Long-term mineral fertilization impact on chemical and microbiological properties of soil and *Miscanthus* × *giganteus* yield

W. Stępień[1], E.B. Górska[1], S. Pietkiewicz[2], M.H. Kalaji[2]

[1]*Department of Soil Environment Sciences, Warsaw University of Life Sciences-SGGW, Warsaw, Poland*
[2]*Department of Plant Physiology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland*

ABSTRACT

This experimental work was undertaken to assess the effect of various fertilization regimes (CaNPK, NPK, CaPK, CaPN, CaKN and Ca) and different soil properties on growth and yield of *Miscanthus* plants and to check the impact of this plant on soil microbial characteristics. Field experiment was set up in 2003 on a long-term fertilization experiment, which had been established since 1923. *Miscanthus giganteus* response to high soil acidity and deficiency of N, P and K was investigated. Some physico-chemical and microbiological properties of soil samples were estimated and microbial characteristics of soil were conducted to investigate the number of the following microorganisms: heterotrophic bacteria, microscopic fungi, and some diazotrophic bacteria. Obtained results showed that, the highest yield of *Miscanthus* was obtained from the field fertilized with the CaNPK; while the lowest one was found for plants grown without nitrogen (CaPK). The high acidity of soil and small amount of phosphorus did not affect the yields in the NPK and CaKN combinations as compared with CaNPK one. The experiments showed that *Miscanthus giganteus* responded positively to mineral fertilization, especially with nitrogen. The rhizosphere of *Miscanthus* plants provides a suitable environment for the growth and development of microorganisms, in contrast to the non-rhizosphere zone.

Keywords: energetic plant; microbial characteristics of soil; nutrients in plant and soil

An important issue in the development of many countries is the gradual replacement of fossil fuels with biomass from renewable sources (Pultowicz 2009). This is an extremely important strategy aimed at slowing down climate changes and strengthening energy security. The 'strategy for renewable energy development' adopted by Poland in 2004 implied that the share of renewable energy in the balance of primary energy at the national level should reach 7.5% in 2010 and 14% in 2020. Cultivation of energy crops, which include *Miscanthus giganteus*, is seen as one of the most important potential sources of biomass (Faber et al. 2007).

Miscanthus giganteus from the grass family, is closely related to species of sugar cane and can freely interbreed with it (Jeżowski 1999, Deuter and Jeżowski 2002). *M. giganteus* performs C4 photosynthesis and is characterized by high biomass production (Chou 2009). The biomass derived from *M. giganteus* plants is used in various industries e.g. in furniture making, construction, the petroleum and cellulose industries, as a fuel (briquettes, oil, gas), in the production of biodegradable plastics, remediation of soils contaminated with heavy metals, for strengthening land areas at risk of erosion, and also as animal food.

This work was undertaken to assess optimal fertilization regime for growth and high yield of *Miscanthus* cultivated in Poland for energy purposes. Moreover, the impact of this species on soil microbial composition on a plantation of *M. giganteus* energy crop under different mineral fertilization regimes was studied.

MATERIAL AND METHODS

The object of chemical and microbiological analyses was soil taken under *Miscanthus giganteus* plants grown in different fertilization combinations. The experiment was set up in 2003 in triplicate on the site of a long-term fertilization experiment in which a fixed fertilization scheme had been applied since 1923: CaNPK, NPK, CaPK, CaPN, CaKN, Ca. The experiment was carried out on a podzolic soil formed from heavy loamy sand. Chemical and microbiological tests were carried out over 2010 and 2011 at three different times of the year (spring, summer, and autumn).

Chemical tests were carried out on plants and non-rhizosphere soil, whereas microbiological tests included both rhizosphere and non-rhizosphere soils. Rhizosphere soil was taken to be the soil that remained within the root system of *Miscanthus* plants after shaking them.

Soil samples were analysed for pH in 1 mol/L KCl (PN-ISO 10390), total nitrogen (PN-ISO 11261), organic carbon (PN-ISO 10694), available P by Egner-Riehm method (Fotyma et al. 2005), and exchangeable forms of K, Mg and Ca (Ostrowska et al. 1991). Plant samples were analysed for total amounts of macroelements (N, P, K, Mg, Ca, S) (Ostrowska et al. 1991).

Microbial characteristics in the soil were assessed on the basis of population sizes of selected physiological groups of microorganisms: heterotrophic bacteria on the Bunt and Rovira medium (1955), and microscopic fungi on the Martin's medium (1950). The most probable number (MPN) of diazotrophic bacteria (*Azotobacter* sp. and *Clostridium pasteurianum*) was determined in the Winogradsky's nitrogen-free medium (Dőbereiner 1961). For this purpose, cultures of *Azotobacter* sp. were established in a short column (5 cm) of the nutrient medium, and those of *Clostridium* sp. in a tall column (15 cm) of the medium. The tests were conducted in triplicate. MPN values for the bacteria were read off using the McCready's tables for three replications (Girard and Rougieux 1967). Population sizes of the physiological groups of the microorganisms under study were expressed in colony forming units (CFU) or MPN (diazotrophs) per 1 kg of dry weight of soil (Filipek et al. 2000).

Measurements were done in three repetitions ($n = 3$). The results of the tests were verified statistically using the analysis of variance (Statgraphics Plus 4.1, Warrenton, USA), homogeneous groups were distinguished with the Tukey's test for $\alpha = 0.05$.

RESULTS AND DISCUSSION

The chemical properties of the tested soils were highly varied, as shown in Table 1. The soils were found to contain 4.5–4.6 g/kg of organic carbon, which resulted from the fact that no organic fertilization had been applied to the experimental field for 87 years. The lowest organic C content was obtained in the combinations without nitrogen fertilization. The amounts of available P and K were the highest in the combination without nitrogen fertilization (CaPK), and the lowest in the plots not fertilized with these nutrients for dozens of years. The higher amounts of P and K on the plots without nitrogen are due to the fact that crop yields in that combination were lower than in the CaNPK combination fertilized with nitrogen, and the uptake of phosphorus and po-

Table 1. Soil chemical properties depending on the applied mineral fertilization and liming

Fertilization combination	C_{org}	N	P	K	Mg	Ca	pH
	(g/kg)		(mg/kg)				
Ca	4.52^{a}	0.49^{a}	10.1^{a}	35.6^{a}	63.1^{b}	881.3^{b}	6.3
CaNPK	4.64^{a}	0.47^{a}	46.7^{b}	66.7^{b}	57.3^{b}	845.3^{b}	6.0
NPK	4.57^{a}	0.46^{a}	44.7^{b}	68.4^{b}	31.1^{a}	382.9^{a}	4.2
CaPK	4.50^{a}	0.50^{a}	79.6^{c}	86.9^{c}	66.1^{b}	879.6^{b}	6.2
CaPN	4.59^{a}	0.49^{a}	60.2^{c}	29.1^{a}	62.3^{b}	881.6^{b}	6.2
CaKN	4.60^{a}	0.47^{a}	8.9^{a}	72.4^{b}	59.1^{b}	819.6^{b}	6.1

Common letters in superscripts indicate the lack of significant differences between mean values ($P < 0.05$) in the same column tassium was also lower, as a result of which more of these elements remained in the soil. The pH in the regularly limed combinations was slightly acidic, and strongly acidic on the un-limed plots.

The results show that the highest yields of *Miscanthus* were obtained in the CaNPK combination with full mineral fertilization (Table 2), and the lowest in the fertilization variant without nitrogen (CaPK). The latter combination gave even lower yields than the control, i.e. the combination with no fertilization since 1923. *Miscanthus* also responded to potassium deficiency with a very large drop in yield. Hence, in the combinations not fertilized with potassium since 1923, the yields of the plant were 17% lower than on the plots with full CaNPK fertilization. *Miscanthus* plants were the least responsive to phosphorus deficiency and soil acidification. The yields on the soil without phosphorus fertilization and on the acidic soil (NPK) were only about 6% lower than on the soil with full (CaNPK) fertilization.

As perennials, *Miscanthus* plants can take advantage of the soil's natural resources to a much greater extent than annuals. Moreover, these plants, while remaining in the field until late autumn and even winter, lose part of the generated biomass, mainly leaves, which, having fallen to the surface of the soil, contribute towards increasing the amounts of some nutrients taken up by crop during the growing season. By harvesting *Miscanthus* late also gives the plants an opportunity to withdraw a portion of the components from the aboveground organs to the roots, which is a characteristic feature of the species. Research results of other authors (Ercoli et al. 1999, Kahle et al. 2001, Kalembasa and Malinowska 2007) indicate that *Miscanthus* can be grown on light soils.

The amounts of elements in *Miscanthus* plants determined at harvest in the different fertilization combinations did not vary much (Table 2). The largest variations were in the amounts of calcium and magnesium, and the smallest ones in those of phosphorus and nitrogen. The lowest amounts of N, P, K were obtained on the plots that had not been fertilized with these components for more than 88 years. The highest phosphorus content was recorded in the CaPK combination, potassium in CaNPK, and nitrogen in CaPN. Sulphur content was also determined. The amount of this element in *Miscanthus* plants was relatively low (less than 0.1% in dry matter). The low sulphur content does not pose a risk of increased SO_2 emissions in the combustion of the biomass for energy purposes (Table 2). Other authors have also indicated that combustion of an ecological fuel also means a significant reduction in the emission of volatile sulphur compounds compared with coal or petroleum products (Chum and Overend 2001, Demirbas 2004).

Fertilization not only changes the physical and chemical properties of the soil, improving them by crop yields, but also produces quantitative and qualitative changes in soil physiological groups of microorganisms. The microbiological tests presented here revealed an effect of mineral fertilization and liming on the number of the physiological groups of microorganisms determined in non-rhizosphere and rhizosphere soils under the

Table 2. Yields of *Miscanthus giganteus* and effect of mineral fertilization on the levels of macroelements in *Miscanthus* plants at harvest; average values for 2010–2011

Fertilization combination	Yields (t/ha)	Content (mg/kg DW)					
		P	K	N	Mg	Ca	S
Ca	22.79[a]	0.09[a]	3.03[a]	4.11[ab]	0.55[b]	1.83[a]	0.55[a]
CaNPK	26.85[b]	0.12[b]	5.42[b]	4.88[c]	0.75[c]	5.27[c]	0.80[b]
NPK	25.19[b]	0.09[a]	5.09[b]	4.70[c]	0.54[b]	3.18[b]	0.85[b]
CaPK	15.16[c]	0.13[b]	5.21[b]	3.23[a]	0.34[a]	2.17[ab]	0.75[b]
CaPN	22.14[a]	0.11[b]	2.55[a]	5.18[c]	0.68[c]	2.56[a]	0.70[b]
CaKN	25.57[b]	0.08[a]	4.9[b]	4.92[c]	0.65[b]	2.45[a]	0.45[a]
Average	22.95	0.10	4.37	4.50	0.59	2.91	0.68

Common letters in superscripts indicate the lack of significant differences between mean values ($P < 0.05$) in the same column. DW – dry weight

Table 3. Number of microorganisms in non-rhizosphere soil under *Miscanthus giganteus* energy crop grown under different mineral fertilization regimes

Fertilization combination	Bacteria (CFU × 10^8/kg DW of soil)	Microscopic fungi (CFU × 10^6/kg DW of soil)	*Azotobacter* sp. (10^3/kg DW of soil)	*Clostridium pasteurianum* (10^5/kg DW of soil)
Ca	180.4^{b}	47.1^{a}	385.2^{ab}	125.7^{b}
CaNPK	88.9^{a}	42.6^{a}	41.9^{ab}	65.3^{ab}
NPK	181.5^{b}	62.5^{b}	34.4^{ab}	20.0^{a}
CaPK	136.1^{ab}	39.4^{a}	528.1^{b}	136.1^{b}
CaPN	103.4^{a}	43.1^{a}	21.3^{a}	17.4^{a}
CaKN	180.7^{b}	47.0^{a}	41.5^{ab}	40.4^{a}

Common letters in superscripts indicate the lack of significant differences between mean values ($P < 0.05$) in the same column. DW – dry weight

Miscanthus crop (Tables 3 and 4). The obtained results are consistent with those described in the literature on the effects of fertilization on soil microbial activity (Barabasz et al. 2002, Kanova et al. 2010, Lazcano et al. 2013). In the rhizosphere soil, the number of heterotrophic bacteria was more than three, and that of microscopic fungi more than two orders greater than the size of these groups in the non-rhizosphere soil (Tables 3 and 4).

Similar relationships were found for the number of aerobic diazotrophic bacteria of the genus *Azotobacter* (Table 4). By comparison, the number of *Clostridium pasteurianum* remained at the same level regardless of the distance from the roots. The large increase in the number of the test bacteria in the rhizosphere soil (Table 4) in relation to non-rhizosphere soil (Table 3) may be caused by organic compounds secreted by the root system of *Miscanthus* plants, such as monosaccharides, amino acids and organic acids (Hromádko et al. 2010, Kaňova et al. 2010, Técher et al. 2011). These compounds can be used by microorganisms for their growth and development, as carbon and energy sources.

In this study, the non-rhizosphere soil taken from the NPK fertilization variant contained the highest number of fungi in comparison with the other fertilization variants (Table 3). The increase in fungal populations has, on the one hand, a positive effect on soil properties because fungi take part in the breakdown of organic matter and thereby in the 'circulation of elements in nature'. On the other hand, fungi synthesize toxins, and thus have a negative impact on soil-inhabiting organisms. The smallest numbers of heterotrophic bacteria, compared with the control (Ca), were found in the CaNPK and CaPN fertilization variants and

Table 4. Number of microorganisms in rhizosphere soil under *Miscanthus giganteus* energy crop grown under different mineral fertilization regimes

Fertilization combination	Bacteria (CFU × 10^{11}/kg DW of soil)	Microscopic fungi (CFU × 10^8/kg DW of soil)	*Azotobacter* sp. (10^6/kg DW of soil)	*Clostridium pasteurianum* (10^5/kg DW of soil)
Ca	778^{c}	850.0^{d}	119.0^{e}	270^{e}
CaNPK	393^{ab}	30.3^{a}	11.0^{d}	113^{d}
NPK	338^{ab}	24.3^{a}	8.0^{c}	8^{a}
CaPK	540^{bc}	47.3^{ab}	3.0^{b}	29^{b}
CaPN	538^{bc}	177.0^{bc}	1.2^{a}	8^{a}
CaKN	159^{a}	300.0^{c}	1.3^{a}	72^{c}

Common letters in superscripts indicate the lack of significant differences between mean values ($P < 0.05$) in the same column. DW – dry weight in the absence of many years of fertilization with mineral nitrogen (CaPK) (Table 3). The number of *Azotobacter* sp. in the non-rhizosphere soil depended on the levels of nitrogen and phosphorus in the soil. The highest count was obtained in the combination without nitrogen fertilization (CaPK), and the lowest in the combination without potassium (CaPN) (Table 3). In the case of *C. pasteurianum*, the highest count outside of the rhizosphere was recorded in the control (Ca) and the combination without nitrogen fertilization (CaPK) (Table 3).

The number of microorganisms in the rhizosphere soil depends not only on the soil properties but also on root exudates. For that reason, the microbial activity in the rhizosphere of *Miscanthus* plants, expressed by the number of physiological groups of microorganisms, proceeds differently than in the soil outside of the rhizosphere. The highest counts of investigated groups of microorganisms in the rhizosphere of the test plant (Table 4) were recorded in the control combination (Ca). Significantly higher numbers of microscopic fungi were also obtained in the soil very low in phosphorus (CaKN). Phosphorus fertilization decreased the fungal count in the soil (Table 4). This can be explained by the fact that the root system of the *Miscanthus* plant releases exudates that are rich in many chemical compounds readily available to microorganisms into the rhizosphere (Hromádko et al. 2010, Técher et al. 2011), which makes it a suitable environment for their growth and development. The applied mineral fertilization decreased the number of diazotrophs in the rhizosphere (Table 4).

In conclusion, *Miscanthus giganteus* responded positively to the addition of mineral fertilization, especially with nitrogen. The highest yield was obtained with full CaNPK fertilization, and the lowest one in the combination without nitrogen fertilization (CaPK). The assessment of the amounts of nutrients accumulated in the aerial parts of the evaluated plant species in full growth (August) and at harvest (February, March) indicates a substantial return of these nutrients to the soil through leaf fall and physiological outflow of nutrients to the underground parts of plants. This aspect should be taken into account in the balancing of nutrients and determination of fertilizer doses. The type of the applied mineral fertilization also affects the microbiological properties of the soil. Strong acidification of the soil increases the number of microscopic fungi in the non-rhizosphere soil. Finally, the rhizosphere of *Miscanthus* plants provides a suitable environment for the growth and development of microorganisms, in contrast to the non-rhizosphere zone.

REFERENCES

Barabasz W., Albińska D., Jaśkowska M., Lipiec J. (2002): Biological effects of mineral nitrogen fertilization on soil microorganisms. Polish Journal of Environmental Studies, *11*: 193–198.

Bunt Y.S., Rovira A.D. (1955): Microbiological studies of some subartactic soils. Journal of Soil Sciences, *6*: 119–128.

Chou C.H. (2009): *Miscanthus* plants used as an alternative biofuel material: The basic studies on ecology and molecular evolution. Renewable Energy, *34*: 1908–1912.

Chum H.L., Overend R.P. (2001): Biomass and renewable fuels. Fuel Processing Technology, *71*: 187–190.

Demirbas A. (2004): Combustion characteristics of different biomass fuels. Progress in Energy and Combustion Science, *30*: 219–230.

Deuter M., Jeżowski S. (2002): State of the knowledge about breeding of gigantic grass from the genus *Miscanthus*. Zeszyty Problemowe Postępów Nauk Rolniczych, *2*: 59–63. (In Polish)

Dobereiner J. (1961): Nitrogen-fixing bacteria of the genus *Beijerinckia* Derx in the rhizosphere of sugar cane. Plant and Soil, *15*: 211–216.

Ercoli L., Mariotti M., Masoni A., Bonari E. (1999): Effect of irrigation and nitrogen fertilization on biomass yield and efficiency of *Miscanthus*. Field Crops Research, *63*: 3–11.

Faber A., Stasiak M., Kuś J. (2007): Preliminary estimation of the productivity of chosen species of energy plants. Postępy w Ochronie Roślin, *47*: 339–346. (In Polish)

Filipek T., Gonet Sł., Kucharski J., Mocek A. (2000): More important units of measures and symbols applied in the soil sciences. Zeszyty Problemowe Postępów Nauk Rolniczych, *472*: 731–739. (In Polish)

Fotyma M., Gosek S., Strączyk D. (2005): New approach to calibration of the Egner-Riehm (DL) soil test for available P and K. Fertilizers and Fertilization, *3*: 111–123.

Girard H., Rougieux R. (1967): Techniques de Microbiologie Agricole. Dunod. Paris.

Jeżowski S. (1999): Miscanthus grass (*Miscanthus sinensis*) – source of renewable and ecological raw materials for Poland. Zeszyty Problemowe Postępów Nauk Rolniczych, *468*: 159–166. (In Polish)

Hromádko L., Vranová V., Techer D., Laval-Gilly P., Rejšek K., Formánek P., Falla J. (2010): Composition of root exudates of *Miscanthus* × *Giganteus* Greef et Deu. Acta Universitatis Agriculturae et Silviculture Mendelianea Brunensis, *58*: 71–76.

Kahle P., Beuch S., Boelcke B., Leinweber P., Schulten H.R. (2001): Cropping of *Miscanthus* in Central Europe: Biomass production and influence on nutrients and soil organic matter. European Journal of Agronomy, *15*: 171–184.

Kalembasa D., Malinowska E. (2007): Effect of doses of sewage sludge on yield and chemical composition of *Miscanthus sacchariflorus* in the field experiment. Fragmenta Agronomica, *1*: 113–118. (In Polish)

Káňová H., Carre J., Vranová V., Rejšek K., Formánek P. (2010): Organic compounds in root exudates of *Miscanthus* × *Giganteus* Greef et Deu and limitation of microorganisms in its rhizosphere by nutrients. Acta Universitatis Agriculturae and Silviculture Mendelianea Brunensis, *23*: 203–208.

Lazcano C., Gómez-Brandón M., Revilla P., Domínguez J. (2013): Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. A field study with sweet corn. Biology and Fertility of Soils, *49*: 723–733.

Martin J.P. (1950): Use of acid rose bengal and streptomycin in the plate method for estimating oil fungi. Soil Sciences, *69*: 215–232.

Ostrowska A., Gawliński S., Szczubiałka Z. (1991): The Methods of Analysis and Estimation of Soil and Plants Properties. Institute of Environmental Protection, 333. (In Polish)

PN-ISO 11261 Determination of total nitrogen – Modified Kjeldahl method. Geneve.

PN-ISO 10390 Soil quality – Determination of pH. Warsaw.

PN-ISO 10694 Determination of organic and total carbon after dry combustion (elementary analysis). Warsaw.

Pultowicz A. (2009): The premises of the market development of renewable energy sources in Poland in the light of the idea of the sustainable development. Problemy Ekorozwoju, *4*: 109–115. (In Polish)

Técher D., Laval-Gilly P., Henry S., Bennasroune A., Formanek P., Martinez-Chois C., D'Innocenzo M., Muanda F., Dicko A., Rejšek K., Falla J. (2011): Contribution of *Miscanthus* × *giganteus* root exudates to the biostimulation of PAH degradation: An *in vitro* study. Science of the Total Environment, *409*: 4489–4495.

Received on December 11, 2013
Accepted on February 18, 2014

Corresponding author:

Prof. Hazem M. Kalaji, Warsaw University of Life Sciences-SGGW, Faculty of Agriculture and Biology,
Department of Plant Physiology, Nowoursynowska 159, 02-776 Warsaw, Poland
e-mail: hazem@kalaji.pl