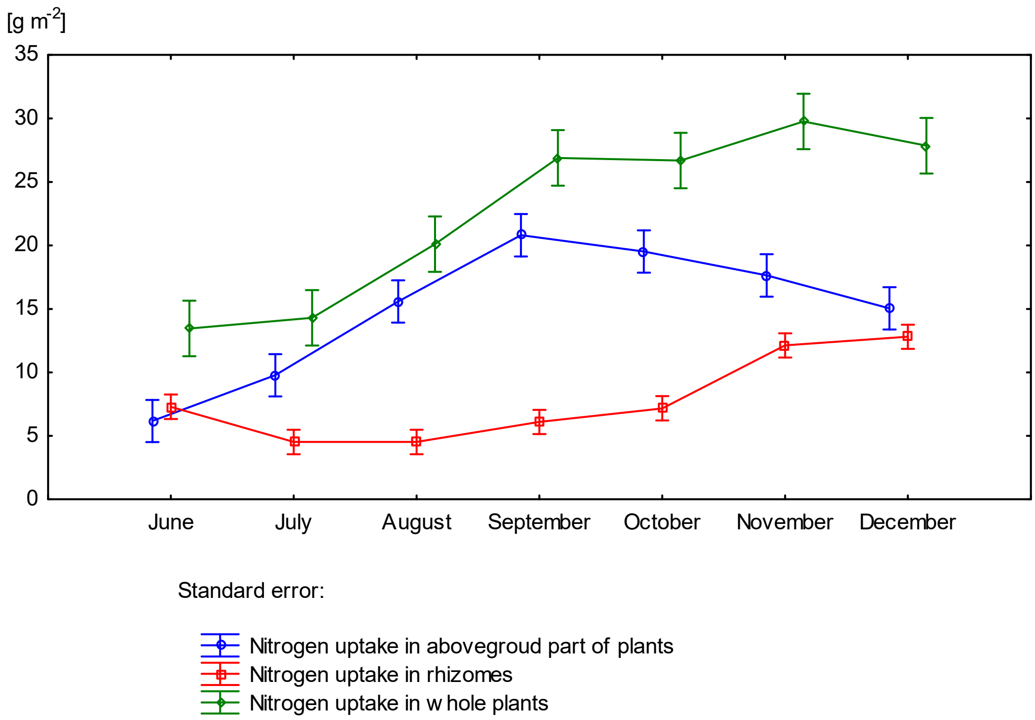

Figure 10. Nitrogen uptake by the whole plants (average for years).

4. Discussion

Nitrogen fertilization is important for biomass production and its components. The results provided statistical evidence to prove that the number of shoots responded positively to N fertilization. Other studies have also shown an increase in the number of shoots after applying N [53–55]. The water concentration in rhizomes and stems, the yield of dry mass leaves, and the nitrogen uptake was dependent on the level of nitrogen fertilization. Higher water content promoted metabolic processes and faster dry mass accumulation [56]. Therefore, research has been undertaken to determine the influence of nitrogen fertilization on the dynamics of the water content changes in rhizomes during the whole vegetation period. According to Drazic et al. (2017) [25], the number of stems per rhizome depended strongly on the soil type and was in strong positive correlation with the yield in all years. In our own research, the number of shoots were not significantly different during the experimental years.

In our research, the application of nitrogen stimulated the number of shoots. The plant height was also increased by N fertilization in various terms of harvesting. The plant height increased after the application of N, which was also reported by Cosentino et al. (2007) [54] and Finnan and Burke (2014) [39].

There have been conflicting results concerning the yield response of *Miscanthus* × *giganteus* to nitrogen fertilization and its yield components. Our positive responses to nitrogen fertilization were in agreement with Arundale et al. 2014 [57]. Moreover, Greef, J.M. (1995) [35] and Lee and Boe (2005) [26] obtained similar results when applying a 60 kg ha^{-1} N dose as appropriate for proper rhizome development and *Miscanthus* × *giganteus* yield increase. In the research of Dierking et al. (2017) [17], a dose of 75 kg ha^{-1} N contributed to the increase in the *Miscanthus* biomass yield, and this amount was applied annually. In the research of Lee and Boe (2005) [26], the dry matter yield visibly increased when the nitrogen fertilization increased up to 60 kg ha^{-1} N. However, increasing the nitrogen dose further did not contribute to an increase in the *Miscanthus* yields. The Miscanthus dry matter yields obtained in this research were 2.55 and 2.49 kg m^{-2} for 60 and 120 kg ha^{-1} N, while in the control plant it was 1.3 kg m^{-2}. Schwarz et al., 1994 [34], conducted an experiment involving nitrogen fertilization that did not have a significant impact on the *Miscanthus* yield. In their second year of cultivation, they obtained a yield of 0.8 kg m^{-2}, and in the third year they obtained 2.2 kg m^{-2}. Moreover, many other studies have shown that nitrogen fertilization is not required to obtain high yields of *Miscanthus* × *giganteus* biomass [58]. Christian et al. (2008) [33] did not find any answer to the applied N in 14 consecutive harvests. This result is supported by other studies that showed no response to N fertilization. However, some experiments have been concerned with soils featuring a large N content [13,21,25,34]. No reaction to nitrogen was found during the first two years after planting. Maughan et al. 2012 [21] reported a small positive reaction in a dose of 100 kg ha^{-1} N of fertilizer. According to Kering et al. (2012) [13], Himken et al. (1997) [58], and Miquez et al. (2008) [21], *Miscanthus* yields are not dependent on the level of nitrogen fertilization, as they determined 2.5–3.0 kg m^{-2} of D.M. and even 3.8 kg m^{-2} of D.M. In our research, the dry matter yield with the nitrogen fertilization of all examined plants was insignificantly higher compared to the control. Only the leaf yields of D.M. depended on nitrogen fertilization.

The ambiguous response to nitrogen fertilization results from several reasons:

1. Most research on *Miscanthus* productivity has been conducted in Europe (different soils, different spatial diversity, and topographic diversity);
2. The studies carried out are generally short-term;
3. The soil type and soil texture [21,37,59];
4. The potential share of nitrogen reserves in rhizomes and soil nitrogen increases the uncertainty of the *Miscanthus* nitrogen requirements [29].

Precipitation is the most important factor that directly and indirectly affects the biomass yield of *Miscanthus* × *giganteus*. Plant biomass production reacts positively to annual rainfall [60], and the seasonal distribution of rainfall is a key factor that determines the formation of perennial grasses and biomass yield [26,60]. In this experiment, the precipitation was variable during the 3-year study period, with much less precipitation than 2015. In our research, the most favorable year with a high and evenly distributed precipitation was in 2016; however, this did not translate into dry matter yields but rather translated to the water content in all the examined plant parts. According to Heaton et al. (2004) [46], the biomass yield may be affected by rainfall during the growing season from April to September.

The nitrogen uptake was significantly affected by the analyzed factors—nitrogen fertilization and the term of harvesting. According to Roncucci et al. (2014) [14], the time of harvest is the most relevant factor in influencing the miscanthus nutrient uptakes. Late harvesting (W) led to a reduction in the nitrogen uptake of about 80% in the aboveground biomass. This nitrogen uptake is observed to be lower than the literature data. In 10 years of research in the UK, Christian et al. (2008) [33] reported that the N is 76 and 6 kg ha^{-1} N. According to Roncucci et al. (2014) [14], N fertilization affected the nutrient uptake mainly in autumn, with no differences in winter. These results are in agreement with those of Himken et al. (1997) [58], who observed a higher N uptake with higher N fertilization rates in November, which is confirmed by our results. Nitrogen fertilization in the fertilizer treatments significantly affected the nitrogen uptake by all plant parts, which is confirmed by Strullu et al. (2011) [30].

Slightly higher results relating to the nitrogen uptake under various N doses in the harvest biomass of giant miscanthus were found in Christian et al. (2008) [33]. In Beale et al. (1997) [29], the rhizome nitrogen uptake decreased until July and then increased until December. Similar conclusions were presented in our research.

5. Conclusions

Nitrogen fertilization did not contribute to the increase in all the examined yield components. The proposed dose caused an increase in all the components of features and the dry matter yield. However, the differences were mostly insignificant. Only the dry mass of leaves increased significantly in the experiment. The water content in the rhizomes and stems increased under nitrogen fertilization. Therefore, we can assume that rhizomes, because of their significant nitrogen uptake, can constitute a nitrogen reserve for elements in the initial growth and development stages of plants. The results coming from our 3-year field experiment suggest that N fertilization is unnecessary for sustainable biomass production.

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