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Effect of White Clover (*Trifolium repens* L.) Undersowing Cultivation and Nitrogen Fertilization on Weed Infestation, Biomass Yield and Its Component, Content and Uptake of Macroelements of Willow (*Salix viminalis* L.)

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Abstract: Fertilization of willow with nitrogen is an important issue with economic and environmental implications. The study was aimed at determining the effect of nitrogen fertilization and white clover on weed infestation, morphology, biomass and chemical composition of willow. A field experiment was established at Wroclaw University of Environmental and Life Sciences (Poland) during the years 2013–2017 with the use of a randomized complete block design (RCBD). The results showed that the number and dry mass of weeds per m^2, the number of willow shoots and the fresh weight yield of willow were smaller where the clover was sown. The plants were found to be higher after applying nitrogen fertilization. The dry mass yield and shoot diameter did not depend on the cultivation method. Nitrogen fertilization increased the ash content. Undersowing willow with white clover, higher nitrogen content was found. Nitrogen fertilization increased the content of ash, and in undersowing cultivation, the willow stems had higher nitrogen content than in monoculture. On average, the phloem had 5.6 times higher crude ash content and 4.6 times higher nitrogen content than wood. On the basis of the conducted research, it can be concluded that in the first years after planting, the undersowing growing of willow with white clover can be an alternative to plantations fertilized and non-fertilized with nitrogen.

Keywords: mixed culture; N-application; crop willow; legumes; chemical composition of plants

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1. Introduction

Short rotation woody crops (SRWC) are important in ensuring a more secure energy future worldwide [1]. The cultivation of energy crops, including short-rotation woody crops (SRWC), leads to an increase in humus content and soil structure improvement [2,3]. Energy crops reduce carbon dioxide emissions both by replacing fossil fuels and by immobilizing organic carbon in soil [4–6]. In the near future, biomass obtained from SRWC will become more important and beneficial [7].

Of all the species cultivated for energy purposes, willow is cultivated in the greatest numbers in Northern Europe [4,7,8]. Due to the numerous environmental benefits, the area of willow cultivation is predicted to grow rapidly over the next decades [9]. This plant is characterized by high biomass increments [10], and due to its natural occurrence in Poland, *Salix viminallis* (L.) is perfectly adapted to the climatic conditions that occur in this country.

Soil erosion and nitrogen leaching on SRWC plantations are lower than on arable land. Nevertheless, it occurs [11–16], especially when plantations are established and on slopes. For instance, Kort et al. [15] stated that the long-term loss of productivity due to soil erosion in Minnesota is 5% but is greater on soils with slopes > 6%. Fertilization of newly established *S. viminalis* in Germany at a level of 75 kg N ha^{-1} per year as calcium nitrate resulted in leaching losses constituting between 23% and 49% of applied N in the first and second years of growth [17].

One way to reduce nitrogen leaching may be to sow white clover during planting energy crops. This plant is a cover crop grown via intercropping with the main crop, maintains soil fertility, and reduces the use of pesticides and mineral fertilizers and minimizes cultivation costs. Mc Laughlin et al. [18] stated that the cultivation of ground cover plants in the field trial with poplars prevented the leaching of fertilizer and nitrogen from the soil. The economic benefits (or losses) from fertilization largely depend on the cost of fertilization and the price received for biomass [19].

Willow is prone to weed infestation in the initial years after planting. It should also be noted that in the first vegetation period, weeds use nitrogen more efficiently than willow, and nitrogen fertilization does not always contribute to the yield increase [20]. In the first year after planting, weeds can limit willow growth from 50% to 95%. Currently, recommendations for the establishment of SRWC involve the use of herbicides and tillage in the autumn to control weeds [21].

Theoretically, undersowing of willow together with white clover can bring a number of benefits. Monoclonal growing systems are considered to be vulnerable to pathogen adaptations. The cultivation of cover crops results in an improvement in biodiversity on willow plantations and may decrease the pressure on willow from pathogens. The compact cover of white clover plants, with a strong root system, prevents erosion. Weed infestation also decreases, which is particularly beneficial for willows when there are high and climbing weeds on the plantation [22]. The symbiotic nitrogen fixation by bacteria coexisting with white clover increases the level of nitrates in the soil. The increase in soil nitrogen content depends on the number of root nodules, the rate of nitrogen fixation from the air and the length of the growing season. Fertilization, other organisms that fix nitrogen from the air and atmospheric lighting may also contribute to the increase in soil nitrate [23]. White clover can supply 100 kg ha^{-1} of atmospheric nitrogen (equivalent to 200 kg of nitrogen in mineral fertilizers) to the soil in chemical compounds [16].

However, white clover competes with willow for nutrients. It has been found that live cover plants integrated with woody plants during the establishment of plantations compete with each other for moisture and possibly light, resulting in reduced growth and leaching [24,25]. Other studies have not confirmed these observations [21,22]

The aim of this study was to compare differences in yield, morphology and chemical composition of willow fertilized and unfertilized with nitrogen and undersowing cultivation (willow with white clover) and then to compare these for 2013–2017. In the working hypothesis, it was assumed that undersowing cultivation with white clover could replace fertilization with nitrogen. The results of the research can be particularly useful for biomass producers. They can also be an inspiration for additional research for the needs of willow growers.

2. Materials and Methods

2.1. Study Sites

A field trial was conducted at Wroclaw University of Environmental and Life Sciences in Poland in 2013–2017. The field trial was located in a suburban area at a distance of 10 km northwest of the center of Wroclaw. The field plots were located at an altitude of 147 m above sea level, in the river Dobra's catchment area, a right-bank tributary of the river Widawa. The geographical position is defined by the following coordinates: 5°10′ N, 17°07′ E.

2.2. Soil Conditions

The field experiments were conducted on a light soil defined as very light alluvial soil, on loose sand and sandy gravel. Each year, soil samples (from each plot) were analyzed. Samples were taken at a depth of 0–30 cm at the start of the vegetation period. Analysis was performed according to the following methods:

- The soil reaction (KCl) by the potentiometric method;
- The available forms of potassium and phosphorus by Egner–Riehm method [26,27];
- Magnesium by Schachtschabel method [28].

The soil is visibly alkaline, the content of phosphorus ranges from medium to high, and potassium from high to very high [26], and the content of Mg is medium [28] (Table 1):

Table 1. Soil pH and macronutrient abundance over the period 2013–2017.

Years	pH	Content of Assimilable Forms (mg kg^{-1})		
	(1 M KCl)	P	K	Mg
2013	7.45	159	154	36.0
2014	7.85	140	160	38.9
2015	7.80	162	153	30.8
2016	7.64	178	186	36.4
2017	7.75	170	193	34.8
Mean	7.70	162	169	35.4
SD	0.159	14.250	18.89	2.963

SD—standard deviation.

2.3. Weather Conditions

The research was conducted in one of the warmest of Lower Silesia's regions. From 1901 to 1950, the average annual air temperature was 8.6 °C, the average temperature of the vegetation period (IV–IX) was 14.7 °C. The groundwater level was 85 cm below the ground level. The mean annual temperature during the study was higher than the long-term trend. The monthly and annual precipitation sums varied. The lowest precipitation was reported in 2015. Specific temperature and precipitation breakdown are described in Tables 2 and 3.

Table 2. Monthly mean air temperature (°C) over the period 2013–2017.

Months	Years					Standard Deviation 2013–2017	Mean Temperature (30-Year Monthly)
	2013	2014	2015	2016	2017		
January	−1.6	0.0	2.3	−1.2	−3.4	2.11	−0.4
February	0.1	3.7	1.5	3.8	0.9	1.67	0.6
March	−0.9	7.0	5.4	4.3	6.8	3.22	3.8
April	9.2	10.6	8.9	8.7	7.9	0.99	8.9
May	14.6	13.3	13.5	15.3	14.2	0.82	14.4
June	17.7	16.6	16.6	18.6	18.5	0.98	17.3
July	20.5	21.2	20.3	19.5	19.0	0.86	19.6
August	19.0	17.3	22.7	17.9	19.4	2.10	18.6
September	12.9	15.5	15.1	16.4	13.3	1.49	13.7
October	10.8	10.7	8.4	8.5	12.0	1.57	9.1
November	5.6	6.6	6.2	3.4	5.5	1.24	4.3
December	3.0	2.3	5.4	1.2	2.9	1.54	0.6
Mean	9.2	10.4	10.5	9.7	9.8	0.54	9.2

Table 3. Monthly total precipitation (mm) over the period 2013–2017.

Months	**Years**					**Standard Deviation 2013–2017**	**Sums of Precipitation (30-Year Monthly Average)**
	2013	**2014**	**2015**	**2016**	**2017**		
January	51.3	35.8	46.0	33.4	16.9	13.26	34.9
February	29.5	1.2	15.6	56.2	24.2	20.30	27.9
March	43.0	40.1	39.5	55.9	31.1	8.98	38.2
April	42.7	55.2	15.8	46.4	57	16.55	33.6
May	135.9	101.4	21.0	5.3	24.1	57.55	54.1
June	171.7	40.2	73.3	44.6	52.5	54.74	67.4
July	36.3	52.9	55.6	114.3	112.2	36.36	78.9
August	68.2	75.0	5.6	27.1	43.6	28.75	65.3
September	105.8	72.2	23.2	44.7	65.7	30.99	44.9
October	7.8	59.4	20.0	83.8	71.4	33.01	33.7
November	25.8	15.5	52.5	36.3	28.4	13.80	36.6
December	13.0	17.5	24.0	36.1	29.6	9.23	36.3
Sum	731.0	566.4	392.1	584.1	556.6	120.29	551.8

2.4. Material and Management Practices

This study was conducted in an experiment with a *Salix viminalis* var. *gigantea* (1047 willow clone). This variety is intended for biomass producers. Research with this variety has been described out by many researchers [29–32]. It was planted with 20 cm ligneous one-year-old cuttings obtained from a nearby plantation (planted in 2010) immediately before planting the plants. The density of cuttings was 16,700 plants ha^{-1}. The row distance was 1 m, and the distance between plants in a row was 0.6 m. White clover of the Romena cultivar was sown in the amount of 10 kg ha^{-1} (hand-sown during plantation setup). The clover was not mown during the studied period. For the first two years in tested undersowing cultivation, clover covered about 95% of the area. Clover dying was observed because of little access to light and drought in the third year of the experiment. To determine the yield, willow plants were harvested after the fifth vegetation period. The chemical composition was determined on four and five-year-old plants.

The preferred crop was winter oilseed rape (OSR), and the forecrop was fallow land. The most frequently weed species in the year preceding plantation establishment included: *Elymus repens* (L.) Gould., *Viola arvensis* Murr. and *Thlaspi arvense* L. In July and September 2012, two sprays of Roundup 360 SL herbicide at a dose of 5 L ha^{-1} were applied, and autumn plowing was performed. In spring, the field was evened out with a cultivator. After these treatments, weeds were no longer controlled until the end of the experiment.

Mineral fertilizers were applied annually in spring (before the start of vegetation). Fertilized plots annually received 35.2 kg P ha^{-1} and 83 kg K ha^{-1} in the form of triple superphosphate and potassium salt. Urea (50 kg N ha^{-1}) was applied on nitrogen fertilized plots.

2.5. Measurement

The number and dry mass of weeds were assessed each year in June. A 50 cm × 50 cm metal frame was placed on the ground between the rows at four places within each treatment plot. The numbers and species of weeds were recorded inside each frame. The weed biomass was assessed by weighing their fresh mass. The dry matter of the weeds was established by the drying method. The fresh mass of weeds was dried at 60 °C for 24 h and then for five hours at 105 °C. After harvest in December 2017, absolute dry mass yield and water content in *Salix* plants were determined based on 4 randomly chosen shoots from every plot. The shoot fragments were cut at a distance of 20 cm on both sides of the gravity center point and subsequently dried at 60 °C for 1 week until a constant weight was reached, and then dried for 5 h at 105 °C (modified norm PN-R-04013:1988). The weight of fresh and dry stem pieces was determined with an accuracy of 0.01 g. Total shoot dry weight per plot was calculated by multiplying the total fresh weight of all sampled shoots for each plot by the dry matter content. Plant shoot dry weight was calculated as total shoot dry weight per plot divided by the number of living plants in each plot.

At the beginning and end of the vegetation period, the diameter and height of the main shoot were measured 20 cm above the ground, and plant losses were determined. The plant was designated as dead if no living tissue was detected at 5 cm above the ground.

2.6. Chemical Analysis

Plant material was collected at the end of the growing season (December 2016 and 2017). Stems were collected from ten randomly chosen plants. Plant material was dried and minced, and chemical analysis was performed:

- Crude ash—determined by burning of 2 g plant material at 600 °C in an electric furnace. After a loss on ignition, the crucibles were placed in a desiccator to cool down and then weighed to the nearest 0.001 g.
- The Kjeldahl method using the Kjeltec apparatus was applied for total nitrogen determination [33].
- Potassium and calcium concentrations were determined by flame photometry using Flapho 4 (Carl Zeiss Jena—GDR).
- Phosphorus concentration was determined by a colorimetric method with vanadium and molybdenum reagents using Spekol 10—Carl Zeiss (Jena—GDR).
- Magnesium concentration was determined by a colorimetric method with yellow titanium using Spekol 10 (Carl Zeiss Jena—GDR).

I separate the phloem from the wood manually. The uptake of crude ash and macroelements was calculated as a multiplication of the dry mass yield and chemical concentration.

2.7. Statistical Analysis

Five years (2013–2017) of one-factor field experiment was established with the use of a randomized complete block design (RCBD). The study was based on rigorous 1-factor field trials. The study compared weed assessments, morphological features, yield, and chemical composition of (a) basket willow in monoculture without nitrogen fertilization, (b) basket willow in undersowing cultivation with white clover and (c) basket willow monoculture fertilized with nitrogen at a dose of 50 kg ha^{-1}. The area of plots was 12 m^2 with 4 replications of each treatment.

All examined parameters were statistically evaluated, using analysis of variation, at a 0.05 level of confidence. Plant mortality variations between treatments were compared using data from square root transformations. The conversion was performed to meet the assumptions of the standard normal distribution. The AWA program [34] was used for the least significant difference (LSD). The results were statistically analyzed using STATISTICA 13.0 PL, Palo Alto, CA, USA. Homogeneous groups were determined by Tukey's multiple range test using consecutive letters starting from "a"—the most beneficial value—to "e"—least beneficial in terms of economic value. ± figures in tables represent the standard deviation. The vertical line at the top of each bar in Figure 1 is the standard deviation for that mean.

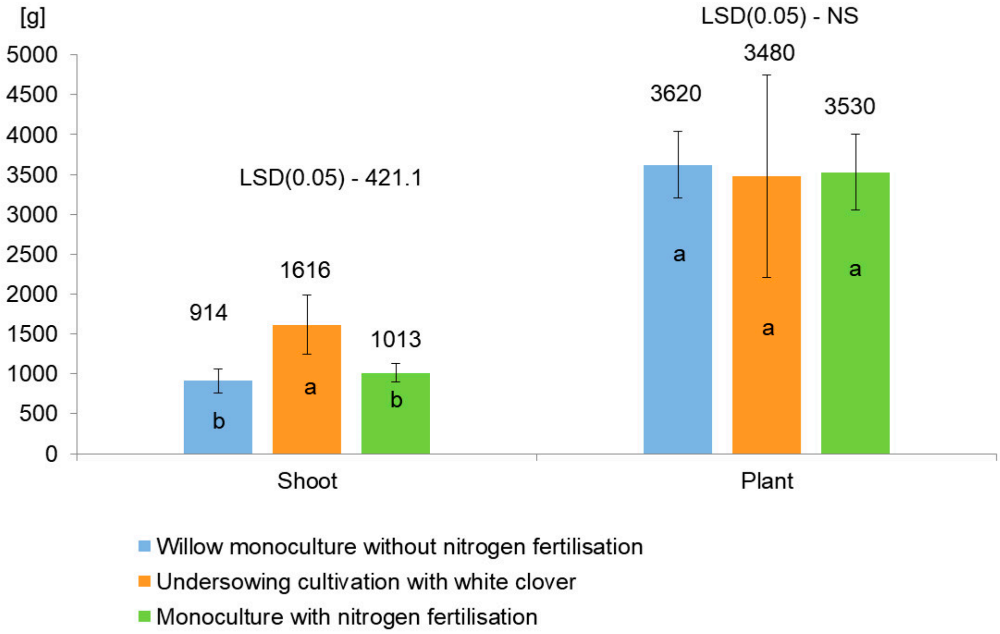

Figure 1. Influence of undersowing cultivation and nitrogen fertilization on the dry mass of 1 shoot and plants of willow plants after harvest in December 2017. LSD—the least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test).

3. Results and Discussion

3.1. Weed Assessments

Weed infestation has a significant effect on yield and plant mortality [35]. In a study carried out by Welc et al. [36], weed competition reduced aboveground biomass production on average by 36%. In our own research for the first two years, the weed flora was dominated by *Elymus repens* (L.) Gould, *Thlaspi arvense* L., *Viola arvensis* Murr. and *Chenopodium album* L. In the next few years, the occurrence of the following perennial weeds increased: *Elymus repens*, *Artemisia vulgaris* L., *Urtica dioica* L., *Solidago canadensis* L., *Achillea millefolium* L., *Taraxacum officinale* F.H. Wigg. Furthermore, Albertsson et al. [37] noticed that the weed flora was initially dominated by annuals for the first two years but became dominated by perennial weeds during the following years. The number and dry weight of weeds, in general, decreased during the 2013–2015 field experiment. In the Edelfeldt et al. [38] study, higher weed biomass was obtained on plots with high nitrogen levels, where they were sown earlier. In our study, the lowest weed count was noted with the undersowing cultivation of willow and white clover (Table 4). Similar results have been reported by other authors [22,39]. The largest dry mass of weeds was achieved in treatment fertilized with nitrogen and the lowest on plots with white clover. The results obtained for a dry matter of weeds showed a high level of variability as measured by the standard deviation. The differences were significant in the first and third years of the study (Table 4). Fertilization may instead benefit weeds more than willow resulting in an increased competitive ability and a high relative growth rate for the weeds. These observations are confirmed by the studies of Balasus et al. [17]. In another study [38], in the first year, fertilization increased weed biomass growth by 46%, suggesting that weeds are more responsive to fertilization than willow.

Table 4. Dry weight and number of weeds.

Years	Cultivation Willow	Number of Weeds (pcs m^{-2})	Dry Weight of Weeds (g m^{-2})
2013	A	21 e ± 4.4	384 ab ± 270.8
	B	13 abc ± 1.2	23 a ± 22.6
	C	19 de ± 1.6	698 b ± 391.2
2014	A	18 cde ± 4.0	244 a ± 77.2
	B	11 a ± 0.6	201 a ± 71.6
	C	17 bcde ± 2.4	325 a ± 133.4
2015	A	16 abcde ± 2.8	255 a ± 41.2
	B	12 ab ± 0.4	30 a ± 7.8
	C	13 abc ± 1.8	279 a ± 94.8
2016	A	15 abcd ± 0.8	223 a ± 7.0
	B	12 ab ± 0.6	168 a ± 22.6
	C	15 abcd ± 1.0	216 a ± 18.6
2017	A	13 abc ± 0.8	207 a ± 197.8
	B	12 ab ± 0.6	252 a ± 74.0
	C	16 abcde ± 1.6	180 a ± 91.0
LSD (α = 0.05)		2.7	189.4
Mean for Cultivation System and Years			
	A	17 b ± 3.9	262 b ± 152.1
	B	12 a ± 0.9	135 a ± 104.2
	C	16 b ± 2.6	339 b ± 257.3
LSD (α = 0.05)		1.2	84.7
2013		18 b ± 4.3	368 b ± 380.7
2014		15 ab ± 4.1	257 ab ± 103.7
2015		14 a ± 2.5	188 a ± 129.1
2016		14 a ± 1.7	202 ab ± 30.0
2017		14 a ± 2.0	213 ab ± 124.0
LSD (α = 0.05)		1.6	109.3

A—willow monoculture without nitrogen fertilization; B—undersowing cultivation with white clover; C—willow monoculture with nitrogen fertilization. ± figures in tables represent the standard deviation. LSD—the least significant difference. Different letters indicate significant differences between treatments (Tukey's multiple range test).

3.2. Plant Mortality Assessments

By the second 3-year harvest, willow survival was still over 80% in the McCracken et al. [40] research. In our own research, in general, 83% of the planted plants survived within 5 years. The number of plants per m^2 decreased significantly in the study years, but no significant differences were found in the number of plants dying in different cultivation systems. Willow loss mainly occurred in the first year after the experiment was established. The lowest rainfall was recorded in 2015 (Table 3), which resulted in a higher number of withered willow plants than in the previous year. At the same time, in that year, the highest standard deviation was recorded for the number of shoots per plant and per 1 m^2. Willow plants produced on average about 3 shoots from one cut (Table 5). In the research by Hangs et al. [41], where the willow plants were cut down after the first year after planting, the number of shoots per plant was 2–3 times higher than that in our own experiment. There were no significant differences between study years and year-by-system cultivation interactions regarding planting density (Table 5).

Table 5. Plant density in undersowing and monoculture cultivation.

Years	Cultivation System	Number of Plants (pcs m^{-2})	Plant Mortality *		Number of Shoots	
			a	b	(pcs m^{-2})	(pcs plant^{-1})
2013	A	1.52 a ± 0.077	8.98	2.93 a ± 0.752	4.98 ab ± 0.720	3.27 abc ± 0.342
	B	1.48 ab ± 0.077	11.38	3.31 a ± 0.735	3.22 bcdef ± 0.552	2.19 bc ± 0.421
	C	1.44 ± 0.161	13.92	3.22 a ± 2.169	4.67 abc ± 0.889	3.23 abc ± 0.284
2014	A	1.50 a ± 0.065	1.27	0.56 a ± 1.125	4.88 abc ± 0.745	3.25 abc ± 0.473
	B	1.46 ab ± 0.046	1.27	0.56 a ± 1.125	3.03 cdef ± 0.660	2.08 bc ± 0.454
	C	1.44 ab ± 0.161	0.0	0.00 a ± 0.000	4.55 abcd ± 0.757	3.16 abc ± 0.181
2015	A	1.36 ab ± 0.126	9.39	2.16 a ± 2.505	5.16 a ± 1.415	3.86 a ± 1.298
	B	1.29 ab ± 0.146	11.7	2.91 a ± 2.067	2.70 def ± 0.890	2.08 bc ± 0.600
	C	1.38 ab ± 0.052	3.74	0.97 a ± 1.935	4.26 abcde ± 0.190	3.10 abc ± 0.223
2016	A	1.36 ab ± 0.126	0.00	0.00 a ± 0.000	4.97 ab ± 0.832	3.65 a ± 0.417
	B	1.25 ab ± 0.181	3.36	1.29 a ± 1.497	2.38 ef ± 0.471	1.91 c ± 0.296
	C	1.35 ab ± 0.045	1.59	0.63 a ± 1.260	4.11 abcdef ± 0.538	3.05 abc ± 0.449
2017	A	1.29 ab ± 0.221	5.00	1.12 a ± 2.235	5.12 a ± 0.734	4.06 a ± 0.987
	B	1.13 b ± 0.336	11.65	2.36 a ± 2.848	2.36 f ± 0.762	2.15 bc ± 0.490
	C	1.27 ab ± 0.077	6.00	1.73 a ± 2.001	4.42 abcd ± 0.262	3.48 ab ± 0.159
LSD (α = 0.05)		NS	-	NS	NS	NS
Mean for Cultivation System and Years						
	A	1.40 a ± 0.151	4.93	1.35 a ± 1.804	5.02 a ± 0.831	3.62 a ± 0.781
	B	1.32 a ± 0.215	7.86	2.09 a ± 1.921	2.74 c ± 0.703	2.08 c ± 0.423
	C	1.37 a ± 0.18	5.05	1.31 a ± 1.873	4.40 b ± 0566	3.20 b ± 0.293
LSD (α = 0.05)		NS	-	NS	0.454	0.329
2013		1.48 a ± 0.107	11.43	3.15 b ± 1.271	4.29 a ± 1.0377	2.89 a ± 0.612
2014		1.47 a ± 0.98	0.84	0.37 a ± 0.876	4.15 a ± 1.067	2.83 a ± 0.661
2015		1.34 ab ± 0111	8.26	2.01 ab ± 2.143	4.04 a ± 1.379	3.01 a ± 1.073
2016		1.32 ab ± 0.128	1.65	0.64 a ± 1.161	3.82 a ± 1.261	2.87 a ± 0.834
2017		1.23 b ± 0.227	7.55	1.74 ab ± 2.224	3.97 a ± 1.352	3.23 a ± 1.018
LSD (α = 0.05)		0.120	-	1.316	NS	NS

A—willow monoculture without nitrogen fertilization; B—undersowing cultivation with white clover; C—willow monoculture with nitrogen fertilization. ± figures in tables represent the standard deviation. LSD—least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test). * a—% of dead plants, b—plant mortality variations between treatments were compared using data from square root transformations. The conversion was performed to meet the assumptions of the standard normal distribution.

A less restrictive test of homogeneous groups showed that willow intercropped with white clover in the last year had the fewest shoots per 1 m^2 compared to the other cropping systems (Table 5). The highest number of shoots per plant and shoots per m^2 was obtained by growing willow in monoculture without nitrogen fertilization and the lowest by growing willow and white clover in undersowing cultivation. Therefore, farmers who decide to cultivate willow with white clover should plant willow in this undersowing system more densely than when growing willow in monoculture., This method of cultivation may turn out to be risky in case of low precipitation.

3.3. Morphological Traits

The stem diameter, plant height and number of shoots were found to be significant factors variable for willow biomass production [38]. The highest plant growth dynamics were observed in the second year of the study. Regardless of the cultivation system, five years after planting, the willow plants reached a height of more than 5 m and a diameter of about 40 mm, and the differences between the cultivation systems were negligible (Table 6). Furthermore, the volume of trees fertilized with easily soluble fertilizers was no different from those fertilized with controlled-release fertilizers in the research by Brown and van den Driessche [42]. Sevel et al. [1] measured the diameter of 3-year-old shoots as being from 18.1 to 27.2 mm compared to the 25.8 mm observed in the own study (Table 6).

Table 6. Influence of undersowing cultivation and nitrogen fertilization on morphological traits of willow plant.

Years	Cultivation System	Height of Plants at the End of Vegetation (cm)	Yearly Increase in the Height of Plants (cm)	Diameter of Shoots After the End of Vegetation (mm)	Yearly Increase in Shoot Diameter (mm)
2013	A	80 f ± 3.5	80 a ± 3.5	10.0 e ± 0.22	10.0 a ± 0.22
	B	75 f ± 10.3	75 a ± 10.3	9.8 e ± 0.14	9.8 a ± 0.14
	C	83 f ± 6.8	83 a ± 6.8	10.2 e ± 0.14	10.2 a ± 0.14
2014	A	212 e ± 42.6	132 a ± 45.7	17.5 de ± 2.38	7.5 a ± 2.25
	B	215 e ± 24.2	141 a ± 31.4	17.4 de ± 5.29	7.6 a ± 5.24
	C	237 e ± 20.9	154 a ± 16.8	20.0 cde ± 1.93	9.7 a ± 2.05
2015	A	287 de ± 39.9	76 a ± 22.5	26.2 bcd ± 2.87	8.7 a ± 0.83
	B	300 de ± 50.8	85 a ± 37.8	24.3 bcd ± 3.73	6.9 a ± 2.27
	C	332 cd ± 12.4	95 a ± 13.0	26.9 bcd ± 3.89	7.0 a ± 2.26
2016	A	365 bcd ± 56.0	77 a ± 78.8	30.0 abc ± 4.04	3.8 a ± 2.39
	B	415 bc ± 80.6	115 a ± 35.1	33.0 ab ± 6.89	8.7 a ± 6.49
	C	440 ab ± 21.6	108 a ± 14.0	34.8 ab ± 7.34	7.8 a ± 10.41
2017	A	510 a ± 30.7	145 a ± 57.8	40.4 a ± 6.20	10.4 a ± 6.67
	B	522 a ± 21.8	107 a ± 82.6	42.0 a ± 10.25	9.1 a ± 6.02
	C	526 a ± 20.0	86 a ± 36.9	42.0 a ± 5.13	7.1 a ± 2.95
LSD (α = 0.05)		NS	NS	NS	NS
Mean for Cultivation System and Years					
	A	291 b ± 152.1	102 a ± 53.7	24.8 a ± 11.2	8.1 a ± 3.83
	B	305 ab ± 164.1	104 a ± 47.3	25.3 a ± 12.89	8.4 a ± 4.32
	C	324 a ± 159.7	105 a ± 31.8	26.8 a ± 12.06	8.4 a ± 4.67
LSD (α = 0.05)		22.9	NS	NS	NS
2013		79 e ± 7.7	79 b ± 7.68	10.0 e ± 0.23	10.0 a ± 0.23
2014		221 d ± 30.2	142 a ± 31.6	18.3 d ± 3.43	8.3 a ± 3.35
2015		307 c ± 39.6	85 b ± 25.4	25.8 c ± 3.40	7.5 a ± 1.94
2016		407 b ± 61.9	100 ab ± 48.7	32.6 b ± 6.03	6.8 a ± 6.90
2017		519 a ± 23.4	113 ab ± 61.6	41.5 a ± 6.85	8.9 a ± 5.13
LSD (α = 0.05)		29.6	34.5	3.98	NS

LSD—least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test). ± figures in tables represent the standard deviation.

Mild temperatures and frequent rainfall are suitable conditions for growing willows. Willows have very high evapotranspiration requirements, and in Sweden, for optimal growth at mid-summer, 5–6 mm of available water per day are needed [43]. Sufficient precipitation was recorded only in June 2013. In 2015 because of the lowest precipitation, the lowest plant height increases were noticed except for the first-year trial (Tables 3 and 6). In the reported experiments [21], it was observed that during the first 2 months after plowing of 34-day old clover, willow grew better in a pure stand. In the own trial, we observed that the plant height in undersowing cultivation was higher than in the non-fertilized plants. However, the highest plant height was observed on plots where nitrogen fertilization was applied (Table 6). Although there were no significant year-by-high of plant differences in plant interactions, the homogeneous groups test showed that in 2015 and 2016, nitrogen-fertilized willow was higher than in the other cropping systems. The highest standard deviation of the height of the plant was recorded in 2016 (Table 6).

There were the least number of shoots per m^2 and shoots per plant in undersowing cultivation (Table 5). However, the higher accessibility of light and nutrients later meant that the dry mass of willow shoots in this cultivation was greater. The dry mass of plants (as a result of multiplying the number of shoots per plant by the mass of one shoot) did not vary between the different cultivation systems (Figure 1).

A regression analysis confirmed the presence of a significant correlation between the diameter of shoots, the height of plants and the dry mass of the main shoot (Tables 7 and 8). Similar to the study by Krzyżaniak [44], a correlation was observed between the height of the main shoot and its diameter. A significant correlation (r^2 = 0.86) between age and diameter was found in one study [45] using regression analysis.

Table 7. Correlation coefficients for the analyzed traits in 2017.

Cultivation System	Mean	Standard Deviation	Correlation Coefficients *		
			Dry Mass of the Main Shoot	Diameter of Shoots	Height of Plants
Dry mass of the main shoot	1187 g	605	1		
Diameter of shoots	39.5 mm	8.99	0.90	1	
Height of plants	510 cm	100	0.74	0.75	1

* All correlation coefficients are significant.

Table 8. Correlation coefficients for the analyzed traits (for the cultivation system) in 2017.

Cultivation System	Mean	Standard Deviation	Correlation Coefficients		
			Dry Mass of the Main Shoot	Diameter of Shoots	Height of Plants
Willow monoculture without nitrogen fertilization					
Dry mass of the main shoot	908 g	545	1		
Diameter of shoots	34.3 mm	6.7	0.98	1	
Height of plants	452 cm	72.7	0.81	0.82	1
Undersowing cultivation with white clover					
Dry mass of the main shoot	1385 g	729	1		
Diameter of shoots	41.8 mm	9.6	0.93	1	
Height of plants	556 cm	123.7	0.71	0.69	1
Monoculture with nitrogen fertilization					
Dry mass of the main shoot	1269 g	468	1		
Diameter of shoots	42.6 mm	8.89	0.81	1	
Height of plants	552 cm	76.3	NS *	0.71	1

* NS—correlation coefficient is not significant.

3.4. Yield of Fresh and Dry Mass of Willow

Willow can yield 20 Mg ha^{-1} under optimal conditions [46]. Based on the experience of other authors, the assumed yield of willow biomass for the first cycle is 10 Mg dry mass ha year^{-1} [17,46]. For example, yields are given 9.1 Mg ha^{-1} in the UK [47], in Swedish 7 Mg dry mass ha^{-1} per year. In other research [48,49], yield in Poland was higher compared to my field experiment. The subsequent rotations grow faster because the willow root system is already established [50]. Some scientists state the increase in biomass yields willow and poplar as a result of N, P and K fertilization [51]. The nitrogen levels had no significant effect on willow yield in a two-year study by Balasus et al. [17]. The total dry mass of willow in undersowing cultivation with white clover was not significantly different from the total dry mass of willow under the control treatment in own study (Table 9). Similar results were obtained by Arevalo et al. [21] after several months of growing *S. sachalinensis* and *S. discolor* with white clover. However, in our own study, the yield of fresh mass was significantly lower in willow with white clover than in monoculture. The water content in the fresh matter of the plants was about 50% (Table 9). In another study [22] at higher precipitation willow–white clover undersowing cultivation had a positive effect on the yield of fresh willow weight.

Table 9. Comparison of yielding in monoculture and willow undersowing cultivation 5 years after planting.

Cultivation System	Dry Mass Yield (Mg ha^{-1})			Fresh Mass Yield (Mg ha^{-1})
	Wood	Phloem	Total	
Willow monoculture without nitrogen fertilization	36.8 a ± 4.02	9.4 a ± 1.06	46.2 a ± 5.08	91.2 a ± 7.12
Undersowing cultivation with white clover	29.0 a ± 4.19	7.3 a ± 1.04	36.3 a ± 5.23	71.1 b ± 1.39
Monoculture with nitrogen fertilization	35.7 a ± 3.54	9.0 a ± 0.88	44.7 a ± 4.42	90.5 a ± 6.33
LSD (α = 0.05)	NS	NS	NS	9.02

LSD—least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test). ± figures in tables represent the standard deviation.

3.5. Chemical Composition and Uptake of Macronutrients in Willow Plants

The main environmental problems associated with short-rotation woody crops in relation to plant nutrients include changes—either depletion or accretion in elemental content of the soil and disposal of ash or other wastes left after burning or other processes using the biomass [12]. Furthermore, a high ash and potassium concentration lower its calorific value [52]. On the other hand, wood ash can be used to fertilize and increase soil pH, thus improving plant growth [12]. Estimation of ash content and macronutrients may, therefore, be important for practical purposes.

Similar to the study by Schroeder et al. [53], the ash content was approximately 16 g kg^{-1} dry mass, and the highest ash content was recorded in willow plants fertilized with inorganic nitrogen (Figure 2).

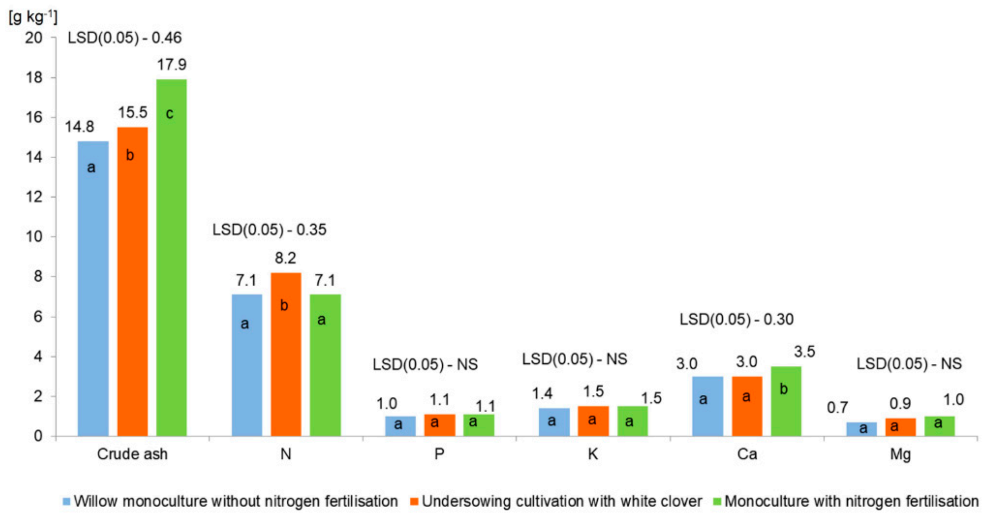

Figure 2. Nutrient content in whole willow plants with two fertilizing treatments and undersowing system (average for 2016–2017). LSD—the least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test).

The nitrogen content was the highest in the undersowing cultivation. Labrecque and Teodorescu [54] suggest that on sites where soil nitrogen content is high, willows have a lower response to fertilization. In contrast to nitrogen, the potassium content was lower in our research than in Sevel [55]. The phosphorus content was comparable to the results obtained by Jama-Rodzeńska et al. [31]. In all instances, the wood had less ash and macroelements than the phloem (Table 10). The ratio of dry wood mass to phloem dry mass in undersowing cultivation and nitrogen fertilization was 3.9:1, and for the control, 3.91:1. Nitrogen fertilization (and production of nitrogenous fertilizers) and combustion of biomass with high nitrogen content adversely affect the climate and human health [56]. Therefore, considering the protection of the environment and human health, the best way of cultivation turned out to be a willow monoculture without nitrogen fertilization.

Table 10. Nutrient content in willow with two fertilizing treatments and undersowing system in 2017.

Cultivation System	Crude Ash	N	P	K	Ca
	g kg^{-1}				
Willow Wood					
Willow monoculture without nitrogen fertilization	7.2	3.9	0.7	0.8	1.4
Undersowing cultivation with white clover	7.3	5.1	0.9	1.0	1.5
Monoculture with nitrogen fertilization	10.2	3.8	0.7	0.9	1.9
Willow Phloem					
Willow monoculture without nitrogen fertilization	43.1	19.0	2.3	3.4	9.3
Undersowing cultivation with white clover	47.5	20.2	1.7	3.6	8.4
Monoculture with nitrogen fertilization	47.9	20.0	2.1	3.7	9.7

Harvesting was the main factor responsible for the net export of nutrients [55] and should be taken into account for the determination of fertilizer doses. In the experiment by Labrecque and Teodorescu [54], after the completion of 3 years of willow rotation and depending on the fertilization of sewage sludge and soil, the nitrogen intake amounted to 50.3–176.2; phosphorus 5.9–20.6; potassium 16.7–71.3; calcium 49.8–96.5 and magnesium 4.3–13.9 kg ha^{-1} per year. Nitrogen and phosphorus uptake on sandy soils was similar, and potassium and calcium uptake were higher [54] than in my own research. In our own studies, the uptake of nitrogen and calcium was similar, magnesium higher and potassium lower than in Several studies [55]. In my field experiment in undersowing cultivation, the accumulation of crude ash and calcium was lower than in monoculture (Figure 3). Less raw ash and calcium elevation may lead to a smaller decrease in soil pH, which is beneficial on light soils in Poland.

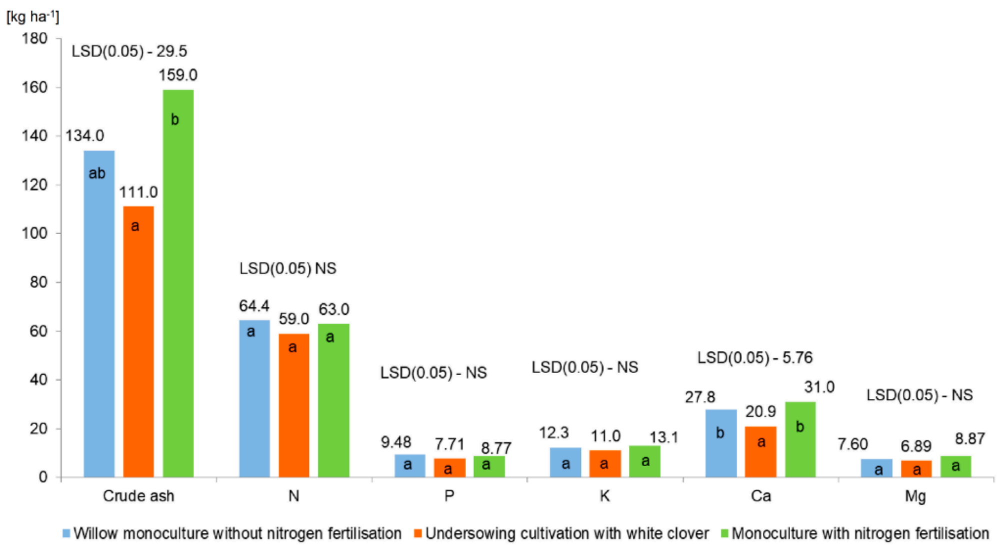

Figure 3. Annual nutrient uptake with two fertilizing treatments and undersowing system. LSD—the least significant difference. NS—difference is not significant. Different letters indicate significant differences between treatments (Tukey's multiple range test).

4. Conclusions

White clover grown together with willow contributed to the reduction of willow shoots per m^2 both and shoots per plant. Undersowing of two selected species also resulted in the highest average dry weight of one willow shoot (g), taking into account the lower competition for daylight and nutrients (least shoot density). The dry matter yield of willow (Mg ha^{-1}) in undersowing system cultivation and nitrogen fertilization did not show positive effects compared to cultivation in monoculture without fertilization. This may suggest that nitrogen fertilization is not an essential factor for willow growth in the first years after planting and partly supports the working hypothesis that intercropping of willow with white clover can replace nitrogen fertilization in the first years after the establishment of the plantation. Willow shoots grown together with clover were characterized by higher nitrogen content compared to monoculture. Lack of nitrogen fertilization in the first years after planting reduces the cost of establishing a plantation and is beneficial to the environment. On the basis of the conducted research, it can be concluded that in the first years after planting, the undersowing growing of willow with white clover can be an alternative to plantations fertilized and non-fertilized with nitrogen.

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