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Response of nitrogen nutritional indices of maize leaves to different mineral-organic fertilization

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Abstract

Maize crop growth rate during the course of vegetation is strongly dependent on nitrogen supply. The most critical stages of maize response to this nutrient are exhibited both in very early growth stages, as related to the stage from five (six) to eight leaves, and in the period from tasselling to the blister stage. Among many indicators of plant nitrogen nutritional status, the most promising include nitrate reductase and acid invertase activity, since the enzymes significantly affect the growth of new tissues. Two series of field experiments were conducted in 2009 and 2010 to evaluate the effect of six fertilization treatments, differing in the prevailing source of nitrogen (from pure mineral to classical organic), on nitrate concentration, nitrate reductase and acid invertase activity and finally on the assimilation area of a single maize plant or the ear leaf. A significant effect of each fertilization treatment on all the examined maize plant characteristics was observed, but it was mostly seasonally variable. Nitrate reductase was linearly affected by the nitrate concentration in maize leaves. Both evaluated indices of acid invertase showed a curvilinear effect on the maize plant morphological characteristics. Therefore it can be concluded that excessive acid invertase activity may inhibit the growth rate of the maize assimilation area in the flowering stage.

Keywords: assimilation area, maize, nitrogen, organic fertilizers

Introduction

The main idea of plant crop balanced fertilization assumes the application of mineral and organic fertilizers in the ratios adjusted to soil agrochemical properties (Ailincăi et al, 2008). On light soils, in order to improve soil organic matter content, mainly organic manures are recommended. On medium and heavy soils, the amount of used mineral fertilizers should be adjusted to the amounts of available nutrients introduced in manure or in other types of organic fertilizers (Agyenim Boateng et al, 2006).

Maize cultivation is generally based on mineral fertilizers, which are considered as a good source of easily available nutrients (Ayoola and Makinde, 2009). Nowadays, manure is of minor importance as a source of nutrients in Poland, despite considerable knowledge on maize response to slurry or farmyard manure. Maize plants are able to use very efficiently nutrients applied in this form (Tejada et al, 2008).

Acid invertase activity is highly related to the growth of young tissues, where it is responsible for maintaining temporal balance between donors and acceptors of carbohydrates (Koch, 2004). Its activity decreases together with plant age, in turn limiting the rate of growth of assimilation plant parts (Roitsch and Gonzales, 2004). In addition, the activity of this enzyme is highly affected by both external and internal growth conditions, such as the amount of delivered carbohydrates, hormone activity, and also drought, low temperatures. For these reasons, acid invertase seems to be a very promising index for determination of maize plant nitrogen status.

The main objective of the conducted field study was to assess the effect of six treatments, differing in nutrient input through mineral and organic fertilizers, on some indices of nitrogen nutritional status of maize plants during the very early and late flowering stages of growth.

Materials and Methods

The field experiments were conducted in two consecutive years – 2009 and 2010 in the Research and Education Unit in Swadzim of the Poznań University of Life Sciences. According to the FAO classification, soil under study was Albic Luvisol, originating from loam sands and lying on sandy loam. According to the Polish agronomic evaluation, this soil type represents good rye complex. The content of main available nutrients, such as P, K, and Mg, was in the medium class. The one-factor experiment comprised six treatments replicated four times, as listed in Supplementary Table 1. The maize variety used as a test crop was Clarica, FAO 280, supplied by Pioneer.

Nitrogen was used in the form of urea; phosphorus in the form of granulated triple superphosphate (46% P_2O_5); potassium in the form of muriate of pot-

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ash (60% K_2O). The used cattle manure contained the following amounts of nutrients: N 4.7, phosphorus 4.9 and potassium 7.8 g kg^{-1} DM. It was assumed that in the year of application, the nutrient utilization was as follows: nitrogen 30%, phosphorus 20%, and potassium 30%. Hence the dose of 30 t ha^{-1} of cattle manure every time provided 42.3 kg ha^{-1} of nitrogen, 29.4 kg ha^{-1} of P_2O_5 and 70.2 kg ha^{-1} of K_2O.

The concentration of nitrates and nitrate reductase (NR) activity in maize leaf blades were determined in two stages of maize growth: (i) the 5(6)-leaf stage (BBCH15/16) and (ii) the late flowering stage (BBCH67). The activity of acid invertase was determined only in the silking stage.

Procedure of determination of nitrate content

250 mg of plant sample (fresh basis) was cut into small pieces, then 20 cm^3 of distilled water was added, and it was cooked for 20 minutes. In the second step of the extraction procedure, the cooled extract was spin-dried at 4,000g for 15 minutes. The concentration of nitrates was determined in the obtained supernatant by the method of Cataldo et al (1975). Then 0.4 cm^3 of 5% salicylic acid in concentrated H_2SO_4 was added to 0.1 cm^3 of supernatant and left for 20 minutes; next, 9.5 cm^3 of 2 M NaOH was added and the samples were cooled at room temperature. Absorbance was measured at 410 nm. The results were expressed as mg of NO_3^- g^{-1} of tissue.

Procedure of determination of nitrate reductase activity (NR)

The method described by Jaworski (1971) was used. Plant samples (200 g) were placed in flasks, then 5 cm^3 of incubation mixture containing nitrates was added and it was left for 1 hour at a temperature of 30°C. In the second step of the procedure, 1 cm^3 of SAA (1% sulfanilamide in 1 M HCl) and 1 cm^3 of NED (0.01% N-(l-naphthyl) ethylenediamine dihydrochloride) were added to 1 cm^3 of the extracted solution and then the mixture was left for 15 minutes. Absorbance of the prepared mixture was measured at 540 nm. The activity of nitrate reductase was expressed as nmole of NO_2^- per 1 g of fresh leaf matter per 1 hour.

Procedure of determination of acid invertase (AI) activity

The modified method by Copeland and Lea (1990) was used. The sample (400 g) was homogenized in 1.6 cm^3 of 50 mM sodium phosphate buffer, pH 7.4, containing 10 μl of mercaptoethanol. The obtained homogenate was than spin-dried for 20 minutes at 15,000 rpm. Next, the AI activity was determined in the obtained supernatant. The reaction mixture contained 0.18 cm^3 of supernatant and 0.57 cm^3 of acetate buffer, pH 5.0, with sucrose. The tubes with the reaction mixture were incubated in water bath at a temperature of 30°C for 45 minutes; then Tricine buffer was added to each tube and the mixture was boiled at 100°C for 3 minutes. Absorbance of the obtained solution was measured on a spectrophotometer at 560 nm. The results were expressed as absorbance per 1 g of tissue and protein.

The assimilation area of a single plant was calculated according to the following formula developed by Szulc (2009):

$$AA = -3.550 + 3.774 \cdot X,$$

where: AA means the assimilation area of a single plant and X is the sum of the areas of the 5th and 6th leaf.

The area of the fifth and sixth leaf was determined using Montgomery's method (quoted after Szulc, 2009): leaf length along the main nerve multiplied by the width measured at the widest place and the result is then multiplied by coefficient 0.75.

Thermal and moisture growth conditions of maize crop in the both vegetative periods are listed in Supplementary Table 2. The total amount of precipitation in the period IV-IX amounted to 452.3 mm in 2009 and 500.7 mm in 2010. The calculated Sielianinov's hydrothermal coefficients of water supply enabled to emphasize that in 2009 water deficits occurred in April and July (hydrothermal coefficients amounted to 0.96 and 0.78, respectively), whereas in 2010 they occurred in April and June (hydrothermal coefficients amounted to 0.49 and 0.42, respectively). The daily mean temperature measured at 2 m was 15.2°C in 2009 and 14.5°C in 2010.

The statistical distribution of the investigated plant characteristics was assessed by means of the Shapiro-Wilk's tests (Shapiro and Wilk, 1965). The obtained data sets were elaborated by using the analysis of variance for each year separately and for the interaction between the years and fertilization treatments with the use of computer software GenStat Release 10.1 (GenStat, 2007).

Results and Discussion

The concentration of nitrates in the both studied stages of maize growth, i.e. in the BBCH15/16 and BBCH67 stages, showed a significant effect of seasonal variability ($p = 0.046$ and $p = 0.020$ for 2009 and 2010, respectively) and also no significant interaction between the years and fertilization treatments ($p = 0.847$ and $p = 0.631$ for the both stages, respectively). These results are a prerequisite for making statistical evaluation separately for each growing season. A significant effect of the fertilization treatments on the nitrate concentration in maize leaf blades was corroborated only in the second year of the study, i.e. in 2010 ($p < 0.001$ and $p = 0.002$ for the BBCH15/16 and BBCH67 stages, respectively) (Table 1). However, in this particular year, the nitrate concentration, averaged over the treatments and irrespective of the examined growth stage, was much higher than in 2009. This phenomenon can be explained only by much lower soil N supply, as indicated by the nitrate content in the leaves of the control plot. In the both years, irrespective of the treatments, a much higher

Table 1 - Nitrate concentration in maize leaf blades, mean ± SD, [mg of NO_3^- g^{-1} of fresh matter]

Fertilization treatments	Stage of 5/6 leaves, BBCH 15-16			Late flowering, BBCH 67		
	Years			Years		
	2009	2010	Mean	2009	2010	Mean
1. C	1.34±0.35	0.99±0.05	1.16±0.30	0.50±0.09	0.41±0.03	0.45±0.07
2. M	1.52±0.29	1.47±0.14	1.49±0.20	0.72±0.08	0.50±0.10	0.61±0.14
3. O	1.41±0.46	1.32±0.05	1.37±0.30	0.71±0.14	0.48±0.06	0.59±0.16
4. M+O	1.74±0.32	1.62±0.15	1.68±0.23	0.88±0.24	0.76±0.17	0.82±0.20
5. 1/2O	1.33±0.19	1.23±0.38	1.28±0.27	0.61±0.17	0.66±0.04	0.64±0.12
6. M+1/2O	1.77±0.02	1.57±0.09	1.67±0.12	0.85±0.40	0.72±0.11	0.79±0.27
$LSD_{0.05}$	ns	0.333	0.316	ns	0.182	0.159
Mean	1.52±0.31	1.37±0.27	1.44±0.30	0.71±0.23	0.59±0.16	0.65±0.20

ns: non-significant statistical difference; SD: standard deviation

nitrate concentration was noted in the early growth stage, than in the late flowering stage. These results support the thesis about extremely high demand of developing maize ear for nitrogen, which occurs during maize flowering (Grzebisz et al, 2008). The effect of individual treatments on the nitrate concentration was significant only in 2010. In the stage of BBCH15/16, the highest values of nitrate concentration were attributed to the M+O treatment, where the amount of the used mineral fertilizer was adjusted to the potential supply of basic nutrients from farmyard manure. However, the same level of the nitrate concentration was found also for the other two treatments, representing pure NPK and the M+1/2O treatment. A slightly different pattern of the nitrate concentration in maize leaves depending on the source of nitrogen was found during late flowering. The highest concentration of nitrates was found in the plants grown with the use of the M+O treatment, followed by M+1/2O and then by ½O. However, a specific maize response to half portion of manure (½O) was not observed in 2009. It may be accounted only for high temperatures, which occurred in 2010 just before flowering, in turn accelerating the rate of organic nitrogen mineralization.

The activity of nitrate reductase (NR) in the examined stages of maize growth did not show any seasonal variability (years) (p = 0.156 and p = 0.790 for 2009 and 2010, respectively). The same observation can be made in the case of the effect of interaction years × fertilization treatments (p = 0.992 and p = 0.975 for the stage BBCH15/16 and BBCH67, respectively) (Table 2). However, the trends of this particular index were strong enough, as presented for data averaged over the years. In the both studied stages of maize growth, a significant effect of fertilization treatments on NR activity was found, when compared to the control treatment (p = 0.034 and p = 0.011 for the stage BBCH15/16 and BBCH67, respectively). Nitrate reductase activity for this particular treatment amounted to 0.24 mmole of NO^{2-} g^{-1} h^{-1} for the stage BBCH15/16 and to 0.30 mmole of NO_2^- g^{-1} h^{-1} for the stage BBCH67. The highest enzyme activity was attributed to the M+O treatment, amounting to 0.45 mmole of NO_2^- g^{-1} h^{-1} and to 0.49 mmole of NO_2^- g^{-1} h^{-1} for the both stages of maize growth, respectively.

The conducted study revealed a significant dependence of NR activity on the nitrate concentration in maize leaves in the both examined stages of plant growth (Figure 1). The linear regression model reflects the best fit of experimental data. The observed relationships are in agreement with other reports, indicating the sensitivity of NR enzyme to the supply of nitrates (Rajasekhar and Oelmüller, 1987; Campbell, 1988). The obtained linear models corroborate the im-

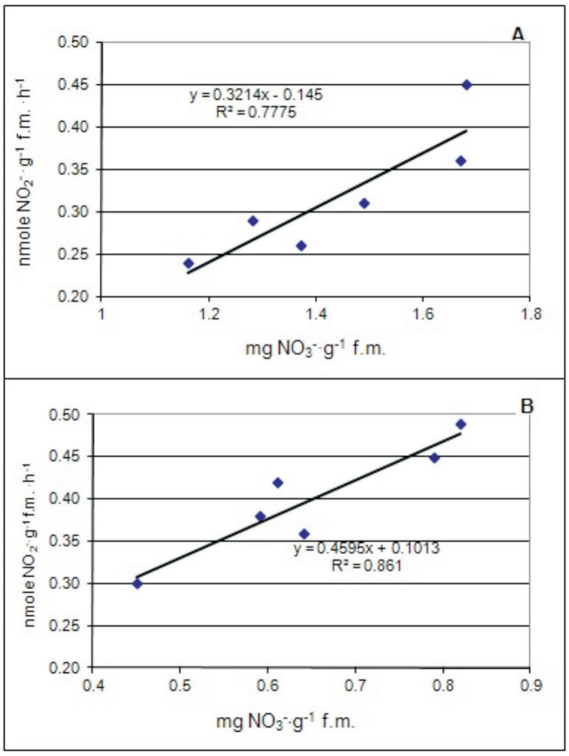

Figure 1 - Nitrate reductase activity as a function of the nitrate concentration in maize leaf blades. A: the stage of 5/6 leaves, BBCH 15/16, B: late flowering, BBCH 67. „(2009-2010)".

Table 2 - Nitrate reductase activity in maize leaf blades, mean ± SD, [mmole of NO_2^- g^{-1} h^{-1}]

Fertilization treatments	Stage of 5/6 leaves, BBCH 15-16			Late flowering, BBCH 67		
	Years			Years		
	2009	2010	Mean	2009	2010	Mean
1. C	0.26±0.14	0.22±0.08	0.24±0.10	0.32±0.22	0.29±0.00	0.30±0.14
2. M	0.37±0.05	0.24±0.22	0.31±0.16	0.42±0.10	0.41±0.24	0.42±0.16
3. O	0.29±0.05	0.23±0.04	0.26±0.05	0.38±0.11	0.39±0.07	0.38±0.09
4. M+O	0.46±0.21	0.43±0.25	0.45±0.21	0.44±0.29	0.54±0.19	0.49±0.23
5. 1/2O	0.34±0.19	0.24±0.04	0.29±0.14	0.38±0.19	0.34±-.12	0.36±0.14
6. M+1/2O	0.39±0.15	0.33±0.24	0.36±0.18	0.43±0.21	0.47±0.02	0.45±0.13
$LSD_{0.05}$	ns	ns	0.205	ns	ns	0.186
Mean	0.35±0.14	0.28±0.16	0.32±0.15	0.39±0.17	0.41±0.14	0.40±0.15

ns: non-significant statistical difference; SD: standard deviation

portance of farmyard manure as a significant source of nitrates for maize crop at its critical stages of yield formation. This particular fertilizer realizes inorganic nitrogen in strong dependence on soil humidity and temperature. Nitrogen mineralization occurs in July under favourable meteorological conditions, which is in accordance with maize development and the highest demand for nitrogen (Grzebisz et al, 2008).

The obtained linear model enables also to calculate the rate of the NR enzyme activity response to an increasing concentration of nitrates in maize leaf blades. In the BBCH15/16 stage, each 1-mg NO_3^- g^{-1} of fresh matter increase in turn resulted in NR activity increase by 0.32 mmole of NO_2^- g^{-1} h^{-1}. However, during late flowering each 1 mg NO_3^- g^{-1} of fresh matter increase in turn resulted in NR activity increase amounting to 0.46 mmole of NO_2^- g^{-1} h^{-1}, i.e. by 44%. These two sets of data clearly demonstrate a much stronger response of developing ear to the supply of nitrates and corroborate the maize crop sensitivity to nitrogen supply (Grzebisz et al, 2008). In this particular stage of maize growth, the supply of nitrogen is responsible for the development of maize kernel endosperm (Cazetta et al, 1999).

The acid invertase (AI) activity in leaf blades of maize plants during late flowering (BBCH67) showed a very similar response to the examined fertilization treatments in the both studied years, as it was previously described for nitrate reductase. Therefore, the only average data for fertilization treatments were statistically analysed. The lowest activity of AI was found for the plants grown on the control plot, as presented by two indices – absorbance per 1 gram of fresh matter and absorbance per 1 mg of protein, amounting to 1.53 A g^{-1} of fresh matter, 8.07 A mg^{-1} of protein (Figure 2). The best sets of treatments, i.e. presenting the highest values but the same level of significance, are slightly different, as based on a given index. For the first one, absorbance per 1 gram of fresh matter, the highest values were attributed to the following treatments:

M+1/2 O ≥ M + O ≥ M

However, the second one, absorbance per 1 mg of protein, showed a slightly different order:

M + O ≥ M+1/2 O ≥ M

Two morphological canopy indices, such as the assimilation area of a single maize plant and the assimilation area of the ear leaf showed a significant response to seasonal variability (year) ($p < 0.001$). The examined interaction years × fertilization treatments did not affect the both indices ($p = 0.329$). However, a significant effect of fertilization treatments on the assimilation area of a single maize plant ($p = 0.041$) and the assimilation area of the ear leaf ($p = 0.030$) was found (Table 3). As expected, the lowest values of both canopy indices were found for the plants

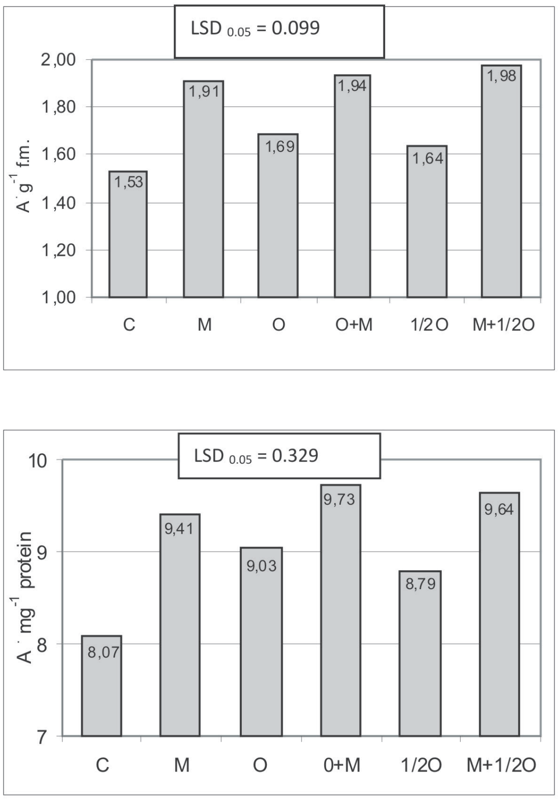

Figure 2 - Acid invertase activity in maize leaf blades during late flowering (BBCH 67). „(2009-2010)".

Table 3 - The assimilation area of maize leaf blades, mean ± SD

Fertilization treatments	Assimilation area of a single plant [cm²] Years 2009	2010	Mean	Assimilation area of the ear leaf [cm²] Years 2009	2010	Mean
1. C	2682.7±276	4080.9±460	3381.8±826	350.0±39.9	549.1±61.2	449.5±116.7
2. M	3809.7±539	4250.0±528	4029.8±547	520.6±67.0	574.6±70.9	547.6±70.9
3. O	3495.9±746	4307.1±109	3901.4±657	453.6±108.7	581.3±19.9	517.5±99.4
4. M+O	3797.2±301	4419.0±56	4108.1±388	496.3±42.6	603.6±18.6	549.9±65.0
5. 1/2O	3154.8±475	4350.9±231	3752.8±727	425.4±74.4	591.0±35.0	508.2±103.6
6. M+1/2O	3537.3±845	4479.9±317	4008.6±776	472.9±109.4	612.7±37.4	542.8±106.4
$LSD_{0.05}$	ns	ns	422.98	ns	ns	58.31
Mean	3412.9±645	4314.6±321	3863.8±679	453.1±89.8	585.4±45.0	519.3±97.0

ns: non-significant statistical difference; SD: standard deviation

grown on the control plot, amounting to 3,381.8 cm² and 449.5 cm², respectively. Significantly higher values of the both indices were found for all the other treatments, indicating a small difference between the tested organic and mineral fertilizer combinations. For the best treatment, i.e. M+O, the two indices were as follows: 4,108.1 cm² and 549.9 cm², respectively. Therefore, it can be concluded that the best ratio of organic and inorganic sources of nitrogen and other nutrients depends on many year to year and site to site variables, responsible for their release in the course of the growing period.

As reported by Grzebisz (2008), leaf carbohydrate status in the course of the growing period can be indirectly described by acid invertase activity. This particular enzyme hydrolysis sucrose transported to young leaf blade to monocarbohydrates. Hence, it can be assumed that the rate of leaf growth is positively correlated with the activity of this enzyme. However, this assumption was not fully corroborated during the conducted study. The both plant canopy morphological indices, as presented in Figure 3, showed a curvilinear dependence on acid invertase activity. The assimilation area of a single maize plant increases linearly up to the AI activity of 1.87 A g⁻¹ of fresh matter, reaching the size of 4,086.2 cm². Up to this level of AI activity, an increase per unit amounted to 1,706.9 cm². With respect to the second plant morphological index, the assimilation area of the ear leaf rose to the AI activity of 1.88 A g⁻¹ of fresh matter, reaching the maximum size of 550.4 cm². These two data sets clearly indicate that an excess of nitrates does not lead to an infinite increase in the assimilation area. This is the main reason for extra nitrate accumulation in older leaves due to temporary lack of available carbohydrates, which could be transformed into new tissues, and also the main reason for the delayed onset of maize crop flowering that in turn shortens the reproductive part of maize vegetation.

Conclusions

1. Fertilization treatments based on both farmyard manure and mineral fertilizers exert a significant effect on nitrogen management by maize plants, as expressed by a significant increase in the nitrate concentration, nitrate reductase and acid invertase activity and the morphological indices of maize crop canopy.
2. Nitrate reductase showed a linear dependence on the nitrate concentration in the blades of maize leaves.
3. The assimilation area of a single maize plant or the

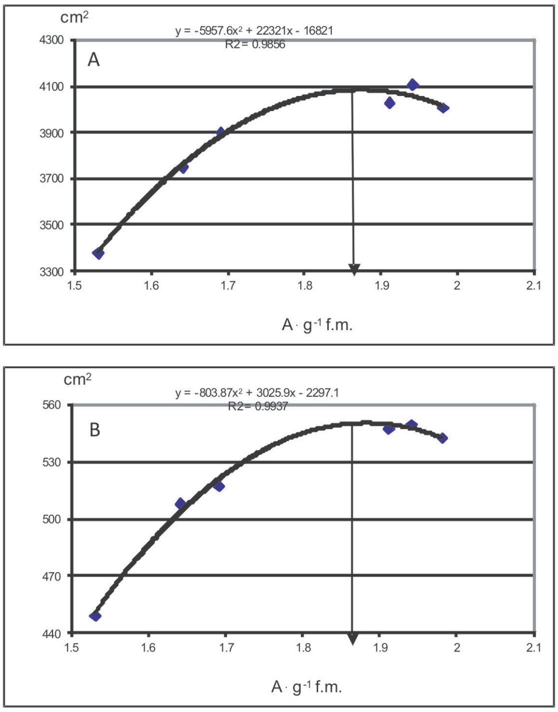

Figure 3 - Dependence of the assimilation area of a single plant (A) and the assimilation area of the ear leaf (B) on acid invertase activity. „(2009-2010)”.

size of the ear leaf showed a curvilinear response to the increasing acid invertase activity, as indicated by a well defined its optimum.

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Supplementary Table 1. Fertilization treatments

Fertilization treatments	Mineral fertilizer [M]			Farmyard manure [O]						Total sum of nutrients [M+O]		
				30 t·ha^{-1}			15 t·ha^{-1}					
	N	P	K	N	P	K	N	P	K	N	P	K
	kg·ha^{-1}											
1. C	0	0	0	0	0	0	0	0	0	0	0	0
2. M	150	90	160	0	0	0	0	0	0	150	90	160
3. O	0	0	0	42.3	29.4	70.2	0	0	0	42	29	70
4. M+O	108	60.6	89.8	42.3	29.4	70.2	0	0	0	150	90	160
5. 1/2O	0	0	0	0	0	0	21.1	14.7	35	21	14	35
6. M+1/2O	129	75.3	125	0	0	0	21.1	14.7	35	150	90	160

Supplementary Table. 2. Meteorological conditions during the study

Months	Years					
	2009			2010		
	T	O	S	T	O	S
IV	12.9	19.2	0.49	9.3	26.8	0.96
V	14.0	109.9	2.53	12.2	110.5	2.92
VI	16.0	113.8	2.37	18.4	43.4	0.78
VII	20.3	75.4	1.19	22.6	97.5	1.39
VIII	20.1	26.2	0.42	19.2	143.5	2.41
IX	15.8	48.6	1.02	13.0	69.9	1.79
X	7.6	59.2	2.51	7.0	9.1	0.42
Vegetation period	15.2	452.3	1.50	14.5	500.7	1.52

T – mean monthly air temperature (°C)

O – monthly amount of precipitation (mm)

S – Sielianinov's hydrothermal coefficient