

## Influence of fertilization on *Miscanthus × giganteus* (Greef et Deu) yield and biomass traits in three experiments in Serbia

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### ABSTRACT

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*Miscanthus × giganteus* (Greef et Deu) is an agro-energy crop of the second generation cultivated in purpose to obtain annually renewable bio-fuel produced from the aboveground biomass. Cultivation is preferred on marginal lands to avoid occupation of arable lands. Influence of fertilization and soil type Gleysol, Planosol and Technosol (open pit coal mine overburden) on yield and biomass traits of miscanthus were investigated during five years' field experiment. Among biometric characteristics: stem height, length and width of leaves, the number of leaves (dry and green) per stem and number of stems per rhizome, only the last one has a strong positive correlation with yield. Fertilization increased yield during fourth and fifth year of development on Gleysol and Technosol. The highest yield on Gleysol was 23.12 t/ha in 2014, on Planosol 10.16 t/ha, and 4.77 t/ha in 2015 on Technosol. The yield of miscanthus, beside fertilization, depends on weather conditions and weeds. Cultivation of miscanthus is possible on marginal soils with minimum application of agricultural measures only in the year of establishment. Gleysol is a type of soil that can be recommended for miscanthus cultivation.

**Keywords:** agro-energy crops; vegetative period; late winter harvest

*Miscanthus × giganteus* (Greef et Deu) is a perennial sterile cultivar grass growing as an agro-energy crop of the second generation, considered to be key renewable raw material for industry and energy production (Jeżowski 2008). High-level biomass production and a possibility of cultivation on lower quality soil (Heaton et al. 2004) make this crop very suitable as annual renewable raw material for biofuel production (Milovanovic et al. 2012). The main challenge is to choose marginal land not suitable for food production, because of low productivity or presence of some substances potentially hazardous for human or animal health, but with characteristics allowing production of energy crops in energy, economy and environmental sustainable manner

(EEA, Gelfand et al. 2013, Lord 2015). Miscanthus plantations have some advantages for the environment in comparison to annual crops: tillage only in the year of establishment, efficient water and nutrients use, resistant to pests and diseases (Cadoux et al. 2012). There are many reports on miscanthus yield obtained in Europe and USA, in a wide range 5–43 t/ha for 3<sup>rd</sup> vegetative period in winter harvest, but it is cropped mainly at arable soils. Irrigation, fertilization and weed control were used to increase yield with responses depending on soil characteristics, geographical area and climate parameters. The question about the influence of fertilization is still an open debate (Cadoux et al. 2012, Lesur et al. 2013, Dierking et al. 2016, Zapater et al. 2016).

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The aim of the research was to determine possibilities for miscanthus cultivation on marginal lands with minimum application of agricultural measures and to investigate fertilization influence on yield and biomass traits.

## MATERIAL AND METHODS

A field experiment was conducted at three locations in the Republic of Serbia. Zasavica (44°54'56.90"N, 19°34'10.44"E) is located in Sremska Mitrovica municipality, near Special Nature Reserve Zasavica. The soil is hydromorphic black, Gleysol type, where the corn was the previous crop. This soil was chosen as unsuitable for food crops cultivation because any application of mineral fertilizers and plant protection agents could jeopardize sensitive wetland ecosystem. The 2<sup>nd</sup> location belongs to the Mine basin Kolubara (44°28'16.41"N, 20°14'59.62"E), Lazarevac municipality. The soil is Technosol. There was no vegetation before this experiment i.e. the lot was created by deposition of overburden from the coal mine in 2010. The 3<sup>rd</sup> location is village Kozjak (44°35'07"N, 19°17'02"E) in Loznica municipality where the soil type is Planosol disrupted by road construction. All locations belong to degraded lands with low productivity.

Plant material, rhizomes of *Miscanthus × giganteus* (with a length of 10 cm, with 3–6 nodes), was manually planted on agro-technically prepared soil (ploughing in the fall of the year before planting and disking and fertilizer application, 50 kg NPK/ha containing 5.14 kg N/ha, 2.42 kg P/ha, 6.23 kg K/ha, just before planting), in April with crop density of 20 000 rhizomes/ha. Each experimental field was divided into two zones (treatments): A – irrigation three times in 1<sup>st</sup> growing season, fertilizing (100 kg NPK/ha: 10.28 kg N/ha, 4.83 kg P/ha and 12.45 kg K/ha) in the middle June of the 1<sup>st</sup> growing season, manual weed removal two times per year during the 1<sup>st</sup> and 2<sup>nd</sup> vegetation period; B – the same treatment without fertilization. In each zone, three plots (5 m × 5 m) were signed as sample units. Attributes of the aboveground biomass development were determined at the end of September (time of maximum growth). Stem height (maximal per plant), length and width of the 4<sup>th</sup> leaf, number of leaves per stem and number of stems per rhizome were defined for 10 randomly

selected plants within the sample unit. Biomass was measured after the hand harvest in March and calculated as yield in DM t/ha (tones of dry matter per hectare).

The field experiment was conducted from 2008 in Kozjak and from 2011 in Zasavica and Kolubara.

Statistical analyses were performed applying the SPSS version 13.0 (SPSS Inc., Chicago, USA). Independent-samples *t*-tests (significance set at  $P < 0.05$ ) were used to test differences in the yield and attributes of the aboveground biomass.

## RESULTS AND DISCUSSION

The yields of miscanthus aboveground biomass were investigated during at least four years in three locations: Zasavica (Gleysol), Kolubara (Technosol) and Kozjak (Planosol) in order to determine the impact of fertilization. Miscanthus yield during the 1<sup>st</sup> year after planting is low and usually is not taken into account (Lesur et al. 2013). Reported results represent annual production of biomass consisted of stems, leaves and inflorescences. During winter nutrients were transferred from the aboveground parts back to rhizomes and used in next vegetative period (Beale and Long 1997), most of the leaves were rip off by the wind and stems were dried, yield decreased about 30% but biomass quality increased (Lewandowski and Heinz 2003).

On Gleysol (Figure 1a) maximal yield was observed 17 DM t/ha in 2014 and 2015 vegetation seasons and 23.122 DM t/ha with fertilization. In 2012 and 2013 the yields were significantly lower. Differences between treatments were not significant in the first two vegetative periods and significant at  $P > 0.05$  during the 3<sup>rd</sup> and 4<sup>th</sup> vegetative periods.

On Technosol in Kolubara, the yields were less than 1.0 t/ha in the beginning of plantations development, but in 2014 and 2015 they reached 4.7 DM t/ha in treatment A with a significant difference to treatment B (3.74 DM t/ha and 3.86 DM t/ha). Differences between years are not significant (Figure 1b).

On Planosol (location Kozjak) the yield increased during canopy development except in 2012 (Figure 1c) with no significant influence of fertilization. In 2014 and 2015, the yield reached more than 10 DM t/ha, with no significant differences between treatments and years.

In all locations, yield was significantly increased after the 2<sup>nd</sup> and 3<sup>rd</sup> vegetative period. The high-

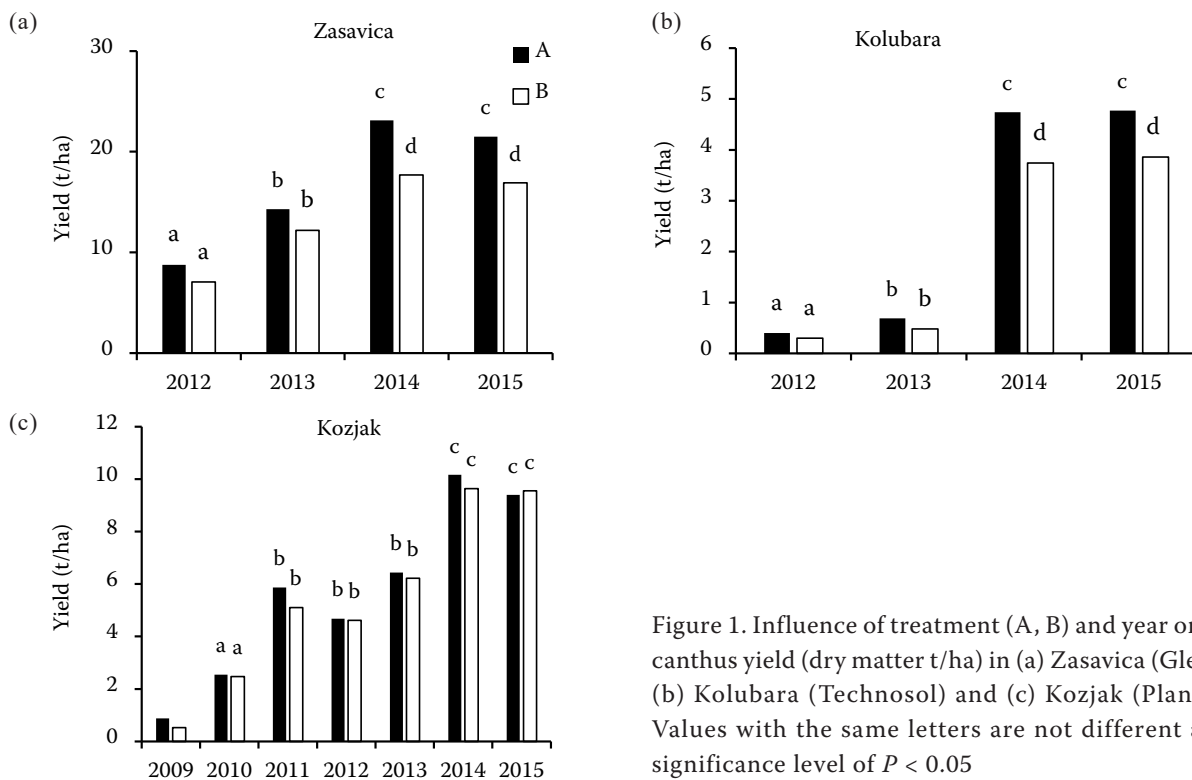


Figure 1. Influence of treatment (A, B) and year on miscanthus yield (dry matter t/ha) in (a) Zasavica (Gleysol); (b) Kolubara (Technosol) and (c) Kozjak (Planosol). Values with the same letters are not different at the significance level of  $P < 0.05$

est yield in all locations after 2014 is probably the result of weather conditions. According to reports of the Republic Hydrometeorological Service of Serbia from the nearest meteorological stations in 2014, the total annual amount of precipitation significantly exceeded the multiannual average and average annual air temperature with no difference to multiannual average was registered. During 2012 and 2013 precipitation was significantly lower than multiannual average and annual temperature was higher compared to the multiannual average. The largest part of production in 2015 was marked by favourable weather conditions, which resulted in very high yields and quality of agricultural crops.

The results are in accordance with literature data (Zapater et al. 2016), and highlighted that the yields of *Miscanthus × giganteus* show a great variability between sites and between years. The crop age has a strong effect on lower yields during the establishment phase compared to the mature crop and biomass production is sensitive to heavy late spring frosts and a lack of water during mid-growing season. In our case, the late frosts were not recorded near the experimental fields but dry summer 2012 and 2013 caused slow biomass development.

The field experiments were designed to compare the effects of application of basic agricultural

measures and additional fertilization (mineral, 10.28 kg N/ha, 2.42 kg P/ha and 12.45 kg K/ha), but weeds significantly affected the initial growth and development of miscanthus plants, especially during the first year. At two locations in Serbia, influence of weeds on miscanthus yield has been investigated recently (Maksimović et al. 2016). Weed infestation causes a strong decrease of miscanthus yield (i.e. 18.60 t/ha to 0.37 t/ha on Luvic Chernozem; 10.33 t/ha to 0.78 t/ha Calcic Gleysol). As opposed to this experiment, weeds were removed from all plots during the 1<sup>st</sup> and 2<sup>nd</sup> growing season, and the differences between treatments were not so pronounced. At location Kolubara (Technosol), weeds in summer 2015 were represented by 43 plant species belonging to families Asteraceae, Fabaceae, and Poaceae, with low cover and no difference between treatments (Stefanović et al. 2016).

Energetic values of pellets formed from miscanthus biomass were 18.88 MJ/kg as upper heating value and 17.6 MJ/kg heating value with no difference between treatments and soil type.

Attributes of the aboveground biomass development were determined at the time of maximum growth. High differences were determined between treatments, soil type and years for stem height

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Table 1. Influence of soil type, year and treatment on miscanthus biomass attributes

Soil type	Year	Stem height (cm)		Stem No.		Green leaves No.		Dry leaves No.		Max. leave length (cm)		Max. leave width (cm)	
		A	B	A	B	A	B	A	B	A	B	A	B
Gleysol	2012	266 <sup>a</sup>	248 <sup>a</sup>	64.0	51.6 <sup>A</sup>	10.1 <sup>aA*</sup>	11.3 <sup>aA*</sup>	4.2	2.9	82.4 <sup>aA*</sup>	82.2 <sup>aA</sup>	2.14 <sup>aA*</sup>	2.11 <sup>aA</sup>
	2013	284	300	70.1	54.4 <sup>A</sup>	11.0 <sup>aA</sup>	11.7 <sup>aA</sup>	3.9	2.2 <sup>A</sup>	81.5 <sup>aA</sup>	81.8 <sup>aA*</sup>	2.10 <sup>aA*</sup>	2.10 <sup>aA*</sup>
	2014	320	362	94.6	84.0 <sup>B</sup>	13.8 <sup>bbB</sup>	14.5 <sup>bbB</sup>	2.4 <sup>A*</sup>	2.0 <sup>A*</sup>	82.8 <sup>aA</sup>	83.0 <sup>aA*</sup>	2.18 <sup>aA*</sup>	2.16 <sup>aB*</sup>
	2015	386	412	92.4	82.2 <sup>B</sup>	14.0 <sup>bbB</sup>	14.2 <sup>bbB</sup>	2.2 <sup>aA</sup>	2.1 <sup>aA*</sup>	82.7 <sup>aA*</sup>	82.8 <sup>aA*</sup>	2.16 <sup>aA*</sup>	2.14 <sup>aB*</sup>
Technosol	2012	173 <sup>A</sup>	189 <sup>A</sup>	6.4 <sup>*</sup>	9.3	9.6 <sup>A*</sup>	11.2 <sup>*</sup>	4.8	3.4	70.2 <sup>bbB</sup>	74.3 <sup>bbB</sup>	1.81 <sup>B</sup>	1.97 <sup>C</sup>
	2013	178 <sup>bA</sup>	182 <sup>ba</sup>	10.8	15.6	9.6 <sup>aA</sup>	10.0 <sup>a</sup>	5.4 <sup>b</sup>	4.8 <sup>b</sup>	70.8 <sup>bbB</sup>	70.3 <sup>bbB</sup>	1.83 <sup>bbB</sup>	1.76 <sup>b</sup>
	2014	174 <sup>ba</sup>	187 <sup>ba</sup>	32.5	38.6 <sup>*</sup>	12.6 <sup>bc</sup>	13.6 <sup>bc</sup>	2.8 <sup>*</sup>	1.2	71.6 <sup>bbB</sup>	72.8 <sup>bbB</sup>	2.08 <sup>aA</sup>	2.11 <sup>aA*</sup>
	2015	188 <sup>b</sup>	192 <sup>ba</sup>	36.2	48.2	12.8 <sup>bc</sup>	13.2 <sup>bc</sup>	2.2 <sup>a</sup>	2.0 <sup>a*</sup>	72.0 <sup>bbB</sup>	71.6 <sup>bbB</sup>	2.21	2.00 <sup>C</sup>
Planosol	2009	242 <sup>dB</sup>	239 <sup>d</sup>	6.2 <sup>*</sup>	7.8	9.6 <sup>aD*</sup>	10.7 <sup>aE*</sup>	2.8 <sup>B</sup>	2.2 <sup>A*</sup>	81.0 <sup>a*</sup>	83.9 <sup>C</sup>	2.15 <sup>A*</sup>	2.45 <sup>D</sup>
	2010	273	315	8.8	14.4	15.4	11.8 <sup>D*</sup>	4.5	2.7 <sup>B</sup>	78.9 <sup>A</sup>	83.3 <sup>C</sup>	2.62	2.41 <sup>D</sup>
	2011	233 <sup>B</sup>	241	13.5	26.9	5.8 <sup>c</sup>	5.10 <sup>c</sup>	6.5	8.5	79.2 <sup>A</sup>	81.2 <sup>A</sup>	2.10 <sup>A</sup>	2.31 <sup>D</sup>
	2012	286 <sup>c</sup>	278 <sup>c</sup>	22.3	38.6 <sup>A*</sup>	10.2 <sup>D</sup>	11.8 <sup>D*</sup>	1.9 <sup>a</sup>	2.2 <sup>aA*</sup>	76.8 <sup>A</sup>	84.2	2.11 <sup>A*</sup>	2.21
	2013	308 <sup>C</sup>	320 <sup>B</sup>	38.9 <sup>C*</sup>	40.1 <sup>A</sup>	9.3	10.4 <sup>E</sup>	3.7	3.1 <sup>B</sup>	78.2 <sup>A</sup>	81.6 <sup>aA*</sup>	2.02 <sup>c*</sup>	2.06 <sup>cC*</sup>
	2014	298 <sup>C</sup>	328 <sup>B</sup>	44.8 <sup>C</sup>	46.4	10.8 <sup>aD</sup>	10.6 <sup>aE</sup>	2.4 <sup>B*</sup>	1.8 <sup>B*</sup>	82.1 <sup>aA</sup>	83.5 <sup>aC*</sup>	2.12 <sup>aA*</sup>	2.13 <sup>aA*</sup>
2015	324	300	42.1 <sup>C</sup>	41.8 <sup>A</sup>	11.8 <sup>b</sup>	12.2 <sup>bd</sup>	2.5 <sup>aB</sup>	2.0 <sup>aA*</sup>	81.9 <sup>aA*</sup>	82.5 <sup>aC*</sup>	2.11 <sup>aA*</sup>	2.15 <sup>aA</sup>	

Lowercase: difference between A and B treatment; capital letter: difference between years in the same treatment; \* difference between soil type in the same treatment and year. Values with the same letters are not different at the significance level of  $P < 0.05$

and the number of stems per rhizome and low for characteristics of leaves (number of green leaves, number of dry leaves, maximal leaf length and width (Table 1).

The highest stems were in Zasavica with a significant fertilization influence, except in 2012, a hot and dry year in the beginning of canopy development when the weeds are still strong and plant roots do not reach underground water table. In Kozjak, stem height was lower and fertilization increased it depending on the year. The lowest stem height was in Kolubara, with no differences between treatments, except in 2012.

Number of stems per rhizome highly depends on soil type: at Gleysol reached 94 in 2014, at Planosol and Technosol it was only 48 and 46, respectively. The differences between treatments were significant in all cases.

Among the investigated biomass attributes, only the number of stems per rhizome is in strong positive correlation with yield in all years and at all locations.

Studies that compared the response of miscanthus to increasing N fertilizer rates were analysed: six

of them concluded a positive response, five of them an absence of response (Cadoux et al. 2012). No nitrogen response during the first two years after planting and then a small positive response at a rate of 100 kg/ha of fertilizer was reported (Maughan et al. 2012). A moderate effect of fertilization was recorded at Gleysol in Zasavica and Technosol in Kolubara after 2014 (wet year). Thus it can be assumed that during hot and dry years (2012 and 2013) the main impact on miscanthus yield had a limited amount of water. Miscanthus yield reached 23.12 DM t/ha in the 4<sup>th</sup> vegetative period on Gleysol with the total application of 15.42 kg N/ha, 7.25 kg P/ha and 18.68 kg K/ha during the year of establishment, which is high in comparisons to other investigated locations and literature data (Lewandowski et al. 2000, Heaton et al. 2004, Jeżowski 2008, Cadoux et al. 2012, Lesur et al. 2013, Haines et al. 2015). High yield can be explained by the high level of underground water and wet conditions in the year of establishment as there was a flood in 2011, which allowed dense and high canopy (Table 1). Increase in biomass yield was related to positive effects of N fertilization

on the number of shoots per plant and on plant height (Finan and Burke 2016). Minimizing N fertilizer input, while still accelerating plant establishment, remains an important agronomic goal because this strategy enhances N use efficiency and improves system net energy balance because synthesis/application of N fertilizer is energy-intensive (Dierking et al. 2016), particularly at the location near protected area as sensitive zone (Cadoux et al. 2012).

At Technosol, the yield was the lowest in the 2<sup>nd</sup> and 3<sup>rd</sup> vegetative period, and after that it reached acc. 5 DM t/ha where fertilization increased yield up to 20%. The results may be explained by the fact that soil has been formed just before the experiment so there was not enough time for the formation of microbial communities (Dierking et al. 2016). For such unfavourable conditions, production of any biomass is a success even with no commercially significant yield. Recommendation for such location is irrigation and fertilization each year during the canopy development.

The main conclusion of the research is that miscanthus cultivation is possible on marginal lands such as Gleysol, Planosol and Technosol with minimum application of agricultural measures only during the year of establishment. Due to favourable water conditions, Gleysol may be recommended for miscanthus production. Additionally, fertilizing with 10.28 kg N/ha, 4.83 kg P/ha and 12.45 kg K/ha yield at Gleysol and Technosol (but not at Planosol) is recommended to increase stand density and height.

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