Willow production during 12 consecutive years—The effects of harvest rotation, planting density and cultivar on biomass yield

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Abstract
Willow biomass produced in short rotation coppice systems can potentially be used as biomass feedstock in Europe, the United States and Canada. However, most researchers focus on data from the first harvest rotation only, whereas multiple rotations have been rarely investigated. The aim of this study was to evaluate the effect of cultivar (5), planting density (12,000–96,000 cuttings/ha) and harvest rotation (annual, biennial, triennial) on willow biomass yields during 12 consecutive years in northern Poland. Every experimental factor and the interactions between factors significantly impacted willow yields. Biomass yield was highest in the triennial harvest rotation (13.3 Mg ha⁻¹ year⁻¹), 15.9% lower in the biennial rotation and 26.9% lower in the annual rotation. The highest average yield (14.6 Mg ha⁻¹ year⁻¹) was noted at a planting density of 24,000 cuttings/ha, and yields were 9.3%–46.0% lower at the remaining densities. Cultivar UWM 095 had the highest average yield (13.0 Mg ha⁻¹ year⁻¹), whereas the yield of the remaining cultivars was 4.6%–32.4% lower. During the 12-year period, yields were higher after the first harvest in annual, biennial and triennial harvest rotations. This above implies that high biomass yields can be obtained after the first harvest rotation if willows are cultivated on fertile soils at higher planting density, well managed and coppiced after the first year. However, yields are unlikely to be higher in successive harvest rotations, and they can even be lower, but more stable than in the first harvest rotation.

KEYWORDS
annual cycle, biennial cycle, dry biomass yield, multiple harvests, Salix, short rotation coppice, triennial cycle
Willow cultivation shows a positive energy balance (Heller, Keoleian, & Volk, 2003; Stolarski, Krzyżaniak, Tworkowski, Szczukowski, & Gołaszewski, 2014; Stolarski, Krzyżaniak, Tworkowski, Szczukowski, & Niksa, 2016), delivers potential profits (Stolarski, Olba-Zięty, Rosenqvist, & Krzyżaniak, 2017b; Stolarski, Rosenqvist, et al., 2015b; Styles, Thorne, & Jones, 2008) and notable environmental benefits (Krzyżaniak, Stolarski, Szczukowski, & Tworkowski, 2016; Londo, Dekker, & Kreus, 2005; Sage, Cunningham, & Boatman, 2006; Volk, Verwijst, Tharakan, Abrahamson, & White, 2004).

The above considerations can encourage farmers to produce willow biomass. However, farmers should focus primarily on stable and high biomass yields to achieve economic, energy and environmental benefits. According to the literature, willow biomass yields are influenced mainly by: (a) selection of optimal species and cultivars (Amichev et al., 2018; Larsen et al., 2014; Serapiglia et al., 2013; Stolarski, Szczukowski, Tworkowski, & Klasa, 2008; Tharakan, Volk, Nowak, & Abrahamson, 2005); (b) soil conditions (Larsen et al., 2014; Stolarski, Szczukowski, Tworkowski, & Klasa, 2011a); (c) type and rate of fertilization (Aronsson et al., 2014; Fabio & Smart, 2018; Sevel, Nord-Larsen, Ingerslev, Jørgensen, & Raulund-Rasmussen, 2014), although fertilization had a limited effect on yield in some studies (Aronsson & Bergstrom, 2001; Quaye, Volk, Hafner, Leopold, & Schirmer, 2011); (d) weather conditions and crop management practices (Tahvanainen & Rytkonen, 1999; Weih, 2004); (e) planting density and harvest rotation (Stolarski, Szczukowski, Tworkowski, Wróblewska, & Krzyżaniak, 2011b; Volk et al., 2018; Wilkinson et al., 2007).

In recent years, the above factors were analysed in numerous studies which are not referenced in this paper. However, the vast majority of authors evaluated only one harvest rotation, usually the first and, in some cases, the second or the third rotation (Georgiadis, Sevel, Raulund-Rasmussen, & Stupak, 2017; Nissim et al., 2013; Sleight & Volk, 2016; Sleight et al., 2016; Stolarski et al., 2008; Walle, Camp, Casteele, Verheyen, & Lemeur, 2007). Willow production systems have a lifespan of up to 25 years (from establishment to closure); therefore, their performance, profitability and energy efficiency should be considered during this period. A higher number of successive harvest rotations have been rarely analysed in the literature. Therefore, the aim of this study was to determine the effects of harvest rotation (annual, biennial, triennial), initial planting density (12,000, 24,000, 48,000 and 96,000 cuttings/ha), planting material (five cultivars) and the interactions between the above factors on willow productivity during 12 successive years. The influence of willow survival rates and the final number of plants on biomass yield was also evaluated across harvest rotations and planting densities. The key factors responsible for changes in willow biomass yield during successive harvest rotations were identified.

## 2 MATERIALS AND METHODS

### 2.1 Location, site preparation and maintenance

The field experiment was established in northern Poland, in the Kwidzyn Valley (53°43′N, 18°53′E) near the town of Kwidzyn. Lucerne was the preceding crop for willows. Glyphosate was applied in late August 2002 to eradicate lucerne and weeds. In the autumn of 2002, the field was ploughed and cultivated twice with a disc harrow to shred plant biomass. The field was harrowed twice in April 2003 immediately before planting. The Azoprim 50 WP herbicide was applied at 3 kg/ha immediately after manual planting of the cuttings. Cuttings with a length of 20 cm were obtained in winter from 1-year-old willow shoots from the collection of the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn (UWM). During the first growing season, the field was weeded mechanically twice to control secondary weed infestation. The field was not mechanically weeded in the remaining years of the experiment. Mineral fertilizers were not applied in the first year of the experiment. In the following years, fertilizers were applied manually at N 90 kg/ha, P 18 kg/ha and K 66 kg/ha after harvest and before the subsequent growing season. Nitrogen was applied as ammonium nitrate at 50 kg/ha at the beginning of the growing season. The remaining portion of nitrogen (40 kg/ha) was applied at the end of May. Phosphorus was applied as triple superphosphate, and potassium was applied as potash salt before the growing season. During each growing season, willows were monitored for pests and diseases.

### 2.2 Experimental factors

A three-factorial field experiment with a strip-split-plot design was set up. The experimental factors were as follows: A—three harvest rotations (annual, biennial and triennial) as the horizontal strip effect: B—four planting densities (12,000, 24,000, 48,000 and 96,000 cuttings/ha) as the vertical strip effect; C—five willow cultivars: UWM 046 (Salix viminalis L.), UWM 095 (S. alba L.), UWM 200 (S. alba L.), Tur (S. viminalis L.) and Turbo (S. viminalis L.) as the subplot effect randomized in four replications. Three willow clones (labelled with the letters “UWM” and a number) and two willow cultivars were studied. The experimental plants are collectively referred to as “cultivars” in this paper. All cultivars were bred from a collection of willow clones established in the early 1990s at
the Department of Plant Breeding and Seed Production of the UWM in Olsztyn. Cultivars Turbo and Tur are protected under the National Plant Breeders' Rights, and they were placed on the national list of varieties in 2007 and 2009, respectively. The plants were grown in four rows in plots with an area of 23.1 m² (7.0 × 3.3 m) each, in a total of 240 plots.

The cuttings were planted at four densities in twin rows. The distance between twin rows was 0.90 m, and the distance between rows in a double row was 0.75 m. Planting density was controlled by modifying plant spacing in rows. The longest distance was 1.0 m (12,000 cuttings/ha), and it decreased to 0.50, 0.25 and 0.125 m with a rise in plant density to 24,000, 48,000 and 96,000 cuttings/ha, respectively.

Despite every effort to select the most uniform experimental site, certain variations in soil conditions, which are characteristic of alluvial soils in river valleys, could not be avoided. The experimental site was relatively flat with minor differences in elevation that did not exceed 1 m. The predominant soil type was complete humic heavy alluvial soil formed from silty clay. One section of the experimental site consisted of sandy subsoil, characterized as medium-compact, shallow, proper alluvial soil on loose sand. This type of soil was predominant in the area where cuttings were planted at a density of 12,000 and 96,000 cuttings/ha and harvested in the annual and, partly, the biennial harvest rotation. Sandy soil had much lighter granulometric composition. The arable layer of sandy soil was underlain by loose sand (from a depth of 37 cm), which indicates that the Vistula River valley had a shallow bottom in the past (Table 1). The aero-hydrographic conditions of sandy soil were far less favourable for willow production and were characterized by greater capillary rise relative to heavy alluvial soil. The above resulted in water deficiency during dry spells. The discussed factors had an adverse effect on the growth and development of plants when rainfall distribution was not favourable. The soil was neutral to alkaline (pH\text{KCl} 6.6–7.3). The organic matter content in the surface horizon of heavy alluvial soil was 6.78%, which contributed to high soil fertility. Alluvial soil contained much less organic matter.

The experiment was established in 2003 which was regarded as the initial year, and data from that year were not included in the study. In early February 2004, one-year-old willows from all plots were coppiced to increase the number of shoots per stump and to stimulate the growth of shoots in the following years. The described experiment covered 12 successive growing seasons (2004–2015). During the entire experiment, willows were harvested 12 times in the annual rotation, six times in the biennial rotation and four times in the triennial rotation (Table 2).

### 2.3 | Determination of biomass yield

Willows were harvested manually with a combustion-engine brush cutter in successive years and harvest rotations. Immediately after harvest, all shoots collected from each plot were cut with a combustion-engine brush cutter.

### Table 1 | The properties and granulometric composition of soils

| Depth (cm) | pH_{KCl} | Organic matter (%) | Granulometric composition % of soil fractions (diameter in mm) | Formation |
|------------|----------|--------------------|-------------------------------------------------------------|-----------|
|            |          |                    | 1.0–0.1 | 0.1–0.02 | <0.02 | <0.002 |           |
| Complete humic heavy alluvial soil formed from silty clay |          |                    |          |          |        |         |           |
| 0–37       | 6.9      | 6.78               | 9.0     | 32.0     | 59.0   | 16.0    | Siilty clay |
| 37–78      | 6.6      |                    | 11.0    | 19.0     | 70.0   | 19.0    | Siilty clay |
| 78–150     | 6.8      |                    | 3.0     | 32.0     | 65.0   | 22.0    | Siilty clay |
| Medium-compact, shallow, proper alluvial soil on loose sand |          |                    |          |          |        |         |           |
| 0–37       | 7.3      | 3.71               | 55.0    | 13.0     | 32.0   | 15.0    | Light loam |
| 37–150     | 7.0      |                    | 96.0    | 4.0      | 0.0    | 0.0     | Loose sand |

### Table 2 | Fertilization and the number of harvests per rotation in each year of the 12-year experiment

| Year of cultivation | Year | Harvest rotation |
|---------------------|------|------------------|
| 1                   | 2003 | Establishment of experiment and shoot cutting |
| 2                   | 2004 | 1^{a} a a |
| 3                   | 2005 | 2^{a} 1 |
| 4                   | 2006 | 3^{a} a 1 |
| 5                   | 2007 | 4^{a} 2 a |
| 6                   | 2008 | 5^{a} a |
| 7                   | 2009 | 6^{a} 3 2 |
| 8                   | 2010 | 7^{a} a a |
| 9                   | 2011 | 8^{a} 4 |
| 10                  | 2012 | 9^{a} a 3 |
| 11                  | 2013 | 10^{a} 5 a |
| 12                  | 2014 | 11^{a} a |
| 13                  | 2015 | 12^{a} 6 4 |

^{a}Application of NPK mineral fertilizers.
were weighed, and their fresh matter yield was determined in Mg/ha. Representative shoots were sampled from each plot to determine the moisture content of biomass. For this purpose, 15 whole shoots were cut with the Scorpion 120 SD wood chipper. Wood chips were packed in plastic bags (to limit natural moisture loss) and transported to the Energy Feedstock Assessment Laboratory at the UWM in Olsztyń. Moisture content was determined in the laboratory by measuring weight loss during drying. Biomass was dried at 105°C until the achievement of a constant weight. The moisture content of biomass and fresh matter yield were used to calculate dry matter yield per ha.

### 2.4 Statistical analysis

The results of the experiment with a strip-split-plot design were processed by ANOVA (Table 3):

$$
Y_{hijk} = m + R_h(A_i + e_{hi}^{(1)} + B_j + e_{hij}^{(2)} + AB_{ij} + e_{hij}^{(3)} + C_k(1) + AC_{ik} + BC_{jk} + ABC_{ijk} + e_{hijk}^{(4)}),
$$

where $Y_{hijk}$ is the observed $i$-th level of factor A (harvest rotation), $j$-th level of factor B (planting density) and $k$-th level of factor C (cultivars) in the $h$-th replication; $m$ is the mean value; $R_h$ is the replication random effect; $AB_{ij}$, $AC_{ik}$, $BC_{jk}$ and $ABC_{ijk}$ are interactions between fixed effects. The errors $e_{hi}^{(1)}$, $e_{hij}^{(2)}$, $e_{hij}^{(3)}$, $e_{hijk}^{(4)}$ were normally distributed with zero mean and variance $\sigma_{hi}^2$, $\sigma_{hij}^2$, $\sigma_{hij}^2$ and $\sigma_{hijk}^2$ (Hoshmand, 2011).

**TABLE 3** The results of ANOVA for dry biomass yield in the experiment with a strip-split-plot design

| Source of variation | $df$ | $F$ | $p$ |
|--------------------|------|-----|-----|
| Replication        | $r-1=3$ | 0.5 | 0.695 |
| Harvest rotation   | $a-1=2$ | 122.7 | <0.001 |
| Error(1)           | $(r-1)$ | $<0.001$ |
| Planting density   | $b-1=3$ | 355.9 | <0.001 |
| Error(2)           | $(b-1)$ | $<0.001$ |
| AxB                | $(a-1)$ | 54.9 | <0.001 |
| Error(3)           | $(a-1)(c-1)$ | 321.2 | <0.001 |
| Cultivar           | $c-1=4$ | 21.0 | <0.001 |
| AxC                | $(a-1)$ | 8.4 | <0.001 |
| BxC                | $(c-1)$ | 18.7 | <0.001 |
| AxBxC              | $(a-1)(b-1)$ | 144.14 | 0.05 |

The significance of differences between the means was tested with Tukey’s HSD procedure at a significance level of $\alpha = 0.05$.

The experimental design had to be decomposed to the evaluated selected harvest rotations (factor A) separately. The repeated measurement model was used, where planting density (factor B) and cultivar (factor C) were the fixed grouping factors, and the year of harvest was the repeated measurement factor (Y). All analyses were performed in the Statistica 13.3 program (TIBCO Software Inc., 2017).

### 3 RESULTS

#### 3.1 Weather conditions, pest infestation and disease incidence

The average air temperature during the growing season (April to October) was 12.9–14.1°C in 2004–2015, and the multiyear average (1982–2015) was 13.5°C (Figure 1). The average annual air temperature (January to December) was 6.6–9.7°C, and the long-term average was 8.0°C. During the 12-year experiment (2004–2015), air temperatures exceeded the long-term average in seven growing seasons and were below the long-term average in five growing seasons.

Total rainfall in the first year of the study (2004) was 577 mm, and it was 12% higher than the multiyear average (Figure 1). The second year was characterized by the lowest precipitation (416 mm) in the experimental period, and it was 19% below the multiyear average. In the third, fifth, 11th and 12th (2015) year of the experiment, total rainfall was 1%–12% lower than the multiyear average. However, total rainfall in 2009–2013 exceeded the multiyear average by 2%–25% in 2013 and 2010, respectively.

An analysis of total rainfall in different growth stages revealed that precipitation was 1%–29% lower in the second (2005), 10th, 11th and 12th (2015) year of the experiment relative to the multiyear average. During the entire experiment, precipitation was lowest in 2005 (261 mm) and 2014 (264 mm). However, the most unfavourable distribution of rainfall across months was observed in the fifth growing season (2008) when total precipitation in May, June, July and September was much lower (by 74%, 36%, 8% and 56%, respectively) relative to the corresponding months of the multiyear period (data not shown). In 2008, total rainfall was highest in the last month of the growing season (October), and it exceeded the multiyear average by as much as 235%. Therefore, the annual average during the growing season of 2008 was only somewhat lower than the multiyear average. However, willows were exposed to considerable water stress at the beginning of the growing season, and they were not able to make effective use of rainwater in the last month of the growing season. As a result, plant growth was inhibited in 2008. Another
critical year was 2014 when precipitation was very low and its distribution was unfavourable during the growing season. In all months of the growing season of 2008, rainfall was 6%–61% lower relative to the multiyear period. In the growing seasons of 2004 and in 2006–2012, total rainfall was 1%–21% higher than in the multiyear period.

The evaluated cultivars differed in their susceptibility to pests and diseases. *S. alba* (UWM 095 and UWM 200) plants were readily colonized by Coleoptera of the Chrysomelidae family, and they were susceptible to fungi (*Glomerella cingulata*). The most common pests were *Phyllodecta vitellinae* L., *Plagiodera versicolor* L. and *Lochmaea capreae* L. These pathogens skeletonized leaves, and they were easily identified despite variations in prevalence.

*S. viminalis* (Turbo, Tur and UWM 046) plants were less frequently colonized by the above pests than *S. alba*. Each year, *S. viminalis* plants were colonized mainly by *Earias chlorana* which were identified twice during the growing season: in late May and in mid-August. However, *Earias chlorana* did not colonize *S. alba*. *Dasyneura marginemtorquens*, and *Pterocomma salicis* were also detected on *S. viminalis* plants during the growing season. It should be noted that the prevalence of foraging pests was much higher in years with less rainfall, especially during periodic droughts. Symptoms of leaf rust (*Melampsora* sp.) were not observed in any of the studied willow cultivars during the experiment.

### 3.2 Dry biomass yield

Dry biomass yield was significantly differentiated by the main factors: harvest rotation (*p* < 0.001), planting density (*p* < 0.001), cultivar (*p* < 0.001) and the interactions between the main factors (*p* < 0.001; Tables 3 and 4).

During the entire experiment, the average dry biomass yield (13 Mg ha⁻¹ year⁻¹) was highest in cultivar UWM 095 (Table 5), and average dry biomass yield in the remaining cultivars, that is, Tur, UWM 046, Turbo and UWM 200, was significantly lower by 4.6%, 9.1%, 12.9% and 32.4%, respectively. In the analysed planting densities, the highest average yield of 14.6 Mg ha⁻¹ year⁻¹ was observed when initial planting density was 24,000 cuttings/ha. The biomass yield was significantly lower by 9.3%, 30.7% and 46.0% at the remaining planting densities of 48,000, 12,000 and 96,000 cuttings/ha, respectively. Willow productivity across harvest rotations was significantly highest in the triennial rotation at 13.3 Mg ha⁻¹ year⁻¹ on average. Biomass yield was 15.9% and 26.9% lower in biennial and annual harvest rotations, respectively.
Cultivars UWM 095 and Tur had the highest yield in annual rotation, followed by cultivars Turbo and UWM 046 in the second homogeneous group, and cultivar UWM 200 in the third group (Table 5).

An analysis of willow productivity in 12 successive annual harvest rotations revealed the lowest yields in willows planted at the lowest density in the first five years (2004–2008; Table 6). In the successive five-year period (2009–2013), lower yields were noted in willows planted at the highest density, whereas willows planted at the highest and lowest density were characterized by similar low yields in the last 2 years of the experiment (2014–2015). Willows planted at a density of 24,000 and 48,000 cuttings/ha were characterized by a much higher yield which was similar in successive harvest rotations. The observed differences in yield between planting densities were also partially attributed to lower soil fertility in the section of the experimental site with the lowest and highest planting densities. An analysis of successive annual harvest rotations revealed that average dry biomass yield (16.4 Mg ha⁻¹ year⁻¹) was also highest in the first rotation (2005). Biomass yield was significantly lower in successive annual harvest rotations: It was 25.8% lower in the second rotation and 46.4% lower in the sixth rotation. Similarly to annual rotations, dry biomass yields also decreased in successive biennial harvest rotations across all cultivars and planting densities. However, the observed changes were more pronounced and had a linear character (Figure 3). For this reason, dry biomass yield in six biennial harvest rotations varied considerably from 3.6 to 22.5 Mg ha⁻¹ year⁻¹, subject to cultivar, planting density and year of cultivation.

In terms of biomass yield, cultivars were ranked in the same order, from the lowest to the highest, in both biennial and annual harvest rotations (Table 5). In six biennial harvest rotations, the lowest yield was noted in plots with the highest planting density (Table 6). Yields were significantly higher in plots with the lowest planting density (12,000 cuttings/ha). However, willows planted at a density of 24,000 and 48,000 cuttings/ha produced even higher yields than willows planted at the lowest and highest density. An analysis of successive biennial harvest rotations revealed that average dry biomass yield (16.4 Mg ha⁻¹ year⁻¹) was also highest in the first rotation (2005). Biomass yield was significantly lower in successive biennial harvest rotations: It was 25.8% lower in the second rotation and 46.4% lower in the sixth rotation.

### TABLE 4 The results of repeated measures ANOVA for dry biomass yield

| Source of variation | df | Annual | df | Biennial | df | Triennial |
|---------------------|----|--------|----|----------|----|-----------|
| Harvest rotation (A)|    |        |    |          |    |           |
| Planting density (B)| 3  | 678.8***| 3  | 405.1***| 3  | 197.8***  |
| Cultivar (C)        | 4  | 79.4*** | 4  | 187.4***| 4  | 40.0***   |
| BxC                 | 12 | 13.1*** | 12 | 22.5***  | 12 | 3.8***    |
| Error(1)            | 60 |         | 60 |          | 60 |           |
| Harvest year (Y)    | 11 | 226.5***| 5  | 805.8***| 3  | 293.1***  |
| YxB                 | 33 | 17.6*** | 15 | 14.1***  | 9  | 8.4***    |
| YxC                 | 44 | 6.5***  | 20 | 17.3***  | 12 | 6.3***    |
| YxBxC               | 132| 3.5***  | 60 | 5.3***   | 36 | 2.8***    |
| Error(2)            | 660|         | 300|          | 180|           |

***p < 0.001.
as much as 24.7 Mg ha\(^{-1}\) year\(^{-1}\), subject to cultivar and planting density. It should be noted that the yield of cultivar Tur was very high in the first biennial harvest rotation. However, its yields decreased steadily and significantly in successive rotations as the production system grew older. In contrast, cultivar UWM 095 was characterized by more stable yields in successive biennial rotations. UWM 200 was the lowest-yielding cultivar at all planting densities.

In the triennial harvest rotation, cultivars UWM 095, Turbo and UWM 046 had significantly higher average yields than cultivars Tur and UWM 200 (Table 5). The highest yield in the triennial harvest rotation was noted in UWM 095 (14.5 Mg ha\(^{-1}\) year\(^{-1}\)), whereas the yields of the remaining cultivars were lower by 2.3%, 5.1%, 8.2% and 24.7%, respectively. The lowest yield in four triennial harvest rotations was noted in plots with the highest planting density (Table 8). Yields were significantly higher in plots with the lowest planting density (12,000 cuttings/ha) than in plots with the highest density. The yields noted in the first (2006) and third (2012) triennial harvest rotation were similar to those observed in plots with a planting density of 48,000 cuttings/ha in the

### Table 5: Average dry biomass yield (Mg ha\(^{-1}\) year\(^{-1}\)) depending on willow cultivar, planting density and harvest rotation

| Cultivar (C) | Planting density (1,000 cuttings/ha) (B) | Harvest rotation (A) | Mean Annual | Biennial | Triennial | Mean |
|--------------|--------------------------------------|----------------------|-------------|----------|-----------|------|
| UWM 046     | 12                                   | 5.4 ± 0.8 h          | 9.7 ± 0.2 f | 14.1 ± 0.5 d | 9.7 ± 4.5 d | 11.8 ± 3.5 e |
|              | 24                                   | 14.1 ± 0.6 d         | 14.2 ± 0.7 d | 15.6 ± 0.5 b | 14.6 ± 0.9 b |
|              | 48                                   | 13.9 ± 0.5 d         | 13.8 ± 0.2 d | 14.6 ± 0.7 c | 14.1 ± 0.6 b |
|              | 96                                   | 5.5 ± 0.6 h          | 9.7 ± 0.3 f  | 10.8 ± 1.2 f | 8.7 ± 2.5 f  |
| Mean         |                                      | 9.7 ± 4.5 d          | 11.8 ± 2.2 e | 13.8 ± 2.0 a | 11.8 ± 3.5 e |
| UWM 200     | 12                                   | 5.2 ± 0.5 h          | 6.6 ± 0.4 h  | 13.0 ± 0.7 d | 8.3 ± 3.6 f  |
|              | 24                                   | 11.2 ± 0.6 e         | 10.0 ± 0.6 f | 13.0 ± 1.0 d | 11.4 ± 1.4 d |
|              | 48                                   | 8.9 ± 1.1 g          | 10.1 ± 1.1 f | 11.3 ± 0.6 c | 10.1 ± 1.3 e |
|              | 96                                   | 4.7 ± 0.5 i          | 4.7 ± 0.6 i  | 6.4 ± 1.5 h  | 5.3 ± 1.2 g  |
| Mean         |                                      | 7.5 ± 2.8 e          | 7.8 ± 2.5 e  | 10.9 ± 2.9 e | 8.8 ± 3.1 e  |
| Tur          | 12                                   | 6.8 ± 0.7 h          | 14.6 ± 0.7 c | 12.4 ± 0.2 e | 11.3 ± 3.5 d |
|              | 24                                   | 13.5 ± 0.8 d         | 16.7 ± 0.2 b | 16.4 ± 0.6 b | 15.5 ± 1.6 a |
|              | 48                                   | 15.8 ± 0.8 b         | 12.9 ± 0.6 d | 14.6 ± 0.6 c | 14.4 ± 1.4 b |
|              | 96                                   | 7.8 ± 0.2 g          | 6.9 ± 0.8 g  | 9.8 ± 1.8 f  | 8.2 ± 1.6 f  |
| Mean         |                                      | 11.0 ± 4.0 e         | 12.8 ± 3.8 b | 13.3 ± 2.7 b | 12.4 ± 3.6 b |
| Turbo        | 12                                   | 6.6 ± 0.8 h          | 8.7 ± 0.6 g  | 14.4 ± 1.2 c | 9.9 ± 3.5 e  |
|              | 24                                   | 13.1 ± 0.4 d         | 13.6 ± 1.5 d | 17.2 ± 0.6 a | 14.6 ± 2.1 b |
|              | 48                                   | 12.0 ± 0.7 e         | 10.9 ± 0.3 f | 15.6 ± 1.0 b | 12.9 ± 2.2 c |
|              | 96                                   | 5.2 ± 1.1 h          | 8.6 ± 0.5 g  | 9.5 ± 0.9 f  | 7.8 ± 2.1 f  |
| Mean         |                                      | 9.2 ± 3.6 d          | 10.5 ± 2.2 c | 14.2 ± 3.1 a | 11.3 ± 3.6 d |
| UWM 095     | 12                                   | 6.9 ± 0.5 h          | 11.9 ± 0.3 e | 15.1 ± 1.0 c | 11.3 ± 3.6 d |
|              | 24                                   | 15.6 ± 0.7 b         | 16.8 ± 0.7 b | 17.5 ± 0.9 a | 16.6 ± 1.1 a |
|              | 48                                   | 13.3 ± 0.6 d         | 15.1 ± 0.4 c | 15.3 ± 0.4 c | 14.6 ± 1.1 b |
|              | 96                                   | 9.2 ± 0.2 f          | 8.7 ± 0.3 g  | 10.2 ± 0.2 f | 9.4 ± 0.7 e  |
| Mean         |                                      | 11.3 ± 3.6 e         | 13.1 ± 3.2 b | 14.5 ± 2.8 a | 13.0 ± 3.4 a |
| Mean         | 12                                   | 6.2 ± 1.0 g          | 10.3 ± 2.9 d | 13.8 ± 1.2 b | 10.1 ± 3.6 e |
|              | 24                                   | 13.5 ± 1.6 b         | 14.2 ± 2.6 b | 15.9 ± 1.8 a | 14.6 ± 2.3 a |
|              | 48                                   | 12.8 ± 2.4 c         | 12.6 ± 2.0 c | 14.3 ± 1.7 b | 13.2 ± 2.2 b |
|              | 96                                   | 6.5 ± 1.9 g          | 7.7 ± 1.8 f  | 9.3 ± 1.9 e  | 7.9 ± 2.2 d  |
| Mean         |                                      | 9.7 ± 3.9 e          | 11.2 ± 3.4 b | 13.3 ± 3.0 a | 11.4 ± 3.7 |

Note. Means ± standard deviation; a, b, c... homogenous groups, main effect of factor A; a, b, c... homogenous groups, main effect of factor B; a, b, c... homogenous groups, main effect of factor C; a,b,c... homogenous groups, interaction AB; a, b, c... homogenous groups, interaction AC; a, b, c... homogenous groups, interaction BC; a, b, c... homogenous groups, interaction ABC.
| Cultivar (C) | Planting density (1,000 ha⁻¹) (B) | 2004 (I) | 2005 (II) | 2006 (III) | 2007 (IV) | 2008 (V) | 2009 (VI) | 2010 (VII) | 2011 (VIII) | 2012 (IX) | 2013 (X) | 2014 (XI) | 2015 (XII) |
|-------------|-----------------------------------|---------|-----------|-----------|-----------|---------|---------|-----------|-----------|-----------|---------|-----------|-----------|
| UWM 046     | 12                                | 9.2 g   | 4.8 i     | 4.1 i     | 4.0 i     | 2.6 j   | 6.0 h   | 7.5 h     | 6.7 h     | 6.2 h     | 5.9 h   | 3.7 i     | 4.2 i     |
|             | 24                                | 17.5 c  | 18.8 b    | 15.5 d    | 15.4 d    | 14.7 d  | 15.7 d  | 15.3 d    | 13.8 e    | 11.9 f    | 9.9 g   | 10.3 f    | 11.0 f    |
|             | 48                                | 18.4 b  | 19.4 b    | 14.6 d    | 14.3 d    | 13.0 e  | 14.3 d  | 15.2 d    | 13.6 e    | 11.7 f    | 9.8 g   | 10.2 f    | 12.2 e    |
|             | 96                                | 12.2 e  | 5.5 h     | 3.7 i     | 3.5 i     | 2.7 j   | 6.2 h   | 7.7 h     | 7.3 h     | 6.3 h     | 5.1 i   | 2.6 j     | 3.2 i     |
| Mean        |                                   | 14.3 a  | 12.1 b    | 9.5 c     | 9.3 c     | 8.3 d   | 10.5 c  | 11.4 b    | 10.3 c    | 9.0 d     | 7.7 d   | 6.7 d     | 7.7 d     |
| UWM 200     | 12                                | 6.6 h   | 5.0 i     | 3.9 i     | 3.7 i     | 2.9 j   | 6.1 h   | 9.0 g     | 8.6 g     | 5.1 i     | 5.1 i   | 2.6 j     | 4.2 i     |
|             | 24                                | 15.8 d  | 15.6 d    | 12.3 e    | 10.6 f    | 8.4 g   | 11.0 f  | 13.6 e    | 13.5 e    | 9.1 g     | 8.5 g   | 6.5 h     | 8.8 g     |
|             | 48                                | 12.7 e  | 11.4 f    | 7.6 h     | 7.4 h     | 5.2 i   | 9.6 g   | 12.6 e    | 9.7 g     | 8.4 g     | 7.8 h   | 6.0 h     | 8.5 g     |
|             | 96                                | 5.8 h   | 4.1 i     | 3.5 i     | 3.4 i     | 2.2 j   | 5.6 h   | 8.9 g     | 5.5 h     | 5.1 i     | 5.0 i   | 3.3 i     | 4.1 i     |
| Mean        |                                   | 10.2 e  | 9.0 e     | 6.8 d     | 6.3 d     | 4.7 e   | 8.1 d   | 11.0 b    | 9.3 c     | 6.9 d     | 6.6 d   | 4.6 e     | 6.4 d     |
| Tur         | 12                                | 10.7 f  | 7.3 h     | 6.5 h     | 5.6 h     | 3.7 i   | 8.1 g   | 10.4 f    | 9.0 g     | 6.2 h     | 6.2 h   | 3.4 i     | 4.3 i     |
|             | 24                                | 18.3 b  | 14.4 d    | 17.3 c    | 14.8 d    | 12.5 e  | 16.1 c  | 13.4 e    | 12.1 e    | 11.4 f    | 10.3 f  | 10.5 f    | 11.6 f    |
|             | 48                                | 18.5 b  | 22.5 a    | 19.4 b    | 18.2 b    | 16.4 c  | 17.5 c  | 17.3 c    | 15.9 d    | 11.5 f    | 10.0 f  | 12.7 e    |           |
|             | 96                                | 13.2 e  | 9.8 g     | 9.5 g     | 8.3 g     | 4.7 i   | 8.3 g   | 10.5 f    | 9.4 g     | 6.0 h     | 5.9 h   | 3.7 i     | 4.7 i     |
| Mean        |                                   | 15.2 a  | 13.5 a    | 13.2 a    | 11.7 b    | 9.3 c   | 12.5 b  | 12.9 b    | 11.6 b    | 8.8 d     | 8.1 d   | 6.9 d     | 8.3 d     |
| Turbo       | 12                                | 9.9 g   | 8.0 g     | 6.3 h     | 5.0 i     | 4.0 i   | 7.6 h   | 10.5 f    | 7.4 h     | 6.1 h     | 6.0 h   | 3.2 i     | 4.8 i     |
|             | 24                                | 18.1 b  | 17.3 c    | 14.6 d    | 13.6 e    | 13.2 e  | 14.1 d  | 12.7 e    | 10.7 f    | 10.2 f    | 10.2 f  | 11.3 f    | 11.5 f    |
|             | 48                                | 18.3 b  | 16.4 c    | 12.6 e    | 10.8 f    | 8.6 g   | 13.5 e  | 13.9 e    | 11.4 f    | 9.6 g     | 9.5 g   | 9.6 g     | 10.3 f    |
|             | 96                                | 10.4 f  | 5.9 h     | 5.1 h     | 4.2 i     | 2.4 j   | 6.2 h   | 6.3 h     | 5.3 h     | 5.3 h     | 5.2 i   | 3.2 i     | 3.3 i     |
| Mean        |                                   | 14.2 a  | 11.9 b    | 9.7 c     | 8.4 d     | 7.1 d   | 10.3 c  | 10.8 e    | 8.7 d     | 7.8 d     | 7.7 d   | 6.8 d     | 7.5 d     |
| UWM 095     | 12                                | 8.0 g   | 6.5 h     | 5.4 h     | 4.2 i     | 3.3 i   | 7.3 h   | 11.2 f    | 10.5 f    | 8.7 g     | 8.9 g   | 3.7 i     | 4.7 i     |
|             | 24                                | 20.7 a  | 21.9 a    | 16.6 c    | 16.0 c    | 15.2 d  | 16.2 c  | 16.0 c    | 14.9 d    | 14.0 e    | 12.8 e  | 10.1 f    | 13.3 e    |
|             | 48                                | 16.9 c  | 14.8 d    | 12.3 e    | 11.8 f    | 8.1 g   | 14.8 d  | 16.2 c    | 14.1 d    | 13.7 e    | 12.0 e  | 10.2 f    | 14.1 d    |
|             | 96                                | 13.3 e  | 13.1 e    | 10.8 f    | 9.5 g     | 6.3 h   | 8.6 g   | 10.2 f    | 9.0 g     | 8.5 g     | 7.9 g   | 5.1 i     | 8.6 g     |
| Mean        |                                   | 14.7 a  | 14.1 a    | 11.3 b    | 10.4 c    | 8.2 d   | 11.7 b  | 13.4 a    | 12.1 b    | 11.2 b    | 10.4 c  | 7.3 d     | 10.1 c    |
| Mean        |                                   | 12.1 b  | 9.2 d     | 7.5 e     | 10.6 c    | 11.9 b  | 10.4 c  | 8.7 d     | 8.1 d     | 6.5 f     | 8.0 e   |           |           |

Note: a, b, c, homogenous groups, repeated measurement factor (Y); a, b, c, homogenous groups, interaction YB; a, b, c, homogenous groups, interaction YC; a,b,c, homogenous groups, interaction Y.
same years. However, the yields in the triennial harvest rotation were highest at a planting density of 24,000 cuttings/ha. An analysis of four successive triennial harvest rotations also revealed the highest average dry biomass yield (17.2 Mg ha$^{-1}$ year$^{-1}$) in the first rotation (2006). It should also be noted that cultivars Tur and Turbo planted at 12,000 ha$^{-1}$ and 24,000 cuttings/ha and cultivars UWM 046 and UWM 095 planted at 24,000 cuttings/ha produced very high yields (in excess of 20 Mg ha$^{-1}$ year$^{-1}$) in the first triennial harvest rotation. Their yields were considerably lower in successive rotations. The average yield in successive triennial harvest rotations was lower by 35.3%, 18.7% and 36.4% in the second, third and fourth harvest rotation, respectively. Similarly to annual and biennial harvest rotations, high biomass yields in the first triennial harvest rotation also decreased in the successive three triennial rotations (Figure 4). However, the average biomass yield in triennial harvest rotations varied significantly. This parameter was 26% higher in the third than in the second triennial rotation, but it was lower and similar to the second rotation in the fourth rotation. Despite a significant decrease in yield in successive triennial harvest rotations, cultivars UWM 095 and Turbo grown at a density of 24,000 cuttings/ha produced high biomass yields at 15.5 and 14.8 Mg ha$^{-1}$ year$^{-1}$, respectively, in the final triennial harvest (Table 8).

The total yields of the analysed cultivars grown at different planting densities in annual, biennial and triennial harvest rotations during 12 successive years were evaluated (Figure 5). Dry biomass yield was significantly highest in excess of 205 Mg/ha in cultivars Turbo and UWM 095 grown at the initial density of 24,000 cuttings/ha in a triennial harvest rotation (homogeneous group a). An analysis of the average total yield across planting densities, harvest rotations and cultivars demonstrated that the triennial harvest rotation with initial density 24,000 cuttings/ha (homogeneous group a) was the optimal option. The second homogeneous group b included willows cultivated at the initial density of 24,000 cuttings/ha in biennial and annual harvest rotations and willows grown at the initial density of 12,000 and 48,000 cuttings/ha in triennial harvest rotations.

### 3.3 The effects of plant survival and final plant density on biomass yield

Willow survival rates were significantly differentiated by harvest rotation, planting density and cultivar throughout the experiment. In most harvest rotations, willow survival rates

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**FIGURE 2** Changes in the biomass yield (Mg ha$^{-1}$ year$^{-1}$) of five willow cultivars planted at four densities in 12 successive annual harvest rotations. Points denote the average biomass yield of every cultivar, and lines denote changes over time.
decreased in successive years with a rise in initial planting density (Figure 6). In annual harvest rotations, cultivar Tur was characterized by the highest survival rate at all planting densities. The highest decrease in survival rates was observed in cultivar UWM 200, in particular in the first 6–7 years of production (Figure 6a). Survival rates were also stabilized in the remaining cultivars after the first 6–7 years, and in the seventh year, they ranged from only 19% in UWM 200 grown at a density of 96,000 cuttings/ha to 95% in Tur grown at a density of 12,000 cuttings/ha. After 12 successive annual harvest rotations, the average survival rates of the studied cultivars were determined at 56%–83%, 51%–67%, 38%–41% and 18%–28% at increasing planting densities, respectively. Plant survival rates decreased considerably in nearly all cultivars in five successive biennial harvest rotations (Figure 6b). Only cultivar UWM 095 grown at a density of 12,000 and 24,000 cuttings/ha was characterized by somewhat higher survival rates in the successive biennial harvest rotations relative to the remaining four cultivars. After six successive biennial harvest rotations, the average survival rates of the studied cultivars were determined at 54%–86%, 31%–61%, 21%–35% and 18%–24% at increasing planting densities, respectively. In triennial harvest rotations, survival rates decreased considerably in the first and second rotation (Figure 6c). In the third and fourth rotations, survival

Table 7: Dry biomass yield (Mg ha⁻¹ year⁻¹) in six successive biennial harvest rotations

| Cultivar (C) | Planting density (1,000 cuttings/ha) (B) | Year and successive biennial harvest rotation (Y) |
|-------------|-----------------------------------------|-----------------------------------------------|
|             | 2005 (I) | 2007 (II) | 2009 (III) | 2011 (IV) | 2013 (V) | 2015 (VI) |
| UWM 046     |          |          |          |          |          |          |
| 12          | 13.2 d   | 11.9 e   | 8.6 f    | 8.6 f    | 7.3 g    | 8.8 f    |
| 24          | 19.7 b   | 14.5 d   | 12.3 e   | 13.9 d   | 12.6 e   | 12.1 e   |
| 48          | 21.4 b   | 13.5 d   | 12.0 e   | 12.5 e   | 11.9 e   | 11.5 e   |
| 96          | 14.8 d   | 9.8 f    | 8.0 g    | 8.4 g    | 8.1 g    | 8.9 f    |
| Mean        | 17.3 b   | 12.4 c   | 10.2 d   | 10.8 d   | 10.0 e   | 10.3 d   |
| UWM 200     |          |          |          |          |          |          |
| 12          | 9.3 f    | 7.3 g    | 5.5 h    | 7.1 g    | 5.3 h    | 5.0 h    |
| 24          | 15.5 c   | 9.3 f    | 7.8 g    | 10.4 f   | 8.7 f    | 8.4 g    |
| 48          | 20.5 b   | 8.9 f    | 7.6 g    | 8.7 h    | 7.6 g    | 7.1 g    |
| 96          | 6.7 h    | 5.2 h    | 4.1 i    | 4.7 i    | 4.0 i    | 3.6 i    |
| Mean        | 13.0 c   | 7.7 f    | 6.2 f    | 7.7 f    | 6.4 f    | 6.0 f    |
| Tur         |          |          |          |          |          |          |
| 12          | 20.2 b   | 17.3 c   | 14.0 d   | 14.5 d   | 11.2 e   | 10.5 f   |
| 24          | 24.7 a   | 18.6 b   | 16.6 c   | 15.0 d   | 12.9 e   | 12.1 e   |
| 48          | 20.0 b   | 15.7 c   | 10.4 f   | 11.7 e   | 10.4 f   | 9.5 f    |
| 96          | 13.2 d   | 9.4 f    | 4.6 i    | 5.1 h    | 4.8 i    | 4.4 i    |
| Mean        | 19.5 a   | 15.3 b   | 11.4 d   | 11.6 d   | 9.8 e    | 9.1 e    |
| Turbo       |          |          |          |          |          |          |
| 12          | 14.3 d   | 9.5 f    | 7.7 g    | 8.2 g    | 7.7 g    | 5.2 h    |
| 24          | 18.0 c   | 15.4 b   | 10.6 f   | 14.7 d   | 12.2 e   | 10.6 f   |
| 48          | 16.8 c   | 11.8 e   | 8.8 f    | 11.0 e   | 8.9 f    | 8.4 g    |
| 96          | 13.9 d   | 9.9 f    | 7.0 g    | 8.2 g    | 7.3 g    | 5.2 h    |
| Mean        | 15.7 b   | 11.6 d   | 8.5 e    | 10.5 d   | 9.0 e    | 7.3 f    |
| UWM 095     |          |          |          |          |          |          |
| 12          | 16.2 c   | 13.8 d   | 10.6 f   | 11.3 e   | 10.0 f   | 9.6 f    |
| 24          | 20.9 b   | 17.9 c   | 14.6 d   | 16.1 c   | 15.8 c   | 15.2 c   |
| 48          | 19.4 b   | 15.2 d   | 12.5 e   | 15.2 d   | 14.5 d   | 14.0 c   |
| 96          | 9.9 f    | 9.1 f    | 7.4 g    | 9.0 f    | 8.8 f    | 7.9 g    |
| Mean        | 16.6 b   | 14.0 e   | 11.3 d   | 12.9 e   | 12.3 e   | 11.7 d   |
| Mean        |          |          |          |          |          |          |
| 12          | 14.7 b   | 12.0 c   | 9.4 d    | 10.3 c   | 8.6 d    | 7.6 d    |
| 24          | 19.8 a   | 15.1 b   | 12.4 c   | 14.0 b   | 12.4 c   | 11.7 c   |
| 48          | 19.6 a   | 13.0 b   | 10.2 c   | 11.8 c   | 10.7 c   | 10.1 c   |
| 96          | 11.7 c   | 8.7 d    | 6.2 e    | 7.1 d    | 6.6 e    | 6.0 e    |
| Mean        | 16.4 a   | 12.2 b   | 9.6 d    | 10.8 c   | 9.6 d    | 8.8 d    |

Note. a, b, c... homogenous groups, repeated measurement factor (Y); a,b, c... homogenous groups, interaction YB; a, b, c... homogenous groups, interaction YC; a, b, c... homogenous groups, interaction YBC.
rates were more stable with a minor decreasing trend. In four triennial harvest rotations, survival rates were highest in cultivar UWM 095 grown at a density of 12,000 cuttings/ha. After four triennial harvest rotations, the average survival rates of all cultivars were determined at 64%–89%, 47%–66%, 26%–39% and 17%–29% at increasing planting densities, respectively.

The observed decrease in willow survival rates in successive harvest rotations influenced biomass yields. Biomass yields increased with a rise in the survival rates of willows grown at nearly all planting densities and harvest rotations (Figure 7). At the lowest initial planting density of 12,000 cuttings/ha, survival rates exceeded 70% in most cases and were below 50% in selected cases only. A similar trend was also noted at the higher planting density of 24,000 cuttings/ha, but survival rates decreased below 50% in a much larger number of cases. Survival rates decreased significantly at planting densities of 48,000 and 96,000 cuttings/ha.

The effect of final plant density (number of plants per unit area at harvest) on biomass yield was also analysed across the analysed harvest rotations and planting densities. Biomass yields increased with a rise in the final plant density at the lowest initial planting density of 12,000 cuttings/ha, in particular in the triennial harvest rotation (Figure 8). Biomass yields also increased with a rise in the final plant density in all harvest rotations with an initial planting density of 24,000 and 48,000 cuttings/ha. In plots with the highest initial planting density of 96,000 cuttings/ha, the correlation between biomass yield and final plant density was lower relative to the plots with an initial planting density of 24,000 and 48,000 cuttings/ha. Our findings indicate that initial planting density in commercial production systems with longer harvest rotations should be around 24,000 cuttings/ha because this variant contributed to a considerable increase in biomass yields. Biomass yields could be maximized at the lowest initial planting density of 12,000 cuttings/ha in a triennial harvest rotation because in this variant, biomass yield increased with a rise in final plant density. An increase in planting density to 48,000 cuttings/ha did not result in a significant rise in biomass yields, and at a planting density of 96,000 cuttings/ha, the increase in biomass yields was negligible (Table 5).
efficiency analyses should be conducted before a commercial production system is established to determine whether higher biomass yields can compensate for higher start-up costs. However, this is a separate problem which is influenced by macroeconomic and microeconomic factors as well as local social, economic and environmental factors.

4 | DISCUSSION

The biomass yield of willows is generally determined by many factors, including plant species, cultivar, soil, weather conditions, planting density, harvest rotation, as well as the timeliness and quality of agricultural treatments. In this study, willow growth was particularly inhibited by periodic dry spells, especially in experimental plots with poorer soil. In a study conducted in south-eastern Sweden, willow yield was 50%–60% lower than in the south-western part of the country due to lower rainfall during the growing season (Börjesson & Berndes, 2006). Groundwater uptake can somewhat compensate for water deficit during the growing season. However, the availability and accessibility of groundwater is determined by the depth of the soil layer and the type and structure of soil. Unfortunately, in the present

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**Table 8**

Dry biomass yield (Mg ha\(^{-1}\) year\(^{-1}\)) in four successive triennial harvest rotations

| Cultivar (C) | Planting density (1,000 cuttings/ha) (B) | Year and successive triennial harvest rotation (Y) | 2006 (I) | 2009 (II) | 2012 (III) | 2015 (IV) |
|-------------|------------------------------------------|-----------------------------------------------|---------|---------|---------|---------|
| UWM 046     | 12                                       |                                               | 16.8 e  | 12.6 e  | 14.4 d  | 12.5 e  |
|             | 24                                       |                                               | 20.6 b  | 13.2 e  | 14.8 d  | 13.7 e  |
|             | 48                                       |                                               | 19.4 b  | 12.7 e  | 13.5 e  | 12.6 e  |
|             | 96                                       |                                               | 13.4 e  | 9.3 g   | 11.1 f  | 9.5 g   |
| Mean        |                                          |                                               | 17.6 a  | 11.9 e  | 13.4 e  | 12.1 e  |
| UWM 200     | 12                                       |                                               | 17.7 c  | 10.0 f  | 14.9 d  | 9.5 g   |
|             | 24                                       |                                               | 16.8 c  | 10.4 f  | 14.7 d  | 10.1 f  |
|             | 48                                       |                                               | 16.0 c  | 7.4 h   | 14.4 d  | 7.3 h   |
|             | 96                                       |                                               | 6.7 h   | 4.8 i   | 9.0 g   | 5.2 i   |
| Mean        |                                          |                                               | 14.3 b  | 8.1 e   | 13.2 e  | 8.0 e   |
| Tur         | 12                                       |                                               | 21.3 a  | 9.1 g   | 10.2 f  | 9.0 g   |
|             | 24                                       |                                               | 23.2 a  | 14.0 d  | 15.4 d  | 13.1 e  |
|             | 48                                       |                                               | 17.8 c  | 13.5 e  | 14.5 d  | 12.5 e  |
|             | 96                                       |                                               | 11.6 f  | 8.2 g   | 11.4 f  | 8.0 g   |
| Mean        |                                          |                                               | 18.5 a  | 11.2 d  | 12.9 e  | 10.7 d  |
| Turbo       | 12                                       |                                               | 20.8 b  | 10.5 f  | 15.9 c  | 10.2 f  |
|             | 24                                       |                                               | 20.4 b  | 16.1 c  | 17.5 c  | 14.8 d  |
|             | 48                                       |                                               | 19.9 b  | 13.8 d  | 15.5 d  | 13.3 e  |
|             | 96                                       |                                               | 13.5 e  | 7.9 g   | 10.1 f  | 6.4 h   |
| Mean        |                                          |                                               | 18.6 a  | 12.1 e  | 14.7 b  | 11.2 d  |
| UWM 095     | 12                                       |                                               | 17.3 c  | 12.9 e  | 16.7 c  | 13.4 e  |
|             | 24                                       |                                               | 21.3 a  | 14.5 d  | 18.5 b  | 15.5 d  |
|             | 48                                       |                                               | 19.8 b  | 12.7 c  | 15.0 d  | 13.7 e  |
|             | 96                                       |                                               | 10.2 f  | 9.2 g   | 12.4 e  | 8.9 g   |
| Mean        |                                          |                                               | 17.2 a  | 12.3 c  | 15.7 b  | 12.9 e  |
| Mean        | 12                                       |                                               | 18.8 b  | 11.0 e  | 14.4 d  | 10.9 e  |
|             | 24                                       |                                               | 20.5 a  | 13.6 d  | 16.2 c  | 13.5 d  |
|             | 48                                       |                                               | 18.6 b  | 12.0 d  | 14.6 d  | 11.9 d  |
|             | 96                                       |                                               | 11.1 e  | 7.9 f   | 10.8 e  | 7.6 f   |
| Mean        |                                          |                                               | 17.2 a  | 11.1 c  | 14.0 b  | 11.0 e  |

Note. a, b, c... homogenous groups, repeated measurement factor (Y); a,b,c... homogenous groups, interaction YB; a, b, c... homogenous groups, interaction YC; a, b, c... homogenous groups, interaction YBC.
experiment, water did not rise from the lower layer of loose sand to the upper layer of light loam in plots characterized by poorer soil on loose sand, which had an adverse effect on growth. Willows cultivated in this segment of the experimental field had less favourable conditions for growth and development than plants growing on humic heavy alluvial soil where water moved more freely.

The effect of water availability on willow yields has been confirmed in this study. Precipitation levels ranged from 261 to 445 mm during 12 growing seasons, with an average of 372 mm. During the entire experiment, total rainfall was highest in the growing seasons of 2004, 2009 and 2011 when it exceeded 420 mm. In those years, rainfall was distributed relatively evenly across months, and groundwater levels did not decrease below 1.5 m. The above contributed to uniform growth, development and satisfactory appearance of willow plants. However, water conditions were particularly unfavourable in 2005, 2008 and 2014 when rainfall during the growing season was only 261–374 mm and was unevenly distributed across months. Groundwater levels decreased below 2.5 m, and water did not rise to higher soil layers, especially in poorer soils. In consequence, willow growth was inhibited in some periods. In successive months, rainfall stimulated plant growth, but biomass yield decreased by more than 50% relative to periods with more favourable weather. Severe water deficit also had a negative impact on plants growing on more fertile soil, where yields were 20%–30% lower relative to the years with much higher rainfall. Periodic droughts were accompanied by high infestation with insects which skeletonized willow leaves and contributed to a further decrease in biomass yield. For this reason, average yield in annual harvest rotations was lowest in 2014 and 2008 (Figure 9). Obviously, adverse weather conditions also affected the plants harvested in biennial and triennial rotations. Therefore, the average yield of willow biomass harvested in these rotations was much lower in the years that followed the dry years, that is, 2015 and 2009. Other authors (Bungart & Hüttl, 2001; Fischer, Prieler, & Velthuizen, 2005; Labrecque & Teodorescu, 2003) also demonstrated that soil type and water availability significantly affected biomass yield.

**FIGURE 4** Changes in the biomass yield (Mg ha\(^{-1}\) year\(^{-1}\)) of five willow cultivars planted at four densities in four successive triennial harvest rotations. Points denote the average biomass yield of every cultivar, and lines denote changes over time.
In this experiment, dry biomass yield was significantly differentiated by cultivar, planting density, harvest rotation and the interactions between these factors. Significantly highest average dry biomass yield was noted in cultivars UWM 095 and Turbo planted at 24,000 cuttings/ha in the triennial harvest rotation (more than 17 Mg ha\(^{-1}\) year\(^{-1}\) on average). The highest yields were noted in plots with higher planting density relative to European and North American standards. The present study revealed that plant survival in successive years of the experiment decreased with an increase in initial planting density in nearly all harvest rotations. The greatest decrease was observed in treatments with the highest initial plant densities, which points to a high degree of competition. Our results indicate that plant survival increases with the allocated space, but higher planting density promotes more effective utilization of space for biomass production. This could be an important observation, but further research is needed to determine whether the profits resulting from higher biomass yield could compensate for the increase in costs associated with a higher number of seedlings. In this study, a shorter harvest rotation and changes in planting density also decreased average biomass yield. Higher rates of mineral fertilizers in shorter harvest rotations (fertilizers were applied after each harvest) did not increase biomass yield. It should also be noted that dry biomass yield in annual rotations decreased in successive years until the fifth growing season (2008), increased in the sixth and seventh year, decreased until the 11th year and increased in the 12th rotation (2015). Similar fluctuations in biomass yield were observed in biennial and triennial harvest rotations, and the highest yield was also obtained in the first harvest rotation. Biomass yield was higher in all first harvest rotations than in the successive rotations, which contradicts the results reported by Sleight et al. (2016) and Sleight and Volk (2016). The above could be attributed to (a) fertile soils where the preceding crop (lucerne) increased the content of organic matter, nitrogen and other micronutrients and macronutrients; (b) very high planting density and very high plant survival rates in the first years of production; (c) good crop management and good plant establishment after the first year of production; (d) variable and adverse weather
FIGURE 6 Survival rates of the analysed willow cultivars grown at different planting densities in (a) annual, (b) biennial and (c) triennial harvest rotations during 12 successive years.
conditions; (e) intensified infestation by beetles which skeletonized leaves, in particular during dry spells; (f) decreasing plant survival rates in successive harvest rotations as plants grew older; (g) interactions and overlaps between the experimental factors, including periodic droughts, pest infestation and plant loss, which could not be easily verified. Therefore, the average yield (10.6 Mg ha$^{-1}$ year$^{-1}$) in the first six annual harvest rotations (2004–2009) was 13% higher than in the following six annual rotations (2010–2015).

Kopp et al. (2001) also observed that the survival of all cultivars planted at very high densities continued to decrease throughout the 10-year experiment. In the cited study, clone SV1 of S. dasyclados produced the highest yield (16.3 Mg ha$^{-1}$ year$^{-1}$). This result is comparable with the best combinations in the first six to seven annual harvest rotations in our study. Over time, biomass yields in annual harvest rotations decreased and began to fluctuate. In the 12th harvest rotation, biomass yield was determined at 3.2–14.1 Mg/ha and was highest in cultivar UWM 09 planted at a density of 48,000 cuttings/ha. In other studies, willow biomass yields in annual harvest rotations also varied considerably from 7.0 to 22.5 Mg/ha (Bullard et al., 2002; Stolarski et al., 2008; Stolarski, Szczukowski, et al., 2017a).

In our experiment, biomass yield in four successive biennial harvest rotations was also highest in the first rotation. The average yield in the first three biennial harvest rotations (12.7 Mg ha$^{-1}$ year$^{-1}$) was also 24% higher compared to the average yield in the following three biennial rotations. In a study performed in the UK, a minor decrease in biomass yield ($-1.3\%$) was reported in the second biennial harvest rotation relative to the first rotation (Bullard, Mustill, Nixon, McMillan, & Britt, 2001). In a different study, a reverse correlation was observed in successive biennial harvest rotations, where the biomass yield of willows grown at a density of 15,000–111,000 cuttings/ha was 7.2% higher in the second biennial harvest rotation (16.6 Mg ha$^{-1}$ year$^{-1}$) than in the first rotation (Kopp, Abrahamson, White, Nowak, & CA, 1997). In the work of Georgiadis et al. (2017), the biomass yield of willows planted at a density of 20,000 cuttings/ha in unfertilized plots reached 8.3 Mg ha$^{-1}$ year$^{-1}$ in the first and second rotation and increased to 9.5 Mg ha$^{-1}$ year$^{-1}$ in the third biennial harvest rotation. However, biomass yield in fertilized plots was more variable and reached 11.7, 10.6 and 12.6 Mg ha$^{-1}$ year$^{-1}$ in the first, second and third biennial harvest rotation, respectively. The biomass yield of cultivar
Tordis grown on sandy soil in a biennial harvest rotation ranged from 8.7 Mg ha$^{-1}$ year$^{-1}$ in the control treatment to 11.9 Mg ha$^{-1}$ year$^{-1}$ in a plot fertilized with 60 kg N ha$^{-1}$ year$^{-1}$ (Sevel et al., 2014). Similar results were obtained in our experiment in willows planted at a density of 12,000–24,000 cuttings/ha, but yields were particularly higher in the first harvest rotation.

In another study, the yield of willows planted at a density of 11,000 cuttings/ha and grown for 2 years was significantly lower at 3.7–6.4 Mg ha$^{-1}$ year$^{-1}$, subject to the applied method of soil enrichment (Stolarski, Krzyżaniak, Szczukowski, Tworkowski, & Bieniek, 2013). The cited authors attributed their findings to the fact that willows were grown on poor sandy soil and were not coppiced after the first year.

In our experiment, biomass yield in triennial harvest rotations which are most popular in commercial production systems was significantly higher than in biennial and annual rotations, and it increased with a rise in planting density from 12,000 to 24,000 cuttings/ha. The productivity of *S. dasyclados* SV1 in the state of New York also increased when the harvest rotation was extended and when planting density was increased from 15,000 to 37,000 cuttings/ha, although the observed differences were not always significant. The highest yield (average of 23.8 Mg ha$^{-1}$ year$^{-1}$) was obtained in a triennial harvest rotation with a density of 37,000 cuttings/ha (Kopp et al., 1997). It should be noted that the biomass yield reported in the first triennial harvest rotation in the above study exceeded the highest yield values in our experiment. In the cited study, biomass yields in plots with a planting density of 15,000 and 211,000 cuttings/ha were also very high at 18.3 and 22.4 Mg ha$^{-1}$ year$^{-1}$, respectively (Kopp et al., 1997). In our experiment, the average biomass yield in plots with similar planting density (12,000 cuttings/ha) was practically identical, whereas the yield in plots with a planting density of 96,000 cuttings/ha was twice lower on average. In other studies, willow yields in triennial rotations increased with a rise in planting density from 10,000 to 15,000, 20,000 and 25,000 cuttings/ha.
ha (Wilkinson et al., 2007). Cultivar Ashton Stott produced much higher yields than the remaining three cultivars (Jorr, Tora and Ulv) at each planting density. Biomass yield at a planting density of 25,000 cuttings/ha was not significantly higher than that reported at a density of 20,000 cuttings/ha, but it was higher by 10 Mg ha\(^{-1}\) year\(^{-1}\) in Ashton Stott at both densities. Meanwhile, the remaining cultivars produced the highest yields at a density of 25,000 cuttings/ha. However, their average yields were lower at 6.9 Mg ha\(^{-1}\) year\(^{-1}\). In our study, the average biomass yield in triennial harvest rotations also increased significantly with a rise in planting density from 12,000 to 24,000 cuttings/ha. The biomass yields of the five analysed cultivars differed significantly in successive harvest rotations in a range of 9.0–23.2 Mg ha\(^{-1}\) year\(^{-1}\). In the United States, significant differences in yield, ranging from 3.5 to 13.6 Mg ha\(^{-1}\) year\(^{-1}\), were also reported in 18 willow clones cultivated in two different sites in a triennial harvest rotation (Serapiglia et al., 2013). In turn, Volk et al. (2018) demonstrated that the yields of the top three willow cultivars across 17 sites in eight US states ranged from 3.6 to 14.6 Mg ha\(^{-1}\) year\(^{-1}\). Biomass yields in a commercial willow production system in Denmark (Nord-Larsen, Sevel, & Raulund-Rasmussen, 2014) also varied considerably (2.4–14.1 Mg ha\(^{-1}\) year\(^{-1}\)), whereas the average yield in a Swedish study reached 7.7 Mg ha\(^{-1}\) year\(^{-1}\) (Mola-Yudego, Díaz-Yáñez, & Dimitriou, 2015). The above results indicate that willow yields are influenced by a variety of factors and that high biomass yields are not easy to achieve. More importantly, biomass yields should be stabilized in successive harvest rotations. In our study, biomass yield was highest in the first triennial harvest rotation. The average yield in the first two triennial harvest rotations (14.2 Mg ha\(^{-1}\) year\(^{-1}\)) was 12% higher than in the following two triennial rotations. Yields decreased across all cultivars and planting densities.

A reverse relationship in three successive triennial harvest rotations was reported in a study by Amichev et al. (2018) where the average 3-year cumulative biomass yield of the top three cultivars was 25% higher in the second rotation (6.7 Mg ha\(^{-1}\) year\(^{-1}\)) than in the first rotation for these same cultivars. The biomass yield of the top five
cultivars was 17% higher, the yield of the top 10 cultivars was 3% higher compared to the first rotation, whereas the yield of all 30 cultivars was 27% lower. In the second crop rotation, biomass yield was 67% higher to 94% lower relative to the first rotation. The yield of willows grown in Canada in several different harvest rotations (3 and 4 years) was higher at 13.8 Mg ha\(^{-1}\) year\(^{-1}\) in unfertilized plots and 19.2 Mg ha\(^{-1}\) year\(^{-1}\) in fertilized plots (Nissim et al., 2013). Moreover, the highest-yielding clone SX64 produced 24.3 Mg ha\(^{-1}\) year\(^{-1}\) of dry biomass in the third triennial rotation, whereas the lowest yield of 8.1 Mg ha\(^{-1}\) year\(^{-1}\) was obtained for clone 5,027 in the first harvest rotation. The variations in the biomass yield of willows grown in two successive triennial harvest rotations (Sleight et al., 2016) and in three successive triennial harvest rotations (Sleight & Volk, 2016) were also studied in the United States. Biomass yields increased by 7.9% between the first (9.6 Mg ha\(^{-1}\) year\(^{-1}\)) and the second (10.3 Mg ha\(^{-1}\) year\(^{-1}\)) triennial harvest rotations of commercial willow cultivars in 360 experimental plots in five locations in north-eastern and north-central United States (Sleight et al., 2016). However, there was minimal agreement between the observed variations in yield. The increase in biomass yield between successive harvest rotations was high (up to 69%) in low-yielding plots in the first rotation, but it decreased when yields in the first harvest rotation were higher (2%), and decreased (~22%) when yields in the first rotation were highest. The authors concluded that the application of a single yield coefficient in all production systems can lead to errors when forecasting yields in successive harvest rotations. Similar observations were made in our study where biomass yields were highest in the first triennial harvest rotation in all analysed combinations. Such high yields were not achieved in successive harvest rotations. These differences were attributed to the seven main factors that were discussed at the beginning of this paper. Our results also suggest that biomass yields in the first harvest rotation are generally low in production systems with low initial planting density, poor soils and extensive (low-input) production methods where plants have to compete for resources. For this reason, yields are more likely to be higher in the second and third harvest rotation than in the first rotation. In production systems with fertile soils (abundant in water and nutrients), higher planting densities, good crop management practices and first-year coppicing, yields are unlikely to be higher in successive harvest rotations and can even be lower than in the first rotation. Therefore, the experimental conditions in this study were more complex than those described.
by Sleight et al. (2016) and Sleight and Volk (2016) where biomass yields were generally lower in the first harvest rotations. However, the above observations are not a golden rule because in our experiment and in other studies, higher yields were sometimes noted in first harvest rotations. The biomass yields noted in our 12-year study in different harvest rotations (12 annual rotations, six biennial rotations and four triennial rotations), cultivars (5) and planting densities (4) constitute valuable inputs because biomass yield directly influences the profitability, energy efficiency and environmental efficiency of willow production systems.

The results described in this paper cover one half of the anticipated 25-year lifespan of a willow production system, and they provide valuable information about cultivars, planting densities and harvest rotations for farmers, researchers and other actors on the biomass market. It should be noted that varied soil conditions, rainfall levels and pest infestation levels exerted a visible influence on willow survival rates during the experiment. Our findings indicate that the planting density of 24,000 cuttings/ha is optimal for commercial production systems with longer harvest rotations because it guarantees a high increase in biomass yield. In contrast, maximal biomass yields cannot be achieved at an initial planting density of 12,000 cuttings/ha.

In this study, higher yields were noted after the first harvest in annual, biennial and triennial harvest rotations. The above implies that high biomass yields can be achieved in the first harvest rotation when willow stands are well managed, coppiced after the first year, established on fertile soils and grown at higher densities. However, biomass yields in these production systems are unlikely to increase in successive harvest rotations, and they can even be lower, but more stable than in the first harvest rotation. The assumption that biomass yields will continue to increase in successive crop rotations is misleading and can result in serious errors when forecasting yields over the entire lifespan of a willow production system. Our results indicate that biomass yields in the first and successive harvest rotations (regardless of their length) are influenced by soil quality, environmental conditions, cultivar and crop management, including first-year coppicing. Planting density should be correlated with harvest rotation. The profitability, energy efficiency and environmental efficiency of willow production systems will be evaluated in the future to generate valuable insights for farmers, researchers, end users of willow biomass (CHPs, power plants, integrated bio-refineries), local communities and decision-makers.

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