EFFECT OF NITROGEN ON THE DISTRIBUTION OF BIOMASS AND ELEMENT COMPOSITION OF THE ROOT SYSTEM OF *MISCANTHUS* × *GIGANTEUS*

Željko S. Dželetović[1,*] and Djordje N. Glamočlija[2]

[1]*University of Belgrade, INEP – Institute for the Application of Nuclear Energy*, Banatska 31-b, p.box 46, 11080 Zemun, Serbia

[2]*University of Belgrade, Faculty of Agriculture*, Nemanjina 6, 11080 Zemun, Serbia

*Corresponding author: zdzeletovic@inep.ac.rs

Abstract: Perennial bioenergy grass crops, despite a relatively similar production of aboveground biomass, show significant differences in the overall root biomass. Rhizomes play a key role in economizing nutrients in miscanthus. The aim of this research was to establish the effect of N (nitrogen) on the distribution of biomass and concentration of major macro- and micronutrients in the miscanthus root system, using simple experiment in pots. After two years of growth, the rhizomes and roots were taken out of the pots, cleaned of earth and analyzed. About 2/3 of the mass of the miscanthus root system consist of rhizome mass. The overall dry biomass of newly formed rhizomes and roots is decreased with the increase in the amount of applied N fertilization. Thereby, the N concentration in the entire root system, as well as in some of its parts, increased with the rise in applied amount of N. Our results show that increasing amounts of applied N consistently negatively correlate with P concentrations in the miscanthus root system, in contrast to Mn concentrations, with which they correlate positively.

Key words: miscanthus; roots; rhizomes; macro- and micronutrients; plant nutrition

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INTRODUCTION

Among the environmental factors that can be modified by farmers, water and N are essential for controlling plant growth (Gonzalez-Dugo et al., 2010) by judicious irrigation and fertilizer application. Sustainable agriculture requires a proper balance between agronomic, economic and ecological aspects of nutrient management. Perennial bioenergetic grass crops, despite a relatively similar production of aboveground biomass, show significant differences in the overall root biomass, root shape and water absorption (Monti and Zatta, 2009). Miscanthus (*Miscanthus × giganteus* Greef et Deu.) is a non-invasive perennial crop originating from eastern Asia. It can be cultivated for 15-20 years in continuity for biomass production. It is a C_4 grass with high efficiency of utilization of radiation and water. The primary economic importance of miscanthus is for energy production. Namely, this species is a potential "energy" crop that can serve as a biofuel

(Dohleman et., 2012; Mishra et al., 2013). Due to efficient biomass production and very good combustion, miscanthus should make a significant contribution to sustainable agricultural production of combustible biomass in the near future (Mishra et al., 2013).

Rhizomes play a key role in economizing with nutrients in miscanthus. Contrary to the majority of agricultural crops, miscanthus is a plant with an extensive root system, which remains dormant during winter, but can respond rapidly to the increased needs of the plant in assimilates, at the beginning of growth in spring (Himken et al., 1997). Miscanthus has a very shallow (surface) root shape, with almost 90% of the total root biomass concentrated in 0-35 cm of surface soils (Monti and Zatta, 2009). However, its roots extend to 2 m depth, so that it can take up soil moisture from considerably depth during drier summer months (Neukirchen et al., 1999). This is more than the rooting depth of most annual crops. Crops that are rooted deeper, such as miscanthus, are more tolerant during periods of drought, due to access to soil layers with higher moisture contents (Chaves et al. 2002). Thus, miscanthus is very productive in a wet environment, but it is very sensitive water deprivation (Monti and Zatta, 2009).

The phase of establishing the crop is 2-5 years to achieve the maximum yield of aboveground biomass. In the first season, miscanthus creates a loose root system, in the middle part of which there is an original rhizome. In the second season, a dense structure of small lateral roots is formed, as well as new rhizome "branches" from the original rhizome. In the third year of cultivation, under agro-ecological conditions of the wider area of Belgrade (Serbia, South-East Europe), Miscanthus reaches a maximum yield (Dželetović et al., 2014, Stričević et al., 2014). As plants age, greater rhizome masses are developed, so it is expected that they increase the amount of assimilates and nutrients translocated from the rhizome to the aboveground parts in spring and from the aboveground parts toward rhizomes in the autumn (Clifton-Brown and Lewandowski, 2002). Analysis of belowground biomass dynamics suggests that while shoot production is at the expense of rhizome mass in the spring, this is recovered by late summer (Dohleman et., 2012).

Both the roots and rhizomes of miscanthus are characterized by high lignin content (Amougou et al., 2011). According to Neukirchen et al. (1999), the concentrations of nutrients in the roots decrease with increased depth. In addition, a greater depth of rooting and higher density of miscanthus roots in the subsurface soil layer enable a potentially higher absorbtion of nutrients from that layer (Neukirchen et al., 1999). This enables the miscanthus crop to overcome periods of low accessibility to nutrients and water, especially during the period of fast growth of the

Table 1. Average dry mass of the miscanthus root system (grams per pot) after the second season of growth (±SD)

Part of root	N_1PK		N_2PK		N_3PK	
	Weight	%	Weight	%	Weight	%
Old original rhizome	10.50 ± 2.03	7.5	6.08 ± 1.37	6.0	8.84 ± 0.90	10.3
Newly-formed rhizomes	84.99 ± 7.65	60.9	62.39 ± 6.18	61.3	48.38 ± 2.41	56.2
Roots and small roots	44.01 ± 3.96	31.6	33.33 ± 3.67	32.7	28.84 ± 1.44	33.5
Σ	139.50	100.0	101.80	100.0	83.06	100.0

aboveground biomass. The density of miscanthus roots in the surface soil layer is lower than the density of roots of annual cultivated crops and it decreases with the increase of distance from the center of the plants (Neukirchen et al., 1999).

Although roots usually contribute with only 10-20% to the overall plant mass, a well-developed root system is necessary for healthy plant growth and development. The plant root growth is genetically controlled, but it is also influenced by environmental factors, and mineral nutrients represent a significant factor influencing the growth of plant roots (Fageria and Moreira, 2011). According to Godin et al. (2013), the biomass compositions are essential to investigate their suitability for bioenergy conversion. The sustainability of the system for the production of energy from the biomass also depends on how the fertilization rates affect the concentration and amount of nutrients in the harvested biomass (Propheter and Staggenborg, 2010). Selection of the N fertilizer rates is one of the most important features in managing N fertilizer.

A well-developed root system is needed to absorb adequate amounts of water and nutrients, especially when plants are under abiotic or biotic stress (Fageria and Moreira, 2011). Because roots are semi-hidden organs, which are hardly separated from the soil, it is difficult to evaluate the effect of certain biotic and abiotic factors on root growth in field conditions (Sainju et al., 2005). The aim of this research was to establish the effect of N on the distribution of biomass and concentration of major macro- and micronutrients in the miscanthus root system by a simple experiment in pots.

MATERIALS AND METHODS

Soil on which the experiment was performed and experimental workflow

For the experiment in pots, the surface layer (0-20 cm deep) was utilized, namely, on soil of carbonate chernozem type (FAO soil classification), at the location INEP-Zemun (44°51´N, 20°22´E).

Table 2. Concentration of macroelements (mg g^{-1}) in miscanthus root at the time of harvest in the second season of growth (±SD)

Treatment	Part of root system	N	P	K	Ca	Mg	Ratio P/K	Ratio Ca/Mg
N_1PK	original rhizome	3.636 ± 0.615	0.515 ± 0.107	1.295 ± 0.187	1.432 ± 0.106	0.659 ± 0.098	0.398	2.17
	newly formed rhizomes	4.088 ± 0.856	0.355 ± 0.059	1.344 ± 0.243	1.355 ± 0.252	0.685 ± 0.076	0.264	1.98
	roots and small roots	6.336 ± 1.368	0.348 ± 0.130	1.403 ± 0.083	2.529 ± 0.042	1.370 ± 0.041	0.248	1.84
	in the entire root system	4.764 ± 1.000	0.365 ± 0.085	1.359 ± 0.188	1.732 ± 0.175	0.900 ± 0.067	0.269	1.92
N_2PK	original rhizome	4.295 ± 0.131	0.348 ± 0.060	0.387 ±0.082	1.597 ± 0.228	0.464 ± 0.046	0.899	3.44
	newly formed rhizomes	6.131 ± 0.812	0.347 ± 0.152	1.441 ± 0.112	1.949 ± 0.137	0.924 ± 0.062	0.241	2.11
	roots and small roots	7.983 ± 1.041	0.353 ± 0.004	1.537 ± 0.018	2.778 ± 0.177	1.352 ± 0.013	0.230	2.05
	in the entire root system	6.626 ± 0.846	0.349 ± 0.098	1.409 ± 0.079	2.199 ± 0.156	1.036 ± 0.045	0.247	2.12
N_3PK	original rhizome	6.602 ± 0.766	0.631 ± 0.102	0.830 ± 0.184	2.027 ± 0.106	0.622 ± 0.068	0.760	3.26
	newly formed rhizomes	4.868 ± 1.830	0.233 ± 0.010	1.365 ±0.017	2.032 ± 0.136	0.837 ± 0.038	0.171	2.43
	roots and small roots	8.357 ± 0.389	0.101 ± 0.016	1.375 ± 0.011	2.378 ± 0.112	1.301 ± 0.018	0.073	1.83
	in the entire root system	6.215 ± 1.238	0.230 ± 0.021	1.313 ± 0.032	2.147 ± 0.125	0.970 ± 0.034	0.175	2.21
Original rhizomes (before planting)		15.211 ± 1.620	0.804 ± 0.374	7.414 ± 0.202	0.432 ± 0.011	0.913 ± 0.218	0.108	0.47

The soil is light clay (39.1% sand, 28.5% silt, 32.4% clay), and regarding chemical properties, it has neutral reaction (pH_{H2O} 7.3, pH_{KCl} 6.5), with average humus percentage (total organic C is 1.71%), and well supplied with N content (total N is 0.141%). It is characterized by a low content of readily available P and average content of available K. The content of heavy metals is in the range of regular values for uncontaminated soils.

Vital miscanthus rhizomes were utilized for the experiment (imported from Austria). The rhizome length was 7.5-15.0 cm, with 2-5 nodes. The experiment in pots was set in three repetitions, on an open and sunny area, at the INEP-Zemun location. Nine plastic pots (three pots at a time for three experimental treatments) were used for the experiment. Each pot had a volume of 12 L. For the vegetative experiment, naturally wet earth was manually fragmented, and then 10 kg of earth was placed in each pot. In the middle part of each pot, at 7-10 cm depth, one rhizome was planted.

For the experiment, laboratory chemicals were used (p.a). These include urea $(NH_2)_2CO$, KH_2PO_4 and K_2SO_4. The experimental treatments were performed by introducing increasing amounts of N component in mineral nutrients in pots: N_1PK, N_2PK and N_3PK. In the N_1PK treatment, the following amounts of nutrients were introduced: 0.325 g N + 0.325 g P_2O_5 + 0.325 g K_2O; so that the ratio between the nutrients was $N:P_2O_5:K_2O$=1:1:1. In the N_2PK, treatment a double amount of N nutrients (0.650 g N) was introduced into the pots, and in the N_3PK treatment a triple amount of N nutrients (0.975 g N) was introduced, while the quantities of P_2O_5 and K_2O remained the same (0.325 g P_2O_5 + 0.325 g K_2O); the ratios between nutrients were 2:1:1 (N_2PK) and 3:1:1 (N_3PK). Nutrients were introduced in the form of solutions. In the second season of growth, nutrients were not introduced, so that the plants utilized nutrients from the previous season for their development.

The moisture of earth in the experimental pots was maintained at an approximate value of 0.6-0.8 of the maximum water capacity. In addition to rainfall, the required level of moisture in the pots was provided by watering from the urban water supply system, according to need, mainly during the summer months. During the winter months, the excess water from pots was not removed, i.e., plants were exposed to excessive wetting and freezing, approximately identical to conditions existing in the field. The experiment in pots ended after almost 2 years.

Analytical methods

The rhizome mass was determined from samples collected at the end of the experiment. Rhizomes and roots were taken from the pots after careful rinsing with water for several hours to obtain a relatively clean sample, without earth granules. The rhizomes and roots were then separated and dried for 7 days in a drying chamber at 60°C. The mass of dried samples of plant material (rhizomes and small roots) was weighed, then ground and kept in small separate paper bags until the time of analyzing.

The concentration of N in the plant material was determined by Kjeldahl's method (Bremner, 1996). The sample of plant material was decomposed by heating with concentrated sulfuric acid in the presence of catalyst (30% H_2O_2). The obtained solution was then quantitatively transferred to distillation flasks, treated with 40% NaOH solution and subjected to vapor distillation. N concentration was calculated after distillate titration.

The concentrations of macro- (P, K, Ca and Mg) and micronutrients (Cu, Fe, Mn and Zn) in the samples of plant material was determined according to procedure described by Jones and Case (1990), by decomposition with HNO_3 at 125°C, with the addition of 30% H_2O_2, for decoloring the solution. P was analyzed spectrophotometrically (Ultro Spec 2000; Pharmacia Biotech, UK). The concentrations of K, Ca, Mg and microelements were determined by atomic absorption spectrophotometry (SP-192 Pye Unicam).

Statistical methods

The data shown in the tables are the arithmetical means of three repetitions of each treatment, with the standard deviation (SD). Dependent variables were analyzed for a significant response to fertilizer treatment using correlation analysis.

RESULTS

The largest part of miscanthus root system biomass, after the second season of growing, consisted of rhizome mass, 66.5-68.4% (Table 1). The old original rhizome was partly dried and a part of the original rhizome was therefore transformed and included in the newly formed rhizomes. The original rhizome differed from the newly formed rhizomes by its darker color and visual appearance, which was light brown. According to the data shown in Table 1, the overall biomass of the miscanthus root system decreased with the increase in the quantity of applied N fertilization, from an average of 139.50 g to 83.06 g. This decrease in the overall biomass was reflected unevenly on the decrease of formed biomass of rhizomes and roots. A decrease in the overall biomass of the root system is intended primarily for reduction of the biomass of newly formed rhizomes, which was, on average, almost cut by half, i.e. from 85 g to 48 g. Thus, by applying increasing amounts of N component, the mass of roots and small roots decreased more slowly, so that their average percentage after the harvest, in the second season, was relatively increased from 31.6% to 33.5% (Table 1).

The obtained concentrations of more important nutrients in the analyzed miscanthus root tissues are given in Tables 2 and 3. N concentrations

Table 3. Concentration of microelements (mg kg^{-1}) in miscanthus root at the time of harvest in the second season of growth (±SD)

Treatment	Part of root system	Cu	Fe	Mn	Zn
N_1PK	original rhizome	12.87 ± 2.81	1097 ± 389	91.0 ± 10.3	15.91 ± 2.40
	newly formed rhizomes	10.92 ± 3.84	515 ± 72	86.2 ± 1.9	15.23 ± 4.11
	roots and small roots	20.54 ± 2.95	3975 ± 241	262.4 ± 19.9	25.36 ± 0.79
	in the entire root system	14.11 ± 3.48	1652 ± 149	142.2 ± 8.2	18.48 ± 2.93
N_2PK	original rhizome	29.53 ± 8.03	839 ± 35	73.0 ± 2.5	36.26 ± 12.33
	newly formed rhizomes	21.87 ± 4.54	1550 ± 397	106.8 ± 20.6	56.82 ± 13.98
	roots and small roots	31.92 ± 4.05	4664 ± 420	348.5 ± 16.6	77.64 ± 4.56
	in the entire root system	25.62 ± 4.59	2526 ± 383	183.8 ± 18.2	62.39 ± 10.80
N_3PK	original rhizome	18.80 ± 6.47	1459 ± 266	105.3 ± 19.4	31.06 ± 1.32
	newly formed rhizomes	22.37 ± 3.98	1093 ± 106	153.4 ± 1.0	35.17 ± 3.39
	roots and small roots	24.46 ± 2.83	4138 ± 75	352.3 ± 9.5	25.63 ± 2.97
	in the entire root system	22.70 ± 3.85	2151 ± 112	215.1 ± 5.7	31.55 ± 3.04
Original rhizomes immediately before planting		66.03 ± 2.43	538 ± 184	25.1 ± 0.9	129.80 ± 52.45

in the roots and small roots after two years of growth were always higher than N concentrations in the rhizomes. The increasing amounts of applied N fertilization after two years of growth produced increasing N concentrations in the rhizomes that were planted (original rhizomes) and in the roots and small roots (Table 4: very strong positive correlation $r_2 = 0.940$), but not in the newly formed rhizomes ($r_1 = 0.378$). Due to this, a lower N concentration was obtained in the entire root system in the N_3PK treatment compared to the N_2PK treatment in the final test result. Namely, the highest N concentrations (Table 2) in the whole root system were obtained in the N_2PK treatment (6.626 mg g^{-1} d.m.), somewhat lower in the N_3PK treatment (6.215 mg g^{-1} d.m.), and lowest in the N_1PK treatment (4.764 mg g^{-1} d.m.).

Increasing treatments with N also demonstrated an uneven effect on the concentrations of P, K, Ca and Mg in the miscanthus root system (Table 2). The concentrations of P in the root system, newly formed rhizomes and roots of miscanthus decreased only in the treatment with the largest quantity of N (N_3PK). Among the applied quantities of N fertilization and P concentrations in the whole miscanthus root system, as well as in some of its parts, we established the existence of a strong negative correlation (Table 4; $r_1 = -0.894$; $r_2 = -0.857$; $r_3 = 0.915$).

On the concentration of K in the miscanthus root system the increasing amounts of N fertilization did not show a clear effect. Namely, in the roots and newly formed rhizomes the K concentration ranged from 1.344 to 1.537 mg g^{-1} d.m. In the roots, the K concentrations were slightly higher than in the newly formed rhizomes, while they were lowest in the original rhizomes. The P/K ratio was reduced in the newly formed rhizomes (Table 2). K concentrations and N concentrations in the newly formed rhizomes showed a very strong positive correlation ($r_1 = 0.984$), which, nevertheless, we did not establish even in the small roots ($r_2 = 0.185$).

The Ca concentration in the rhizomes increases with the increase in the amounts of applied N (Table 2): from 1.432 mg g^{-1} d.m. in the original rhizome and 1.355 mg g^{-1} d.m. in the newly-formed rhizome in the treatment N_1PK, up to 2.027 mg g^{-1} d.m. in the original rhizome and 2.032 mg g^{-1} d.m. in the newly-formed rhizome in the treatment N_3PK. The Ca concentration in the roots and small roots was higher than in the rhizomes, but the increasing amounts of N did not show a clear effect on it. Among the applied amounts of N fertilization and Ca concentrations in the newly formed rhizomes we established the existence of a very strong correlation ($r_1 = 0.917$), which was also strong on the level of the whole root system ($r_3 = 0.811$). Thereby, the correlation between Ca concentration and N concentrations ($r_3 = 0.994$) was also very strong on the level of the entire root system.

The Mg concentrations in the roots and small roots were, on average, up to 2 times higher than those in the rhizomes (Table 2), but the increasing amounts of applied N did not show a clear effect on them. However, the ratio of Ca/Mg in the newly formed rhizomes, with the increase of applied amounts of N, expanded from 1.98 for the N_1PK treatment to 2.11 for the N_2PK treatment and to 2.43 for the N_3PK treatment (Table 2). In the miscanthus roots we established the existence of a very strong negative correlation between the Mg concentrations with applied amounts of N fertilization ($r_2 = -0.964$) and a very strong positive correlation with P concentrations ($r_2 = 0.963$). We also established the existence of a very strong correlation between the Mg and N concentrations in the entire miscanthus root system ($r_3 = 0.957$) and in the newly formed rhizomes

($r_1 = 0.958$), while in the roots and small roots, on the contrary, a strong negative correlation ($r_3 = -0.815$) was established. The Mg and Ca concentrations correlate in the newly formed rhizomes ($r_1 = 0.887$) and entire root system ($r_3 = 0.919$).

Higher Ca and Mg concentrations in the miscanthus root system, compared to the original planted rhizome, are evidently absorbed from the soil that is rich in these macronutrients (carbonate chernozem). In the entire root system, as well as in the newly formed rhizomes, the Ca concentrations are in correlation with the applied N (Table 4). The Mg concentrations in the roots correlate negatively with applied N and positively with P concentrations (Table 4). The assumption that roots and rhizomes of miscanthus react differently on the introduction of increasing amounts of N fertilization is based on the established correlations between the Mg and N concentrations, which is positive in the newly formed rhizomes ($r_1 = 0.958$), and negative in the roots ($r_3 = -0.815$). The Ca/Mg ratio in the newly formed rhizomes expanded with an increase in the applied N (Table 2), so that the Mg and Ca concentrations correlate both in the newly formed rhizomes and on the level of the entire root system.

Analysis of the average Mn concentrations in the newly formed rhizomes indicated that its concentration increases with the increase of applied N. Mn concentrations increased both in the rhizomes and roots with the increase in the amount of applied N from 86.2 mg kg^{-1} d.m. in the newly-formed rhizomes and 262 mg kg^{-1} in the roots and small roots in the N_1PK treatment, to 153.4 and 352.3 mg kg^{-1} in the N_3PK treatment (Table 3). A very strong positive correlation was established between the Mn concentrations and applied amounts of N fertilization in the newly formed rhizomes ($r_1 = 0.976$) and in the whole miscanthus root system ($r_3 = 0.997$), and in the roots and small roots Mn concentrations showed a strong correlation ($r_2 = 0.884$). In the newly formed rhizomes and in the entire miscanthus root system, Mn concentrations also showed a strong correlation with P concentrations ($r_1 = -0.970$; $r_3 = -0.879$), Ca ($r_1 = 0.808$; $r_3 = 0.856$) and copper ($r_1 = 0.762$; $r_3 = 0.772$).

The increasing amounts of applied N fertilization, after two years of cultivation, did not show clear effects on the concentrations of Cu, Fe and Zn in the miscanthus root system (Table 3). Generally, the concentrations of Cu and Fe in the small roots were higher than those in the newly formed rhizomes. In the newly formed rhizomes and in the whole miscanthus root system, the Cu, Fe and Zn concentrations showed a mutually strong correlation, as well as a strong correlation with the concentrations of N, Ca and Mg (Table 4). In addition, K concentrations in the newly formed rhizomes and small roots showed a very strong correlation with Fe concentrations ($r_1 = 0.927$; $r_2 = 0.925$) and Zn ($r_1 = 0.957$; $r_2 = 0.986$) in these organs.

In relation to original rhizome, the root system was characterized by an altered chemical composition (Table 2). The rhizomes, developed on chernozem, were characterized by considerably higher concentrations of Ca (Table 2), Fe and Mn (Table 3), while the concentrations of other analyzed elements (P, K, Cu and Zn) were lower.

The concentrations of micronutrients in the miscanthus root system have not been studied in detail to date. In relation to the original rhizome, after 2 years of cultivation on chernozem lower Cu and Zn concentrations were obtained, and higher Fe and Mn concentrations in the miscanthus root system (Table 3). The established positive correlations between the applied amounts of N and Mn concentrations in the entire miscanthus root system and in some of its parts (Table 4) clearly indicate the existence of influence. We did not establish the effect of the increasing amounts of applied N on Cu, Fe and Zn concentrations. However, the Cu, Fe and Zn concentrations in the miscanthus root system were in mutual correlation, and they also correlated with N, Ca and Mg concentrations (Table 4).

The total content of some elements in the whole root system (Table 5), calculated on the basis of the dry mass of some parts of the root system (Table 1) and the concentrations of analyzed elements in them (Tables 2 and 3), give completely different numerical and correlation indicators. Namely, with the increasing amounts of applied N fertilization the total content of P, K, Ca, Mg and Mn in the entire miscanthus root system decreased, while the total content of N, Cu, Fe and Zn did not show a clear effect. The applied quantity of N fertilization showed a linear negative correlation with total P content in the miscanthus root (r_4 = -1.000), while it had a very strong negative correlation with K contents (r_4 = -0.996), Ca (r_4 = -0.969), Mg (r_4 = -0.998) and Mn (r_4 = -0.996). A very strong positive correlation exists between the overall Ca contents in the miscanthus root with the total N, P, K, Mg and Mn contents. The total K content showed a linear positive correlation with the total Mn content in the miscanthus root and had a very strong correlation with the total P content (r_4 = 0.995) and Mg (r_4 = 0.989).

Due to a decrease in the overall biomass of the root system with increasing amounts of applied N fertilization (Table 1), the total content of P, K, Ca, Mg and Mn in the entire miscanthus root system decreased (Table 5), although, for example, the levels of Ca and Mn concentrations in some parts of the root system increased, the concentrations of P, K and Mg remained at the same level.

DISCUSSION

Distribution of the root system biomass

Root growth is under multi- or polygenic control and is influenced by environmental factors, including soil temperature, soil moisture content, solar radiation and the physical, chemical, and biological properties of soil (Fageria and Moreira, 2011). One of the most efficient ways of improving the efficiency of N utilization in agricultural crop production is the good provision of N. The response of root growth to chemical fertilization is similar to the response of growth of the aboveground part, although the magnitude of the response differs (Fageria and Moreira, 2011). However, according to Kering et al. (2012), in perennial grasses the yield of dry matter of the biomass does not show a strong intercorrelation with N fertilizer rates. It is known that perennial plant species, such as those identified as bioenergy raw materials, form a larger quantity of biomass under the soil (Neukirchen et al., 1999; Dohleman et al., 2012). Thereby, grass root mass is under little influence of N application compared with treatment without N application; application of other nutrients with N increases the dry matter of the root mass (Malhi et al., 2010). For this reason, the setting of our experiment was conceived in such a way to monitor the effect of increased application of N with the application of P and K (K).

In miscanthus, the growth rates depend on environmental conditions, such as (Miguez et al., 2008): type of soil, rainfall and temperatures, as well as on crop management practice, such as harvest time, planting density and the application of N fertilizers. The dry biomass yield per unit area is the main factor that controls the total amount of chemical components, digestible organic matter, bioethanol and thermal energy that can be expected to be harvested per unit area (Godin et al., 2013). There are conflicting opinions about the need to fertilize miscanthus. Numerous studies demonstrate that the *M. × giganteus* response to N fertilization is poor. High N utilization efficiency in miscanthus results in a biomass with low N concentration, which is very desirable for combustion with the aim of minimizing potential pollution (Miguez et al., 2008).

Table 4. Mutual correlations of element concentrations in the miscanthus root with applied amounts of N fertilization. Correlation intensity - r: (a) very strong (0.901 - 1.000); (b) strong (0.751 - 0.900); and (c) medium and weak (≤0.750)

	applied amounts of N fertilization	N	P	K	Ca	Mg	Cu	Fe	Mn
newly formed rhizomes (r_1)									
N	0.378 ^{c}								
P	-0.894 ^{b}	0.077 ^{c}							
K	0.206 ^{c}	0.984 ^{a}	0.255 ^{c}						
Ca	0.917 ^{a}	0.717 ^{c}	-0.640 ^{c}	0.580 ^{c}					
Mg	0.628 ^{c}	0.958 ^{a}	-0.213 ^{c}	0.891 ^{b}	0.887 ^{b}				
Cu	0.885 ^{b}	0.766 ^{b}	-0.582 ^{c}	0.638 ^{c}	0.997 ^{a}	0.919 ^{a}			
Fe	0.557 ^{c}	0.979 ^{a}	-0.126 ^{c}	0.927 ^{a}	0.843 ^{b}	0.996 ^{a}	0.880 ^{b}		
Mn	0.976 ^{a}	0.167 ^{c}	-0.970 ^{a}	-0.013 ^{c}	0.808 ^{b}	0.444 ^{c}	0.762 ^{b}	0.363 ^{c}	
Zn	0.479 ^{c}	0.994 ^{a}	-0.034 ^{c}	0.957 ^{a}	0.790 ^{b}	0.984 ^{a}	0.833 ^{b}	0.996 ^{a}	0.276 ^{c}
roots and small roots (r_2)									
N	0.940 ^{b}								
P	-0.857 ^{b}	-0.630 ^{c}							
K	-0.162 ^{c}	0.185 ^{c}	0.647 ^{c}						
Ca	-0.374 ^{c}	-0.034 ^{c}	0.798 ^{b}	0.976 ^{a}					
Mg	-0.964 ^{a}	-0.815 ^{b}	0.963 ^{a}	0.419 ^{c}	0.607 ^{c}				
Cu	0.339 ^{c}	0.640 ^{c}	0.194 ^{c}	0.874 ^{b}	0.746 ^{c}	-0.076 ^{c}			
Fe	0.226 ^{c}	0.546 ^{c}	0.308 ^{c}	0.925 ^{a}	0.819 ^{b}	0.041 ^{c}	0.993 ^{c}		
Mn	0.884 ^{b}	0.991 ^{a}	-0.517 ^{c}	0.318 ^{c}	0.103 ^{c}	-0.728 ^{c}	0.739 ^{c}	0.655 ^{c}	
Zn	0.004 ^{c}	0.346 ^{c}	0.511 ^{c}	0.986 ^{a}	0.926 ^{a}	0.262 ^{c}	0.942 ^{a}	0.975 ^{a}	0.471 ^{c}
in the entire root system (r_3)									
N	0.742 ^{c}								
P	-0.915 ^{a}	-0.408 ^{c}							
K	-0.479 ^{c}	0.234 ^{c}	0.792 ^{b}						
Ca	0.811 ^{b}	0.994 ^{a}	-0.505 ^{c}	0.125 ^{c}					
Mg	0.515 ^{c}	0.957 ^{a}	-0.125 ^{c}	0.506 ^{c}	0.919 ^{a}				
Cu	0.718 ^{c}	0.999 ^{a}	-0.376 ^{c}	0.267 ^{c}	0.990 ^{a}	0.966 ^{a}			
Fe	0.569 ^{c}	0.974 ^{a}	-0.189 ^{c}	0.449 ^{c}	0.943 ^{a}	0.998 ^{a}	0.981 ^{a}		
Mn	0.997 ^{a}	0.794 ^{b}	-0.879 ^{b}	-0.406 ^{c}	0.856 ^{b}	0.583 ^{c}	0.772 ^{b}	0.634 ^{c}	
Zn	0.290 ^{c}	0.857 ^{b}	0.121 ^{c}	0.701 ^{c}	0.795 ^{b}	0.970 ^{a}	0.874 ^{b}	0.952 ^{a}	0.367 ^{c}

The rhizomes of perennial plants, as storage organs, are responsible for the mobilization of nutrients toward other parts of the plant at the beginning of the growth period. It has been established in the literature that the highest content of nutrients in miscanthus emerges at the end of summer. After that, nutrients are efficiently translocated toward the rhizomes and then remobilized during the extension of shoots in the next season (Himken et al., 1997; Smith and Slater, 2010). A high efficiency of N utilization is mainly the result of the capability of miscanthus to recycle N (Christian et al., 2006), to translocate N to the aboveground part in spring, and the rhizomes at the end of the growth season when plant senescence begins, as well as of C_4 photosynthesis. At the end of the growth season, nutrients are translocated to the rhizomes and recycled toward the soil in fallen leaves. Due to this separation, the nutrient content in the aboveground part is low.

The effect of different rates of N fertilizer on the production of miscanthus dry matter depends on the quantity of available N in the soil, climatic conditions and miscanthus development in the year of study. In our research, after a two-year period, the largest part of the mass of miscanthus root system consisted of rhizome mass, i.e. 66.5-68.4%, of which only ≤10.3% consisted of the old original rhizome (Table 1). As a comparison, Hansen et al. (2004), in 9- and 16-year-old miscanthus stands on loam sand near Hornum in Denmark (56°50´N, 09°26´E), established that at the depth of 0-20 cm the rhizome mass was 10.9-12.6 t d.m. ha^{-1}, and big roots 3.2-3.7 t d.m. ha^{-1}. The percentage of rhizomes in the overall dry matter of the root system without small root mass, according to Hansen et al. (2004), was approximately 77%. They did not notice rhizomes and big roots in the deeper soil layers.

Overall biomass of the miscanthus root system decreased with the increase in the amount of applied N fertilization. This indicates that the applied amounts of N were in excess in the N_2PK and N_3PK treatments. Himken et al. (1997) suggested that N fertilization has no significant effect on dry rhizome matter. In our experiment in pots, we established that the increasing amounts of applied N had an effect on the distribution of formed biomass of the root system. The specific N-stimulating influence on root biomass production in grasslands (Tomaškin et al., 2013) is apparently not expressed in miscanthus. We have found, with the increase in the quantities of applied N, that the percentage of the mass of roots and small roots after harvest, in the second season of miscanthus growing, increases, to the detriment of the rhizome mass (Table 1). In addition, the results obtained by Behnke et al. (2012) show that N fertilization of *M.* × *giganteus* mainly leads to the release of N_2O, increases the fluxes of inorganic N (primarily NO_3^--N) through the soil profile and enhances the harvested N without significant increase in produced biomass. Unlike most cultivated grass, the response of *M.* × *giganteus* to N fertilization is unlikely to be economically profitable (Arundale et al., 2014). Our results confirm the modest demands of this plant for N and argue against increased application of N fertilization.

Concentrations of nutrients in the miscanthus root system

Nitrogen nutrition of the aboveground organs of *M.* × *giganteus* depends on N reserves in the underground organs, but precise quantities are unknown (Strullu et al., 2011). The exact needs of miscanthus in nutrients and mineral fertilizers are still under discussion (Cadoux et al., 2012). Although there is no significant response in the yield of formed aboveground biomass on N fertilization rates in the first two years of growing *M.* × *giganteus*, the amount of N in the harvested biomass is considerably higher in the treatment with applied N fertilization (Behnke et al., 2012). The data presented by Christian et al. (1997) indicate that 1-year-old miscanthus has low requirements for N fertilization. They established that only 38% N, applied in spring, was adopted by a 1-year-old crop, of which more than one half was established in the rhizomes. In a second study, Christian et al. (2006) established 55.4% of marked ^{15}N in 2-year-old plants. This research shows that *M.* × *giganteus* becomes more efficient with N fertilizer, when a crop is at least 2 or 3 years old, compared with 1-year-old crops. Smith and Slater (2010) showed that in a mature crop of miscanthus the date (time) of harvest has a substantial impact on N dynamics. In this way, postponed (delayed) harvest can reduce N concentrations in the harvested biomass, reducing the needs for N fertilizers (Heaton et al., 2009).

N fertilization has no effect on crop yield in late harvest treatments, but spurs yield in early harvest treatments due to the lower N content in the underground biomass (Strullu et al., 2011). N content in the aboveground part of the biomass is widely variable, mainly due to variable production levels and, to a lesser extent, variations in the concentrations (Cadoux et al., 2012). The accumulation of N in the aboveground biomass showed that it depends primarily on the initial reserves of N in the underground biomass, and secondarily, on N absorption by the whole crop (Strullu et al., 2011). This high effectiveness of N utilization results in aboveground biomass with low N concentration, which is preferable for direct combustion with the view of minimizing pollution (Miguez et al., 2008). It is estimated that from aboveground parts, 21-46% N, 36-50% P, 14-30% K and 27% magnesium are translocated to the rhizomes (Himken et al., 1997). In the spring, these reserves are also mobilized due to the return to new aboveground parts, making miscanthus partly independent of actual supply of N to the soil (Christian et al., 1997).

Table 5. Total content of macroelements and microelements in the entire miscanthus root system at the time of harvest, in the second season of growth (mg per pot), calculated on the basis of dry mass of some parts of the root system (Table 1) and the concentration of analyzed elements (Tables 2 and 3)

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
N_1PK	664.6	50.9	189.6	241.6	125.6	2.0	230.5	19.8	2.6
N_2PK	674.5	35.5	143.5	223.9	105.5	2.6	257.1	18.7	6.4
N_3PK	516.2	19.1	109.1	178.4	80.6	1.9	178.6	17.9	2.6
Mutual correlation of the total element contents with applied amounts of N fertilization (r_4):									
applied amounts of N fertilization	-0.837 [b*]	-1.000 [a]	-0.996 [a]	-0.969 [a]	-0.998 [a]	-0.132 [c]	-0.650 [c]	-0.996 [a]	0.000 [c]
N		0.847 [b]	0.788 [b]	0.946 [a]	0.869 [b]	0.653 [c]	0.960 [a]	0.784 [b]	0.548 [c]
P			0.995 [a]	0.974 [a]	0.999 [a]	0.150 [c]	0.664 [c]	0.994 [a]	0.018 [c]
K				0.945 [a]	0.989 [a]	0.048 [c]	0.584 [c]	1.000 [a]	-0.084 [c]
Ca					0.983 [a]	0.372 [c]	0.817 [b]	0.943 [a]	0.246 [c]
Mg						0.193 [c]	0.695 [c]	0.988 [a]	0.061 [c]
Cu							0.839 [b]	0.042 [c]	0.992 [a]
Fe								0.578 [c]	0.760 [b]
Mn									-0.091 [c]

*correlation intensity: (a) very strong (0.901 - 1.000); (b) strong (0.751 - 0.900); and (c) medium and weak (≤0.750)

The effectiveness of N utilization decreases with the increase of N fertilization rates (Lewandowski and Schmidt, 2006). The response of *M. × giganteus* to N fertilization is limited, although it varies between sites (Smith and Slater, 2010). Cosentino et al. (2007) did not establish more significant differences in N content in the miscanthus biomass between treatments that received nutrients through fertilization (60 and 120 kg N ha^{-1}). In providing 100 kg ha^{-1} per year, according to Lewandowski and Schmidt (2006), the effectiveness of N utilization for miscanthus is 0.35 Mg of dry biomass kg^{-1} N. Miscanthus can satisfy its needs in N through recycling, atmospheric precipitation and mineralization, without the need for constant introducing of additional N through fertilizers (Riche et al. 2010). From the total amount of N being lost from the aboveground part of crop until the final harvest at the end of winter, half is N translocated to the rhizomes, and half for N in fallen leaves.

Neukirchen et al. (1999) state that N content in the root ranges from 0.7-1.4%. According to Himken et al. (1997), N concentration in rhizomes remains rather stable during the year and it is not affected by N fertilization. By N application, according to Schwarz et al. (1994), its content in a crop increases almost linearly, though different quantities of N fertilizers do not show a clear effect on the yield. In our study, N concentration in the whole root system correlate with the increase in applied amount of N. Thereby, in the roots and small roots we have obtained considerably higher N concentrations than in the rhizomes. On the contrary, Christian et al. (2006) obtained during harvest a higher N content in the rhizomes (1.7%), and lower in the roots (1.3%). According to Himken et al. (1997), N application mainly tends to increase N concentration in the dry matter of rhizome. In the absence of N application in the second season of growing, we obtained considerably lower N concentrations in the miscanthus root relative to usual concentrations (Table 2).

In the research conducted by Neukirchen et al. (1999), in the miscanthus root system concentrations of N (0.7-1.4%) and K (0.6-1.2%) are considerably higher than concentrations of P (0.06-0.17%). Our results comply with this observation, although we have obtained, due to absence of nutrient introduction in the second season of growing, considerably lower values of N, P and K concentrations. In our study of P concentration in the entire root system, as well as in some of its parts they consistently negatively correlate with the increase in applied N. On the other hand, the increasing amounts of N do not show a clear effect on K concentrations. Only in the newly formed rhizomes do the K concentrations correlate with N concentrations. According to Himken et al. (1997), the concentrations of P, K and Mg in the rhizomes, like N concentrations, remain rather stable during the year and they are not affected by N fertilization. In our study, the rhizomes and roots reacted differently to absorption and accumulation of specific macro- and micronutrients with increasing amounts of applied N fertilization. The established correlations among the concentrations of some elements in miscanthus roots and rhizomes can mutually and considerably differ. We assume that this is the result of the different reactions of the roots and rhizomes to.

The applied amount of N shows a linear negative correlation with the total P content in the miscanthus root, while it has a very strong negative correlation with K, Ca, Mg and Mn contents. On the other hand, the overall K content shows a linear positive correlation with the total Mn content in the miscanthus root; therefore, the total content of N, Cu, Fe and Zn did not show a clear influence.

CONCLUSIONS

After two years of growth, the overall biomass of the miscanthus root system decreases with the increase in the amount of applied N fertilization. Most of the mass of the miscanthus root system consists of the rhizome mass (66-69%). With the increase in the amounts of applied N, the percentage of the mass of the roots and small roots of miscanthus increases linearly, to the detriment of the rhizome mass. The concentration of N in the root system increases with the increase in applied N, its concentration in the roots being higher than in the rhizomes. Our results show that increasing amounts of applied N negatively correlate with P concentrations in the miscanthus root system, contrary to the concentration of Mn with which they correlate positively.

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