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Willow Short Rotation Coppice Trial in a Former Mining Area in Northern Spain: Effects of Clone, Fertilization and Planting Density on Yield after Five Years

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Abstract: A willow short rotation coppice (SRC) trial was conducted on former mining land in northern Spain over a period of five years, with the purpose of evaluating the effects on yield of two planting densities (9876 and 14,815 cuttings ha^{-1}), three treatments (control, two levels of nitrogen, phosphorus and potassium compound fertilizer (NPK) plus weed control) and three willow clones (Björn, Inger, Olof). The area was subsoiled, ploughed, harrowed and fertilized with NPK before trial establishment. A randomized block design was applied, with three replications of each treatment in a total of 54 plots, each of an area of 400 m^2. The effects of the interactions between the various factors on yield and other growth parameters were also studied. The clone factor significantly affected the number of shoots per stool (greatest for the Inger clone) and the Olof clone, which showed the lowest mortality rate and produced the largest trees and largest quantity of biomass. The combined application of fertilizer and herbicide also significantly increased the values of all response variables considered, except the mortality rate. The planting density did not significantly affect the response variables. Clone × treatment interactions were significant for the shoots per stool, height, diameter and biomass variables, and the Olof clone displayed the highest height and diameter growth and yield. The results obtained in the first rotation indicate that the Olof clone adapted well to the trial conditions and therefore would be appropriate for producing biomass in abandoned mine land in Asturias. These findings will help in the development of strategies for the establishment and management of SRC on marginal land.

Keywords: biomass productivity; yield; growth; marginal areas; SRC; willow clones

1. Introduction

The European Commission's 2020 Energy Strategy aims to increase the use of renewable resources [1]. The main global prospect for energy production worldwide is the use of renewable resources in general and biomass in particular [2]. The Intergovernmental Panel on Climate Change (IPCC) predicts that biomass will be used to produce between 25% and 46% of global energy by 2100 [3]. One of the best ways of ensuring the long-term availability of biomass for renewable energy production is to establish and grow new perennial energy crops, which also adds value to marginal land [4].

The region of Asturias in northwestern Spain has abundant natural energy resources, including both renewable and fossil fuels. The region was a major coal producer during several decades. Although coal mining peaked during the last century, it continues to be one of the most important sources of employment and income in Asturias [5]. However, the sector is currently in recession, and large areas of mine land have been abandoned. The Hunosa Group, the most important coal-mining company in the region, currently owns up to 3759 ha of former mine land. At least 700 ha of this land is characterized by slopes of less than 30% and is therefore suitable for mechanized establishment of energy crops. Because of the unfavorable soil structure/properties in these areas (nutrient depletion, low organic matter content, etc.), establishing other crops would also be a challenge [6].

Energy crops can be used to produce biofuels or can be combusted directly to generate heat and/or electricity [7]. Fast-growing energy crops such as willow or poplar can be planted at high densities and managed as short rotation coppice (SRC). Planting energy crops as SRC is considered one of the most energy-efficient methods of reducing coal production and reconverting the sector to other productive activities, as the growth of these crops is understood as a means of reducing greenhouse gas emissions [8].

The most noteworthy features of SRC include high yield biomass production, high height growth through short rotation and high planting density, prolonged vegetative growth, resistance to pests and diseases and good healing of cut surfaces after biomass harvesting [9]. Furthermore, production of biomass with high calorific value and low ash contents and other components that may act as atmospheric pollutions or cause technical problems in boilers is an attractive prospect as biomass properties vary and are specific to different types of plants [10]. Short rotation coppice can be established on a wide range of types of land, including marginal land [11]. Thus, Zurba et al. (2013) [12] recommended planting SRC on marginal land, in parallel with other sustainable land management options. The use of SRC also contributes in the long term to improving soil quality and biodiversity, protecting ground water and preventing soil erosion. Taking into account the wide adaptability of members of the genus *Salix* spp. to extreme conditions and to nutrient-impoverished and polluted soils [13], short rotation willow coppice (SRWC) can be established in soils that are not suitable for agricultural exploitation [14]. The use and recovery of former mining land is therefore a realistic possibility [15,16]. Indeed, SRWC is one of the most promising bioenergy cropping systems for use in temperate regions of Europe [17], as well as in Canada and the USA [18,19], together with *Populus* sp., *Eucalyptus* sp. and *Robinia pseudoacacia* L.

Previous studies have shown that SRC biomass production can be influenced by many factors, including edaphic properties such as nutrient and water availability [20–22], climatic conditions [23,24], the plant material used [24–26], pest and weed control [27], plant density [28,29] and rotation length [16,30].

Increased growth response is often observed following fertilization [31]. The application of fertilizers has been shown to increase production from 30–70% within three years, and the greatest increases are achieved in the least fertile areas [26,32]. Planting density should also be taken into account, as crop structure greatly influences biomass yield [33]. In other studies carried out in similar climatic zones, several authors have reported optimal planting densities for coppiced willow of between 10,000 and 15,000 plants ha^{-1} [34]. In Europe, the crop rotation in perennial energy crops grown as short rotation coppice is usually 3–5 years.

In SRC plantations, successful establishment (i.e., survival in the first year of rotation) is usually considered to have occurred when approximately 90% of the plants survive. It is understood that plants should be replaced if more than 30−40% of the stock dies. One way of assessing the adaptability of plants to a study area is to monitor their growth, which is directly influenced by clone, climate and site conditions [35]. To ensure plant survival, exhaustive weed control should be carried out, along with routine follow-up of the pests and diseases that potentially affect energy crops.

SRC trials have not previously been established in the region of Asturias to evaluate the potential for biomass production. Landowners wishing to establish SRC plantations for this purpose therefore do not have any information on which to base the planting design.

The goals of this study were as follows: (i) to evaluate the viability of establishing SRC willow crops on restored coal mining land in Asturias and (ii) to evaluate the effects of clone, fertilization and planting density on yield.

2. Materials and Methods

2.1. Site Description

The experiment trial was established in May 2008 in the recultivated area of the Mozquita opencast coal mine in Asturias (northern Spain). The study area is characterized by an average annual temperature of 13 °C and an average annual precipitation of 1115 mm, of which 345 mm falls during the growing season (May–September). The climate is oceanic with high annual precipitation, and although summer precipitation is relatively low in some areas, physiological drought does not occur in any part of the region, which is located entirely within the European Biogeographic Atlantic Region [36] (Figure 1 and Table 1).

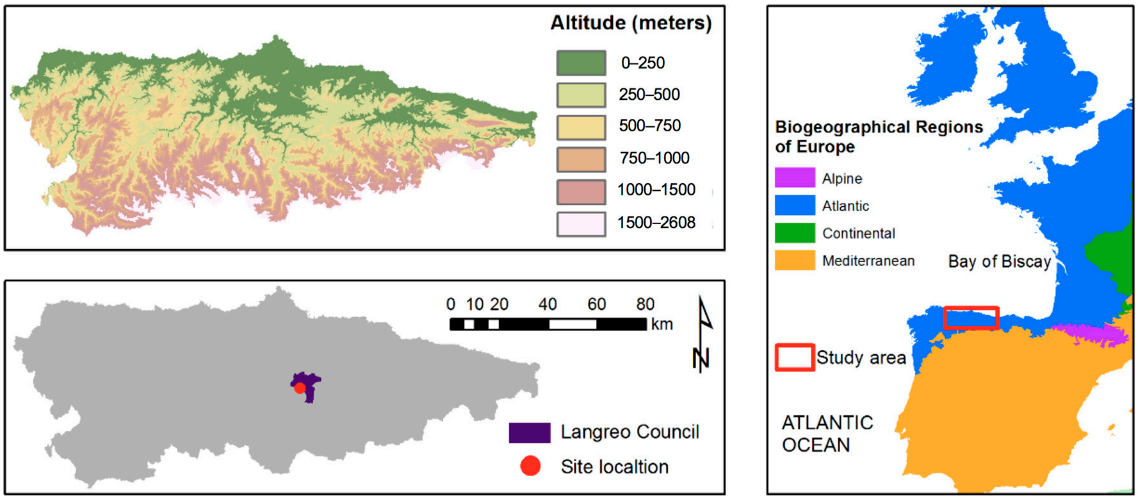

Figure 1. Location of the study area in Asturias (northwestern Spain).

The clay loam substrate (with a high content of coarse elements, approximately 30%) was dumped and ameliorated in 2003 by constructing a drainage and treatment system for run-off water and spreading a layer of topsoil (of a thickness of approximately 25 cm) over the entire surface. Soil formation is at an early stage, and the soil structure is still unstable. The steep terrain minimizes any groundwater effects.

The effective soil depth was measured in order to determine the area in which the plant roots can penetrate unhindered to obtain water and nutrients essential for normal growth. Thus, the depth was measured, with a Dutch auger, at a minimum of three randomly-selected points in each plot. Five soil samples were collected before planting, with the same auger, from the upper soil layer (0–20 cm) and combined to form one sample. The samples were air-dried, crumbled, finely crushed and sieved with a 2-mm screen before analysis in duplicate. The particle-size distribution was determined by the pipette method after the samples were dispersed with sodium hexametaphosphate and Na_2CO [37]. The pH was measured in H_2O with a glass electrode in a suspension of soil and water (1:2.5), and the electrical conductivity was also measured in the same extract (diluted 1:5). Organic matter was determined by loss on ignition. Total N was determined by the Kjeldahl method [38]. Available P was determined colorimetrically with Mehlich 3 reagent [39]. Exchangeable cations (K, Mg, Na and Ca), extracted with 1 M NH_4Cl, and exchangeable aluminum, extracted with 1 M KCl, were determined by atomic absorption spectroscopy. The effective cation exchange capacity (ECEC) was calculated as the sum of the values of the latter two measurements (sum of exchangeable cations and exchangeable Al). The physical and chemical characteristics of the soils are shown in Table 1.

Table 1. Description of the Mozquita experimental trial.

Site Description	Parameters	Units	Mozquita Trial
Site conditions	Location		Mozquita (Langreo-Asturias)
	Latitude		43°16′15.90″ N
	Longitude		5°41′55.75″ N
	Elevation	m	597
	Mean slope	%	19
	Mean soil depth	m	0.36
	Previous crops		Herbaceous
	Annual average temperature	°C	13
	Annual max. temperature	°C	18
	Annual min. temperature	°C	7
	Annual precipitation	mm	1115
Initial soil properties	pH	H_2O (1:2.5)	6.68
	Electrical conductivity	dS m^{-1}	0.23
	Sand	%	35.34
	Clay	%	27.32
	Organic Matter	%	1.61
	N total	%	0.11
	C/N ratio		8.80
	Available P (Mehlich 3)	mg kg^{-1}	7.34
	Extractable Na	$cmol_{(+)}$ kg^{-1}	1.48
	Extractable K	$cmol_{(+)}$ kg^{-1}	0.39
	Extractable Ca	$cmol_{(+)}$ kg^{-1}	5.71
	Extractable Mg	$cmol_{(+)}$ kg^{-1}	3.57
	Extractable Al	$cmol_{(+)}$ kg^{-1}	1.75
	ECEC	$cmol_{(+)}$ kg^{-1}	12.91

Soil texture was determined by the pipette method; total nitrogen was measured by the Kjeldahl method; available P was determined with Mehlich 3 reagent; and exchangeable cations (K, Mg, Na and Ca) and exchangeable aluminum were determined by atomic absorption spectroscopy. ECEC: effective cation exchange capacity.

2.2. Experimental Design

The experimental trial was established in an area of 2.16 ha. In the winter of 2008, the surface was subsoiled, ploughed to a depth of 30–40 cm and harrowed before the willow cuttings were planted. The soil was tilled, and the area was fertilized with an N-P-K fertilizer (8-24-16) applied at a rate of 500 kg ha^{-1}. The control plots were fertilized only once (F0 = control). The fertilizer was incorporated into the soil with a disc harrow.

Dormant hardwood cuttings (length 20 cm and diameter, 1–2 cm) were stored at 3 °C. To promote optimal rooting, the cuttings were soaked in water 48 h prior to planting, which was carried out by hand in early spring 2008. Immediately after the cuttings were planted, oxyfluorfen (5 l ha^{-1}) was applied over the whole area to prevent weeds emerging during the first months of growth. Three commercially available willow clones were selected for study. As willows adapt well to extreme soil conditions (e.g., nutrient poor and polluted soils) [13] and have shown good growth and potentially good biomass productivity (hereafter, yield) when grown as short rotations crops [9], we initially considered various clones that have been tested in several countries around the world, at a wide range of latitudes and under different climate conditions [40,41]. We finally selected three clones for testing after taking into account the following: (i) clones should be adapted to the ecological conditions of the plantation site according to the recommendations of the supplier and/or outlined in published research; and (ii) at least one of the progenitors of the selected clones must be native (*Salix triandra* L.) or naturalized (*Salix viminalis* L.) in the study region.

The willows were planted according to the double row planting design, by leaving a space of 0.75 m between each set of double rows, a space of 1.5 m to the next set of double rows and a space between plants of 0.9 or 0.6 m depending on the stocking level considered. The experiment was established following a randomized complete block design (3 blocks), and three factors were considered: clone (3 levels: Björn, Inger and Olof), planting density (2 levels: low density = 9876 plants ha^{-1} and high density = 14,815 cuttings ha^{-1}; Figure 2) and treatment (fertilization and weed control applied annually, 3 levels: F0 = control, F1 = 300 kg ha^{-1} + 4 l ha^{-1} a^{-1} and F2 = 600 kg ha^{-1} + 4 l ha^{-1} a^{-1}), as shown in Table 2. The basic design was repeated in three blocks, with a total of 54 plots of an area of 400 m^2, and constituted by 9 double rows with 22 or 33 cuttings per row (depending on the stocking densities). No irrigation, or pest, or disease control was performed during the cultivation period throughout the study area.

Table 2. Experimental plantation.

<table>
<tr><th colspan="2">Characteristics</th><th colspan="3">Mozquita Trial</th></tr>
<tr><td colspan="2">Total area</td><td colspan="3">2.3 ha</td></tr>
<tr><td colspan="2">Experimental design</td><td colspan="3">Randomized complete blocks</td></tr>
<tr><td colspan="2">Number of replicates</td><td colspan="3">3</td></tr>
<tr><td colspan="2">Species</td><td colspan="3">Salix spp.</td></tr>
<tr><td colspan="2">Clones tested</td><td colspan="3">Björn (B), Inger (I) and Olof (O)</td></tr>
<tr><td colspan="2">Origin of clones tested</td><td colspan="3">Sweden</td></tr>
<tr><td colspan="2">Progenitor</td><td colspan="3">B: Salix schwerinii E. Wolf × Salix viminalis L.
I: Salix triandra L. × Salix viminalis L.
O: Salix viminalis L. × (Salix schwerinii E. Wolf × Salix viminalis L.)</td></tr>
<tr><td rowspan="4">Treatments</td><td>Planting density</td><td colspan="2">Low
9876 plants·ha^{-1}</td><td>High
14,815 plants·ha^{-1}</td></tr>
<tr><td></td><td>F0 (control)</td><td>F1</td><td>F2</td></tr>
<tr><td>Fertilization</td><td>None</td><td>N-P-K 6:20:12
300 kg ha^{-1} a^{-1}</td><td>N-P-K 6:20:12
600 kg ha^{-1} a^{-1}</td></tr>
<tr><td>Herbicide</td><td>None</td><td>Application of glyphosate 4 l ha^{-1} a^{-1}</td><td>Application of glyphosate 4 l ha^{-1} a^{-1}</td></tr>
</table>

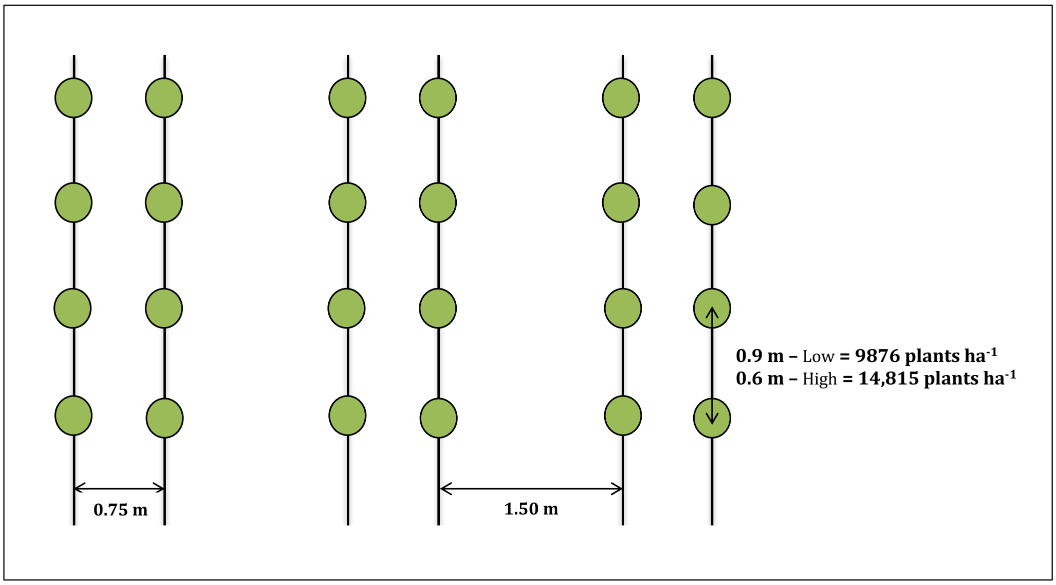

Figure 2. Diagram illustrating the experimental design.

2.3. Field Measurements and Yield Estimates

Several variables were measured after the growth period. In order to prevent the edge effect, the measurements were made in a subplot in the center of each of the plots. A total of 40 stools (live or dead) were measured in each of the 54 subplots in this study. Growth data were obtained following the protocol described by the U.K. Forestry Commission [42] for data collection in short rotation willow plantations.

Within each of the subplots, shoot diameters were measured at 20 cm and 130 cm above ground level (D20 and D130) with a digital caliper, and the total heights (H) were measured from ground to the top of the shoot with a Vertex III hypsometer (Haglöf Sweden AB, Långsele, Sweden).

The mortality rate (expressed as a percentage) was also recorded at the end of each vegetative season (Table 3). The crop was harvested in autumn 2012, five years after establishment of the trial, and 5 stools were randomly selected and subsequently cut in each of the above-mentioned subplots. A total of 270 stools were harvested manually with pruning shears (Electrocoup F3010) or a chainsaw. The height of the stumps was about 10 cm higher than the stumps remaining after commercial harvesting. The fresh weight (FW) of each stool was measured with an electronic balance (precision ±10 g). A representative subsample (300–500 g) of each stool was taken to the laboratory and weighed immediately with a precision balance (precision ±1 g). The subsample was dried to constant weight at 70 °C: the dry weight was recorded, and the dry biomass of the plot was calculated by multiplying the FW by the ratio of dry to fresh weight of the subsample.

The yield per hectare of each subplot was estimated as Mg ha^{-1} by multiplying the average dry biomass of the 5 stools by an expansion factor of 14,814.8 (0.6 m plant spacing within row) or 9876.5 (0.9 m plant spacing within row). The yield was subsequently corrected by the estimated mortality rate. The basic statistics for these variables are shown in Table 3.

Table 3. Summary statistics for each variable studied (shoots per stool, height (H), diameter at 20 cm above ground level (D20), mortality rate and dry weight (DW) for the three *Salix* clones used in the trial.

Clone /Variables	*n*	Minimum	Maximum	Mean	Standard Deviation
Björn					
Shoots per stool (*n*)	18	1.0	3.4	1.5	0.5
H (cm)	18	99	397	228	97
D20 (cm)	18	0.8	3.2	1.7	0.7
Mortality rate (%)	18	2.5	100.0	42.5	25.5
DW (Mg·ha^{-1})	18	0.2	10.2	3.9	3.1
Inger					
Shoots per stool (*n*)	18	1.3	3.2	2.3	0.5
H (cm)	18	67	417	241	87
D20 (cm)	18	0.6	2.6	1.8	0.5
Mortality rate (%)	18	0.0	77.5	17.7	21.0
DW (Mg·ha^{-1})	18	0.2	15.4	6.8	4.8
Olof					
Shoots per stool (*n*)	18	1.1	1.8	1.4	0.2
H (cm)	18	177	697	468	164
D20 (cm)	18	1.3	3.8	2.6	0.8
Mortality rate (%)	18	0.0	27.5	11.7	7.6
DW (Mg·ha^{-1})	18	1.1	30.8	14.1	9.1

2.4. Statistical Analyses

The standard model for the trial is as follows:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + \lambda_l + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + (\alpha\beta\delta)_{ijk} + e_{ijkl} \tag{1}$$

where y_{ijk} is the observed value of the response variable (shoots per stool, height, diameter, mortality or biomass) of clone i in block l, considering a planting density level k and applying treatment j; μ is the overall mean; α_i, β_j, δ_k and λ_l are the clone, treatment, planting density and block effects, respectively; $(\alpha\beta)_{ij}$, $(\alpha\delta)_{ik}$, $(\beta\delta)_{jk}$ are the bifactorial interactions; $(\alpha\beta\delta)_{ijk}$ is the interaction of the three factors; and e_{ijk} is the random experimental error term.

A multifactor analysis of variance (ANOVA) was performed for each response variable (shoots per stool, diameter, height, mortality and yield) to test the overall effect of the different factors considered (clone, plantation density and fertilization treatment) within the framework of the randomized block design.

When a significant factor effect was observed ($p \leq 0.05$), Tukey's honestly significant difference (HSD) multiple range test was used to determine homogeneous groups according to the similarity of the response variable. Prior to the ANOVA, data were tested for normality and homoscedasticity of residuals. Tests were performed using SPSS 23.0 statistical software (IBM Corp, Armonk, NY, USA) [43]. When necessary, data were log-transformed in order to meet the criteria of normally distributed data with equal variances.

3. Results

The soil in the study area was slightly acidic to neutral (≅pH 6.7), with low electrical conductivity, low depth and low contents of exchangeable base cations and of Mehlich 3 available P. The organic matter content of the upper horizon was low, and the C/N ratio was slightly low (Table 1).

The results of the analysis of variance (Table 4) showed that clone and treatment factors were significant ($p < 0.05$), while the density effect was not significant ($p = 0.649$) for any of the response variables considered. As expected, the block effect was not significant at $p < 5\%$. The first order interactions were significant, with the exception of the density × treatment interaction, which was not significant ($p = 0.104$) for the number of shoots per stool. The second order interaction was not significant for any of the response variables. The results of the Tukey HSD test, applied when a significant factor effect was observed ($p \leq 0.05$), are shown in Table 5.

Table 4. A multifactor analysis of variance (ANOVA) result. *F*-values and significance level (*p*) for each of the factors and their first order interactions.

	Shoots per Stool (*n*)		**H (cm)**		**D20 (cm)**		**Mortality Rate (%)**		**DW (Mg·ha^{-1})**	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Clone	27.797	<0.001 **	14.806	<0.001 **	7.337	0.002 **	12.309	<0.001 **	9.084	<0.001 **
Planting density	0.036	0.850	0.115	0.736	0.188	0.667	0.503	0.481	0.041	0.840
Treatment	3.317	0.044 *	8.763	<0.001 **	10.644	<0.001 **	0.519	0.598	10.070	<0.001 **
Block	0.446	0.643	0.745	0.480	0.798	0.456	2.814	0.069	0.506	0.606
Clone × planting density	11.173	<0.001 **	6.393	<0.001 **	3.545	0.008 **	0.519	0.630	3.699	0.007 **
Clone × treatment	10.303	<0.001 **	17.721	<0.001 **	9.646	<0.001 **	0.540	0.711	8.108	<0.001 **
Planting density × treatment	1.952	0.104	3.546	0.008 **	4.538	0.002 **	0.399	0.695	4.008	0.004 **

* Indicates significance at $p \leq 0.05$, and ** indicates significance at $p \leq 0.01$.

3.1. Number of Shoots per Stool

The mean number of shoots per stool of the *Salix* hybrids clones ranged from 1.3–2.2 (Table 5). The Tukey HSD test for the mean comparisons for both clone and treatment factors identified two different groups in relation to the number of shoots per stool (Table 5). The Inger clone produced the highest number of shoots per stool, whereas Björn and Olof produced significantly lower numbers of shoots. Taking into account the treatment factor, the number of shoots was lowest in the control plots and significantly higher in the fertilized plots (Table 5).

Regarding the first order interactions, the effect of planting density on the number of shoots per stool depended on the clone (Table 4). For clones Björn and Olof, the number of shoots per stool was highest at the highest planting density, and for Inger, it was highest at the lowest planting density (Table 5).

The effect of the fertilizer treatment on the number of shoots produced also depended on the clone. Although the amount of fertilizer did not affect the number of shoots produced by the Björn clone, it did affect the number of shoots produced by the Inger and Olof clones (Table 5).

Table 5. Mean values of the Tukey HSD test of the different variables in relation to the factors clone, planting density and fertilizer treatments and the groupings according to the results of the Tukey HSD test.

	Shoots per Stool (*n*)			**H (cm)**			**D20 (cm)**			**Mortality Rate (%)**			**DW (Mg·ha^{-1})**		
	F	**M**	**G**	**F**	**M**	**G**	**F**	**M**	**G**	**F**	**M**	**G**	**F**	**M**	**G**
Clone	B	1.4	b	B	206	b	B	1.6	b	B	42.5	b	B	1.3	b
	I	2.2	a	I	222	b	I	1.7	b	I	17.9	a	I	3.6	ab
	O	1.3	b	O	434	a	O	2.5	a	O	11.9	a	O	8.6	a
Treatment	F0	1.4	a	F0	181	b	F0	1.3	b	-	-	-	F0	1.1	b
	F1	1.7	a	F1	315	a	F1	2.1	a	-	-	-	F1	5.4	a
	F2	1.8	a	F2	344	a	F2	2.3	a	-	-	-	F2	6.6	a
Clone × Density	BN1	1.3	b	BN1	236	b	BN1	1.8	a	-	-	-	BN1	1.6	b
	IN1	2.3	a	IN1	218	b	IN1	1.6	a	-	-	-	IN1	3.3	ab
	ON1	1.0	b	ON1	394	a	ON1	2.3	a	-	-	-	ON1	7.0	a
	BN2	1.5	b	BN2	177	b	BN2	1.3	b	-	-	-	BN2	1.0	b
	IN2	2.1	a	IN2	226	b	IN2	1.7	b	-	-	-	IN2	3.8	ab
	ON2	1.4	b	ON2	478	a	ON2	2.6	a	-	-	-	ON2	10.7	a
Clone × Treatment	BF0	1.2	a	BF0	142	b	BF0	1.1	b	-	-	-	BF0	0.4	b
	BF1	1.4	a	BF1	236	ab	BF1	1.7	ab	-	-	-	BF1	1.3	ab
	BF2	1.7	a	BF2	290	a	BF2	2.1	a	-	-	-	BF2	3.3	a
	IF0	1.8	b	IF0	174	a	IF0	1.4	a	-	-	-	IF0	2.2	b
	IF1	2.4	a	IF1	276	a	IF1	1.9	a	-	-	-	IF1	8.6	a
	IF2	2.6	a	IF2	274	a	IF2	2.0	a	-	-	-	IF2	6.8	ab
	OF0	1.2	b	OF0	275	b	OF0	1.8	b	-	-	-	OF0	3.1	b
	OF1	1.6	a	OF1	571	a	OF1	3.1	a	-	-	-	OF1	17.6	a
	OF2	1.4	ab	OF2	559	a	OF2	3.0	a	-	-	-	OF2	16.8	a
Density × Treatment	-	-	-	N1F0	180	b	N1F0	1.4	b	-	-	-	N1F0	0.9	b
	-	-	-	N1F1	324	a	N1F1	2.3	a	-	-	-	N1F1	6.7	a
	-	-	-	N1F2	347	a	N1F2	2.4	a	-	-	-	N1F2	5.7	a
	-	-	-	N2F0	183	a	N2F0	1.3	b	-	-	-	N2F0	1.2	b
	-	-	-	N2F1	287	a	N2F1	1.9	ab	-	-	-	N2F1	4.4	a
	-	-	-	N2F2	366	a	N2F2	2.3	a	-	-	-	N2F2	7.6	a

F = factor; M = mean; G = group.

3.2. *Tree Dimensions (H and D20)*

The influence of the clone and the treatment on the tree dimensions was highly significant at the end of the rotation. The mean stem height of the *Salix* hybrids clones ranged from 206 cm for Björn to 434 cm for Olof, and D20 ranged from 1.6 cm for Björn to 2.5 cm for Olof. Comparison between means using the Tukey HSD test for both variables identified the same two groups for the clone and treatment factors. Thus, Olof produced the highest H and D20, respectively, whereas Inger and Björn were included in a different group with significantly lower values of these variables. As occurred with the number of shoots, Tukey HSD defined fertilizer treatments F2 and F1 as belonging to the same group, which yielded significantly higher values of both H and D20 than the control plots (F0) (Table 5).

Analysis of the clone × planting density and clone × treatment interactions produced some interesting results (Table 5). The effect of planting density depended on the clone factor for both H and D20. Thus, the highest mean H was reached by Olof, at both planting densities (low and high). However, for D20, the clone factor was only influential at the high planting density, with Olof producing the highest D20. Regarding the clone × treatment interaction, the different treatments influenced the variables considered. For Björn and Olof, both H and D20 were influenced by the different treatments applied, relative to the control plots. However, for the Inger clone, neither H, nor D20 were affected by the different doses of fertilizer applied.

The planting density × treatment interaction shows that the treatment only affected H at the lowest planting density, and there were no significant differences at the highest planting density (Table 5). However, the treatment × planting density interaction affected D20. In this case, for both planting densities, the control plots showed the lowest yields relative to the plots to which fertilizer was applied, and the highest yields were obtained with the highest dose of fertilizer at both planting densities (Table 5).

3.3. Mortality

The data indicate high interclonal variability in mortality rates during the trial (Table 3). The mortality rate throughout the rotation ranged between 11.9% (for the Olof clone) and 42.5% (for the Björn clone). The mortality rates of the Olof and Inger clones were significantly lower than those of the Björn clone (Table 5). However, there were no significant differences in mortality rates in relation to the other factors considered or their interactions (Table 4). Thus, the mortality rate was not influenced by planting density or treatment (herbicide + fertilizer) or by the interactions between these factors and the clone factor. The analysis of variance revealed that the block factor was almost significant, indicating the importance of site conditions in the results of the trial.

3.4. Yield (Dry Weight)

Highly significant differences in aboveground woody biomass were detected between clones at the end of the rotation. Comparison between means using the Tukey HSD test identified Olof as the most productive clone (8.6 Mg ha^{-1}), with about 50% higher production than Inger (3.6 Mg ha^{-1}) and 70% higher than Björn (1.3 Mg ha^{-1}). The soil treatment had similar effects on yield and on the other response variables. Thus, the yield was lowest in the control plots and was significantly higher in the fertilized plots, but did not differ significantly in relation to the fertilizer dose (Table 5).

Again, the yield was influenced by the three genotypes tested (Table 5). The Björn clone produced the lowest yield (1.6 and 1.0 Mg ha^{-1}, for the low and high planting densities, respectively), followed by the Inger clone (low planting density = 3.3 Mg ha^{-1} and high planting density = 3.8 Mg ha^{-1}). The Olof clone produced a much higher yield (7.0 Mg ha^{-1} at the lowest planting density and 10.7 Mg ha^{-1} at the highest planting density) than the other willow clones (Table 5). The clone × treatment interaction was highly significant, and the different combinations between clone and treatment produced the greatest differences in yield. Thus, for all three clones, the lowest yields were obtained in the control plots, highlighting the importance of the combined weed control + fertilization treatment. The effects of the planting density × treatment interaction were only statistically significant at the high planting density, and differences in yield were observed between the control plots and the fertilized plots.

4. Discussion

In this paper, we discuss the growth and yield of three hybrid clones of *Salix viminalis* planted at two different densities and with three fertilizer treatments on marginal land in a former mining area.

4.1. Clone Factor

Use of willow clones that are well adapted to the climatic conditions of the planting area is essential for biomass production in cultivation of willow SRC [34], which is why different clones were tested. The three clones performed differently in relation to growth and yield, with the Olof clone producing the best results for all variables analyzed. Considering the number of shoots per plant, the Inger clone produced the highest values up to the end of the first rotation. The Olof clone, for which yield was highest, produced a significantly lower number of shoots than Inger, the second most productive clone. One of the traits that increases the quality of biomass as an energy resource is a low ash content. Taking into account that the ash content of bark is much higher than that of wood [44] and that the bark to wood ratio decreases with increasing tree diameter [45], the smaller number of shoots per stool of the Olof clone enables the production of stems with larger diameters and therefore better quality woody biomass. Comparison of the tree dimension variables in the willow clones (i.e., shoot diameter and plant height) showed that both height growth and diameter growth were significantly higher in the Olof clone than in the other two clones. The mortality rate was also lowest in the Olof clone. The differences between clones were even more pronounced in relation to yield; the least suitable clone, Björn, produced a mean yield of 0.3 Mg ha^{-1} year^{-1}, followed by the Inger clone, with 0.7 Mg ha^{-1} year $^{-1}$. The Olof clone produced the highest yield, of 1.7 Mg ha^{-1} year^{-1}. The yield produced by Olof was similar or slightly lower than those reported for *Salix viminalis* planted in former mine land in other studies. For example, Bungart and Huttl [46] reported yields of between 1 Mg ha^{-1} year^{-1} and 7.8 Mg ha^{-1} y^{-1} in former mine sites, regardless of the treatment or planting density.

Other examples of woody biomass yields obtained in marginal sites in Germany are given by Gruenewald et al. [16,47], who reported yields of between 2 and 4 Mg ha^{-1} year^{-1} for a short rotation willow crop cultivated on a six-year rotation period, and Stolarski et al. [48], who reported an average willow yield (oven-dry matter) of around 8 Mg ha^{-1} year^{-1} after the first rotation (four years) in northern Poland.

As expected, the productivity of the willow varieties tested in the present study in marginal abandoned mining land was lower than obtained in other experimental studies in commercial plantations established on former or current agricultural land [49]. For example, Rosso et al. [4] reported growth rates between 3.8 and 13.3 Mg ha^{-1} year^{-1} for three years for different *Salix* clones, under good and optimal growing conditions in Northern Italy. Similar growth rates (14.1 Mg ha^{-1} year^{-1}) were obtained under optimal conditions in Poland [50] and in the U.S. and Canada [22,32], although they are usually in the range 10–12 Mg ha^{-1} year^{-1} [51]. Early experimental short-rotation plantations of *Salix viminalis* on former arable land in Europe (Germany) have yielded growth rates of between 2 and 4 Mg ha^{-1} year^{-1}, for first rotation of a duration of five years [20], and of up 15–20 Mg ha^{-1} year^{-1} [52], for *Salix viminalis* crop trials on former agricultural land. Similar values are expected to be obtained in the U.K. by using selected genetic material [53]. However, as pointed out by Volk et al. [51] for the U.S. and Canada, more recent figures suggest that an average growth rate of 10 Mg ha^{-1} year^{-1} is more realistic for areas with good growing conditions in northern Europe [54].

Considering the above-mentioned findings for experimental plantations on marginal land, we can state that our trial site (marginal, former mine land), shows acceptable potential for biomass production. Moreover, as pointed out by Grünewald et al. [47], biomass yields are expected to increase significantly in subsequent rotations. Therefore, regardless of economic or environmental considerations and taking into account that former mine land is unsuitable for growing food crops due to the possible presence of contaminants [26], producing woody biomass seems to be a good option for valorization of the land.

The trial results show differences in the patterns of mortality rates in the three clones tested. The survival rate should clearly be considered a key parameter for clone selection and breeding. The mortality of plants in willow SRC stands may be due to several factors. The climate is usually one of the most important factors. The low frost resistance of *Salix viminalis* clones may negatively affect the stability of the energy crop. Frost can cause irreparable damage to the plantation, as observed by Tahvanainen and Rytkönen [18] in a study in which different clones of *Salix viminalis* were tested in 35 different plantations in southern Finland and in which the winter frost caused a reduction in seedling survival. In addition, although some varieties of willow are not killed by late spring frosts, the young shoots of other varieties may be affected, by not being capable of adapting to the possible lack of water or heat stroke [35]. The intake of water cannot compensate for loss by transpiration. However, the high mortality of the Björn clone was not associated with the climatic conditions, as the other clones tested were not affected.

Pests and diseases can also affect survival. For example, *Melampsora* spp. cause a serious fungal disease [53]. Nonetheless, the plantation was not affected by any pests or diseases throughout the trial.

Good weed control is very important, especially during plantation establishment. Weed control was successfully carried out in the present trial. In other studies, successful weed control was consistently found to lead to improved biomass production [27]. By contrast, unsuccessful weed control led to decreased biomass production and even to the total destruction of the plantation [18].

Poor site conditions, such as shallow rooting depth, unsuitable texture, etc., can prevent the plants from adapting to the terrain. In addition, knowledge of the rooting characteristics of willow clones may contribute to predicting clone performance in changing environments and help in the choice of clone to use in marginal environments. For example, in the present trial, the Björn clone displayed a poor ability to adapt to the site conditions. Some experimental plantations may have been established in such poor soils that they failed to survive [55].

4.2. Planting Density Response

An adequate combination of rotation length and the optimal planting density can increase yields by as much as 33% [25,56]. Choosing the best planting density is key to ensuring high production and survival over time. However, the choice is difficult. It is well known that maximum annual biomass growth (ABG) is reached earlier in dense plantings because the trees occupy the site more quickly than in less dense plantings. However, less dense plantings quickly catch up and maintain high ABG longer than their dense counterparts because inter-tree competition is less severe [57,58].

In our experimental trial, the planting density significantly affected the number of shoots per stool, the tree dimension variables and yield, although it did not significant affect mortality. In general, studies of the effect of plantation density on growth and yield have produced contradictory results. This is not surprising, as growth and yield of a particular genotype are the result of complex interactions between different factors such as site quality, silvicultural practices, pest and diseases, rotation length and initial planting density [32]. Thus, different studies involving short rotation coppice have also found that yield increases with initial density of the plantation [23,56,59,60]. In a study carried out in Sweden, Bergkvist and Ledin (1998) [23] found that the yield of *Salix viminalis* during the first cutting cycle (five years) was positively correlated with the number of plants per hectare for up to 20,000 plants ha^{-1}, but not for the higher planting density of 25,000 plant ha^{-1}. Tubby and Armstrong [60] reported increased yield as planting density increased from 4500 up to 15,625 stools per hectare for *Salix viminalis* “Bowless Hybrid” harvested after three years. In different studies carried out with willow, researchers found that the annual biomass yield increased with increasing planting density at a range of planting densities of 10,000–25,000 plants ha^{-1}, in the U.K. [56], and two planting densities of between 7500 and 22,500 plants ha^{-1}, in Finland [59]. In a laboratory trial conducted with willow by Cao et al., in which different planting densities were tested in pots, the total biomass, as well as the biomass of stem or leaves, of each treatment, increased with increased planting density [61].

On the other hand, some studies of *Salix* spp. [62,63] and *Populus* spp. [57,64–68] or combinations of alder, poplar and willow in coppice and single plantations [69] have reported no positive effect of increasing density on biomass yield. A wide range of initial planting densities (i.e., 3000–40,000 plants ha^{-1} for hybrid poplar) produces similar yields [70]. Moreover, Verwijst and Telenius [71] reported that the total yield remains independent of density and may even decrease as a result of increased (excessive) natural mortality.

The most frequent planting density used in various European countries (U.K., Denmark, Sweden) and in the U.S. ranges from 10,000 up to 25,000 plants ha^{-1} with a usual plantation layout in double row design with a distance of 0.75 m between the double rows and of 1.5 m to the next double row, with the distance between plants ranging from 1–0.4 m. More specifically, planting densities of about 10,000 cuttings per ha [72] or 15,000 cuttings per ha [23] seem to be optimal for SRC plantations to obtain a good compromise between biomass yield and cost of establishment, as a high number of cuttings markedly increases the cost of crop establishment.

Therefore, taking into account our findings and considering the complex interactions between multiple factors affecting the yield, the range of optimal initial plantation densities will vary in different situations. Although we only analyzed data from one experimental trial and our results must be interpreted with caution, they suggest that planting more than 10,000 trees per hectare in SRC plantations with *Salix* clones in former mine land in north Spain is an unnecessary expense.

4.3. Response to Fertilization and Herbicide

The yield from willow plantations is largely determined by the silvicultural treatments applied. In this experimental trial, all plots (including the control plots) were fertilized at the start of the study to improve the initial nutrient content. The treatment factor (fertilization + herbicide) was applied annually, and two doses of NPK fertilizer were considered (F1 = 300 kg ha^{-1} or F2 = 600 kg ha^{-1}), both in combination with weed control (4 l ha^{-1}). Concerning the pH, the site presented an optimal value for willows (6.68), and thus, it may represent a particularly valuable marginal land as it does not need liming [73]. Moreover, irrespective of whether fertilization is followed by weed control, growth of weeds may lead to strong competition for water, which may be reflected by a slight decrease in growth of the plantation relative to the control plots, as observed by Barrio et al. (2007) [74] in poplar. Therefore, weed control during the establishment phase (the first three years) is essential for optimal willow rooting and growth [75,76].

We observed that fertilization produced a significant increase in the number of shoots per stool (between 17 and 22% more shoots), although there were no significant differences in relation to fertilizer dose. This was true for the most and the least productive clones, Olof and Inger, respectively, but there were no significant differences for Björn, and a reduction in the number of shoots was obtained by increasing fertilization dose. Sevel et al. [77] also observed a strong correlation between number of shoots per stool and the fertilizer dose and attributed this finding to the amounts of nutrients available.

Regarding the effect of fertilization on yield, many studies have shown a significantly increased growth response following fertilization [31,78–80], although other studies did not find any such effect [81,82]. The lack of response mainly appears in plantations in which the initial nutrient status of the soil is high, as in former agricultural land, otherwise a significant effect may be detected. Thus, in an SRWC plantation in Estonia, the shortage of nutrients was suggested to be limiting for plant growth [83].

In our experimental trial, the lack of nutrients and the strong weed competition led to very low yield in the control plots and an increase on average of the 80% and 84% after application of treatments F1 and F2 relative to the control, with no differences between the two treatments. Many other authors have found a significant increase in growth with fertilization, but with a limited response when the dose of fertilizer is increased [31,81,84]. Thus, on the basis of these results, Adegbidi et al. [31] and Holm and Heinsoo [83] recommended annual application of 100 kg N ha^{-1} for willow. However, it can be difficult to establish the optimal amounts of fertilizer, i.e., the amount of N required for maximum biomass growth, due to the risk of supplying surplus N [81]. The interaction between weed control and fertilization and other environmental and economic considerations must also be taken into account in order to increase the effectiveness of the treatments.

Trials conducted on abandoned farmland have shown that growing willow can rapidly increase the amount of soil organic matter [22], which may also occur in abandoned mining sites. Incorporation of annual leaf litter fall can make a large contribution to soil N, increasing soil fertility and organic matter [85]. Thus, the increased yield over time is probably due to improved soil fertility [86]. Higher yields than those reported for mine sites could be achieved when water and nutrient availability are sufficient. Comparison of data obtained in different trials must take into account the variable and in many cases cultivation-related conditions.

As Bullard et al. [25] has pointed out, the necessity, appropriateness and cost-effectiveness of nutritional intake in this type of plantation generate many uncertainties, as crop response depends on many factors that are not always taken into account.

5. Conclusions

Acceptable yields can be achieved in five years in SRC with highly productive *Salix viminalis* clones planted on former mine land. Selecting the most suitable genotypes for growing under poor soil conditions is of particular importance for the valorization of marginal land. The Olof clone appeared to be most suitable for planting in the suboptimal conditions of the trial for the purpose of producing biomass, with a survival rate of around 90%. Fertilization and weed control are important management operations and always necessary in marginal land in which the initial nutrient status of the soil is poor. Although the results regarding planting density are preliminary, they indicate that planting around 10,000 trees per hectare may be suitable for SRC plantations with *Salix* clones in the study area.

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