Journal of Elementology ISSN 1644-2296

Przygocka-Cyna, K. and Zawieja-Roszak, A. (2024)
'Sulfur added to cattle slurry as a means to improve the nitrogen economy of maize during the grain filling period',
Journal of Elementology, 29(2), 433-451,
available: https://doi.org/10.5601/jelem.2024.29.1.3286

RECEIVED: 9 February 2024
ACCEPTED: 30 April 2024

ORIGINAL PAPER

Sulfur added to cattle slurry as a means to improve the nitrogen economy of maize during the grain filling period

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Abstract

Mineral additives to cattle slurry based on sulfur compounds improve the nitrogen (N) economy of grain maize during the grain filling period (GFP). This hypothesis was validated on the basis of a field experiment with maize, conducted in three consecutive seasons (2017-2019) on soil low in available sulfur and calcium. The field experiment was conducted at Lipie (51°51′34″ N, 17°5′5″ E, Poland) on soil formed from sandy loam, classified as an Albic Luvisol. The two-factor experiment included two forms of sulfur fermented with cattle slurry: elemental sulfur (S-0) and calcium sulfate dihydrate (Ca-S), applied in four doses of S: 0, 22.5, 45 and 90 kg ha^{-1}. The N contained in cattle slurry was 133 kg ha^{-1}. The average grain yield (GY) after applying slurry only (control S) was 9.6, 10.1 and 8.6 t DW ha^{-1} in 2017, 2018 and 2019, respectively. The increase in GY in response to S application was revealed in two of three years of the study, and amounted to 0.75 t DW ha^{-1} (+10% compared to S control) in 2017 and 1.4 t DW ha^{-1} (+16%) in 2019. In the latter season, GY depended on both N sources, i.e. on its remobilization from plant resources and uptake from the soil. However, the driver of GY increase in response to the applied S was post-flowering N uptake. The study showed that the application of cattle slurry enriched with sulfur is a simple agronomic solution to increase the productivity of N in maize, especially in years with drought, as in 2019. For this reason, this treatment should be considered as a preventive measure.

Keywords: grain yield, critical growth stages, nitrogen, accumulation, remobilization, post-flowering uptake

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The publication was financed by the Polish Minister of Science and Higher Education as part of the Strategy of the Poznan University of Life Sciences for 2024-2026 in the field of improving scientific research and development work in priority research areas.

INTRODUCTION

Natural fertilizers, including animal slurry, are a valuable source of nutrients for crops (Grzebisz et al. 2022). Slurry is a natural fertilizer, a waste product generated by animal husbandry in a bedding-free system. It is a mix of feces and urine in natural proportions and water used to clean livestock stalls (Sanchez et al. 2005). A 2014 report by AMEC for the European Environment Commission estimated that total slurry production in the EU-27 was around 600 million tons per year. The fertilizing value of fermented slurry is mainly determined by the content of ammonium nitrogen ($N\text{-}NH_4$) – Soroko et al. (2009). Animal slurry can be considered as N fertilizer or soil amendment. If slurry is to be treated as N fertilizer (for spring application) its C:N ratio and the $N\text{-}NH_4:N_f$ ratio should be as wide as possible. The main reason is that NH_4^+ ions are directly taken up by a plant. In cattle slurry, the share of $N\text{-}NH_4$ in the total N (N_t) is in the range of 40-60%. As a result, slurry is considered a natural, fast-acting fertilizer in this respect, largely similar to mineral N fertilizers (Fangueiro et al. 2015).

The fertilizing value of slurry decreases due to NH_3 volatilization both during storage (fermentation) and application. NH_3 emissions from slurry have long been counteracted by the addition of various compounds. The additives introduced into animal slurry can act in two ways (i) limiting water evaporation or (ii) lowering the pH of slurry (Jugowar et al. 2017). Ammonia losses due to evaporation are controlled simply by adding water or covering the tank with different types of covering materials, including straw. One of the practical solutions to reduce NH_3 losses is to acidify slurry to pH < 5.5, using mineral acids (Fila 2017).

Animal slurry is the most commonly used natural fertilizer in maize production, and is the primary source of all nutrients required by this crop. This fertilizer can fully cover the maize demand for N, without affecting the size and quality of the crop (Menezes et all 2017). Sulfur is taken up by maize in a high amount, but significantly impacts the N economy of this crop in Poland (Podleśna et al., 2017, Kulczycki 2021). The use of sulfuric acid requires the construction of appropriate technological infrastructure, which increases the costs of slurry application (Fangueiro et al. 2015). In the search for simple, practical and cheap methods of increasing the N productivity of slurry, it is worth paying attention to (S) compounds other than sulfuric acid. The classic examples of those type of S carriers are elemental sulfur (S^0) and calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$) – Grzebisz et al. (2023). It is well known that adding magnesium and calcium salts to animal manures effectively reduces ammonia emissions. The concentration of $(NH_4)_2CO_3$ in the suspension formed during the hydrolysis of urea is lower due to the precipitation into $CaCO_3$ (Rank et al. 1988).

Because of its versatility, maize is one of the most valuable crop in the world, grown for food, fodder and industrial uses (Erenstein et al. 2022).

It is well documented that the first critical stage of yield formation by maize is the stage of the 5th leaf. At this stage, the initials of flowers are formed, a process decisive for the final grain yield (Ritche et al. 2003). The rate of N uptake by maize at this particular stage affects the development of yield components during the growth period, extending from the onset of flowering to the watery stage of kernel growth, known as 'a critical window' (Grzebisz et al. 2008). The N content in the photosynthetic organs of maize is the main causative factor of plant activity in the period after flowering (Lamptey et al. 2017). In varieties from the stay-green group, this process is supported by N taken up by the plant from the soil (Ciampitti, Vyn 2013).

The main conceptual assumption of the undertaken research was to answer the question: will the addition of inorganic sulfur, regardless of its chemical form, to cattle slurry increase the productivity of N in maize? The key objective of the study was to evaluate the impact of the application of cattle slurry with the addition of inorganic sulfur carriers on (i) grain yield of maize, (ii) N accumulation by maize in critical stages of yield formation, (iii) indicators of N management in maize during the grain filling period (BBCH 60 - BBCH 89).

MATERIALS AND METHODS

Experimental site

A field experiment was conducted at Lipie (51°51′34″ N, 17°5′5″ E, Poland) on soil formed from sandy loam, classified as an Albic Luvisol (Neocambic) – Kabała et al. (2017). The presented study is based on three main seasons of maize fertilized with cattle slurry enriched with sulfur mineral additives: the first season in 2017 (referred to as the first season), the second one in 2018 (second), and the third in 2019 (third). The content of organic matter (C_{org}) in a 0.0-0.3 m layer was low, ranging during the study from 15±0.6 to 16±0.3 g kg^{-1} soil (losses on ignition). Soil reaction (pH) and the content of available nutrients, measured before the application of cattle slurry and mineral fertilizers, is shown in Table 1. Soil reaction (pH) was in the slightly acid range in the first two seasons and in a neutral range in the third one. The content of available nutrients, measured before the application of cattle slurry and mineral fertilizers, was in the medium class for P in the topsoil and in the low class in the subsoil. The content of available potassium (K) and sulfur (S) was very low in both soil layers. The calcium content in the topsoil was in the low class in the first two seasons and in the very low class in the subsoil. In the third season, it was in the very low class in both soil layers. The content of available magnesium (Mg) in the topsoil was in the medium class in the first season and in the high class in the remaining two seasons. In the subsoil, a medium class was only noted in the second season. The total amount of the mineral N (N_{min})

Table 1

Soil characteristics of the experimental plots during the consecutive growing seasons

Soil characteristics	2017		2018		2019	
	Soil layers, cm		Soil layers, cm		Soil layers, cm	
	0-30	30-60	0-30	30-0	0-30	30-60
Soil reaction (pH in 1M KCl)	5.83	5.65	5.96	5.85	6.65	7.06
N mineral (kg ha^{-1})[1]	14.9	12.6	14.8	14.3	21.3	21.7
Phosphorus (mg P kg^{-1})[2,,3,4]	144 M	60.8 L	131 M	50.3 L	181.7 H	92.8 L
Potassium (mg K kg^{-1})[2,3,4]	90.1 L	70.8 L	89.5 L	77.6 L	78.5 L	59.6 L
Calcium (mg Ca kg^{-1}) [2,5]	1082 L	920 VL	1012 L	903 VL	991 VL	611 VL
Magnesium (mg Mg kg^{-1})[2,3,4]	63.1 M	94.0 H	70.6 H	60.2 M	85.5 H	89.5 H
Sulfate sulfur[6] $S\text{-}SO_4$ (mg S kg^{-1})	1.2 L	1.0 L	0.9 L	0.5 L	1.6 L	1.6 L

[1] 0.01M $CaCl_2$ (Łukowiak et al. 2017); [2] (Mehlich 3 1984); [3] availability classes: VL – very low, L – low, M – medium, H – high, [4,5] (Kęsik 2016, Trávník 1999); [6] (Zbíral 2018)

determined in the 0.0-0.6 m layer was low, below 30 kg N ha^{-1}, in the first two seasons. In the third season, it slightly exceeded 30 kg N ha^{-1}.

Weather conditions

Weather conditions were highly variable in the consecutive growing seasons (Tables 2, 3). The beginning of spring vegetation favored the maize growth (April, May and June). Air temperatures, especially in 2018, were exceptionally high compared to the long-term averages. The course of precipitation during the early stages of maize growth was highly variable in the subsequent seasons. In 2017, low precipitation were noted in May and July. In other seasons, a shortage of precipitation also occurred during this period. The positive trend of temperatures was maintained in July and August, which are critical months for the formation of the yield components by maize. As mentioned above, the highest temperatures were measured in 2018. In July, the shortage of precipitation was revealed in the second and third season. August was wet in 2017 and dry in the other two seasons. Temperatures in September, with the exception of 2018, were within the range of long-term averages.

Experiment design

The field experiment with maize fertilized with cattle slurry enriched with sulfur was set up as a two-factor completely randomized blocks design, replicated 4-fold. The experimental factors were:

Table 2

Meteorological conditions during the study; monthly mean temperature in the maize growing season (°C)

Growing season	Subsequent months							
	April	May	June	July	August	Septem-ber	October	mean
2017	8.1 (+0.60)#	14.3 (+0.5)	18.4 (+1.7)	18.8 (+0.4)	19.7 (+1.6)	13.8 (-1.0)	11.1 (+1.2)	14.9 (+0.7)
2018	13.6 (+6.1)	17.0 (+3.2)	18.9 (+2.2)	20.7 (+2.3)	21.7 (+3.6)	16.5 (+1.7)	11.3 (+1.4)	17.1 (+2.9)
2019	10.5 (+3.0)	12.3 (-1.5)	22.5 (+5.8)	19.6 (+1.2)	20.9 (+2.8)	14.7 (-0.1)	11.7 (+1.8)	16.0 (+1.8)
Long-term mean 1957-2018	7.5	13.8	16.7	18.4	18.1	14.8	9.9	14.2

Source: Meteorological Station Szelejewo Drugie; # (+0.60) – deviation from the long-term average

Table 3

Meteorological conditions during the study; monthly mean precipitation in the maize growing season (mm)

Growing season	Month							Total
	April	May	June	July	August	September	October	
2017	50 (+16)#	38 (-20)	44 (-21)	80 (0)	74 (+8)	53 (+11)	68 (+27)	407 (+21)
2018	17 (-17)	24 (-34)	36 (-28)	80 (0)	15 (-65)	41 (-2)	35 (-6)	248 (-138)
2019	12 (-22)	68 (+9)	7 (-58)	46 (-34)	28 (-37)	66 (+23)	31 (-10)	258 (-128)
Long-term mean 1957-2018	34	58	65	80	65	43	41	386

Source: Meteorological Station Szelejewo Drugie; # (+16) – deviation from the long-term average

1. Two inorganic carriers of sulfur:
   a) elemental sulfur (acronym; S^0),
   b) calcium sulfate dihydrate (acronym: Ca-S).
2. Sulfur doses, kg S ha^{-1} (acronym SD):
   a) 0 – raw slurry (without S), 22.5, 45, 90 kg S ha^{-1}.

Elemental sulfur, ground to a fine powder, contained 99.9% pure S. Calcium sulfate dehydrate ($CaSO_4 \cdot 2H_2O$) with a crystalline structure contained 17.0% of S and 21.3 of Ca (own data). Both mineral supplements were added and mixed with raw cattle slurry and then incubated/fermented for 21 days in 1000 liter containers filled to 800 liters. After the incubation period, slurry was applied to the plots and immediately mixed with the soil. The total dose of applied N was 133 kg ha^{-1}. Before setting up the experiment, 150 kg K_2O ha^{-1} in the form of potassium chloride (60% K_2O) and 80 kg P_2O_5 ha^{-1} in the form of triple superphosphate (46% P_2O_5) were applied to the entire field.

Triticale was both the preceding crop and at the same time a catch crop for maize. It was plowed in spring before the experiment was set up. The maize variety ES Hubble was used. It is an early variety (FAO 230), two-line hybrid, flint type. The sowing density was 90,000 plants ha^{-1}. Maize was sown 14 days after slurry application. Sowing dates in individual seasons were as follows: 8.04.2017, 16.04.2018, and 8.04.2019. A single plot was 30 m^2 in total, while the harvested area covered 15 m^2. Plant protection was carried out according to the code of good practice. Harvest dates in individual seasons were as follows: 04.10.2017, 28.09.2018, and 25.09.2019. The plants were harvested at the stage of physiological maturity of grains (BBCH 89).

Plant material sampling and analysis

The plant material used for dry matter and N content determination was collected at BBCH 15 (five leaf stage), BBCH 60 (the beginning of flowering), and BBCH 89 (physiological maturity) from an area of 2.0 m^2. The sampled material was then divided, depending on the maize stage of growth, into subsamples of leaves (LE), stems (ST), cob core leaves (CL), cob core (CC), and grain (G). The standard macro-Kjeldahl procedure was used to determine the N content in the plant parts, and is presented in % of dry weight.

Indices of nitrogen management during the grain filling period

The following set of equations describing nitrogen management during the grain filling phase of maize growth were employed (Grzebisz et al. 2021):

1. Nitrogen Remobilization Quota (NRQ), kg N ha^{-1}:

$$NRQ = Na60 - Nav89.$$

2. Nitrogen uptake by maize during the grain filling period (GFPN), kg N ha^{-1}:

$$GFP\text{-}N = Na89 - Nc60.$$

3. Nitrogen Harvest Index (NHI):

$$NHI = GN/Na89 \cdot 100\%.$$

4. Contribution of remobilized N in grain N (CRN-G), %:

$$CRN\text{-}G = NRQ/GN \times 100\%.$$

5. Contribution of N uptake during the GFP in grain N (CGFPN-G), %:

$$CGFPN\text{-}G = GFPN/GN \cdot 100\%$$

where: Na60 – total N accumulation in maize at BBCH 60, kg N ha^{-1};
Na89 – total N accumulation in maize at BBCH 89, kg N ha^{-1};
Nav89 – total N accumulation in vegetative parts of maize at BBCH 89, kg N ha^{-1};
GN – nitrogen accumulation in grain at BBCH 89, kg N ha^{-1}.

Statistical analysis

The influence of the experimental factors on the tested crop's characteristics was assessed by analysis of variance for completely randomized blocks design. When the F-test showed significant factor effects at $p<0.05$, means were separated by honest significant difference (HSD) applying the Tukey method. In order to analyze the relationships between the examined characteristic, the Pearson correlation and linear regression were used. The optimum set of variables for a specific plant trait was determined using stepwise regression analysis. All of the statistical analyses were carried out using Statistica 12 software (StatSoft Inc., Tulsa, OK, USA, 2013).

RESULTS AND DISCUSSION

Grain yield

The key factor affecting yields of maize was the weather (Figure 1, Table 4). The lowest grain yield (GY) was recorded in 2017 (80% of that in 2019 = 100%). The impact of the type of S carrier on grain yield was revealed in 2017 and 2018, but it turned out to be significant and in favor of S^0 only in 2018. The effect of S doses was visible in every year, but showed variable trends (Figure 1). In 2017, the grain yield on the control S was 7.60 Mg DW ha^{-1}. The influence of S was observed only on the plot where 45 kg S ha^{-1} was applied and amounted to 8.35 Mg DW ha^{-1} (+10%). In 2018, GY on control S was the same as on the plot which was fertilized with 22.5 kg S ha^{-1} and amounted to 10.2 Mg DW ha^{-1}. In 2019, GY on control S was 8.61 Mg

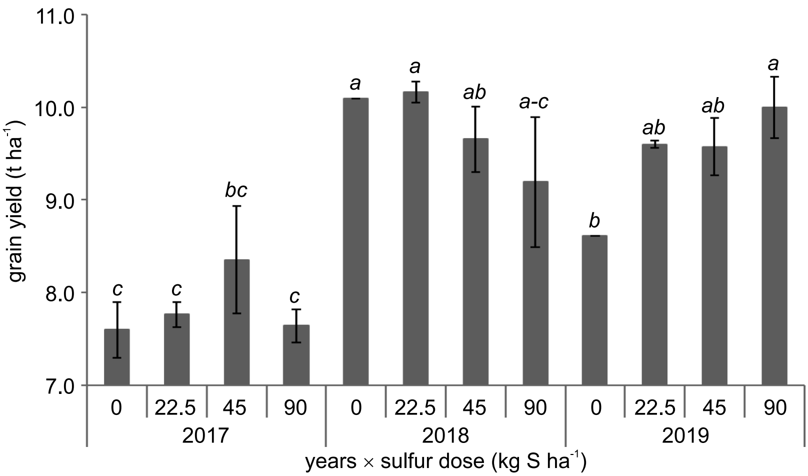

Fig. 1. Grain yield of maize in response to the interaction of the years and sulfur dose. Labelling with the same letters suggests that there are no big differences between experimental treatments according to the Tukey's test. The standard error of the mean is expressed by the vertical bar in the column

DW ha^{-1}. The strongest and most significant increase due to S was recorded on the plot with 22.5 kg S ha^{-1} and amounted to +1.2 Mg ha^{-1} (+11.5%). The GY increased up to the S dose of 90 kg ha^{-1}, amounting to 10.0 Mg DW ha^{-1} (+16.1%).

Nitrogen accumulation in critical stages of grain yield formation

The amount of N accumulated in maize at the 5th leaf stage (Na15) in each year was significantly dependent on the experimental factors and interactions between them (Table 4). However, no significant relationship with GY was found (Table S1). In 2019, the year with the highest GY, Na15 was significantly lower than in the other years. The N accumulation in maize at the onset of flowering (Na60) was the largest in 2018, and the lowest in 2019. However, only in 2019 was there a significant impact of the S carrier on the N accumulation in the crop (+15.1% in favor of S^0) – Table S2. The variability of Na60 in the successive years of the study is explained by the Y × SD interaction (Figure 2). In 2017, the N accumulation in maize rose

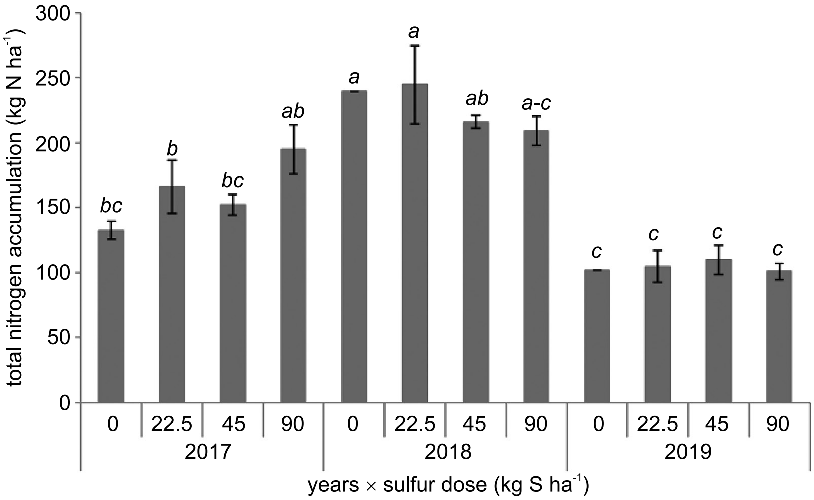

Fig. 2. Nitrogen accumulation in maize at the onset of flowering in response to the interaction of the years and sulfur dose. Labelling with the same letters suggests that there are no big differences between experimental treatments according to the Tukey's test. The standard error of the mean is expressed by the vertical bar in the column

along with the increasing doses of S applied. The application of 90 kg S ha^{-1} resulted in an increase in N uptake by 46.6% (195 vs. 133 kg N ha^{-1}). In 2018, the highest N uptake was recorded on the plot fertilized with 45 kg S ha^{-1}. However, this was a negligible increase compared to the S control. The higher S doses resulted in a considerable decline in N uptake. In 2019, the N accumulation in maize was the lowest, and no major impact of the applied S was registered. The trends for N60L were almost the same

Table 4

Accumulation of nitrogen in maize in critical stages of grain yield formation, kg N ha^{-1}

Factor	Level of factor	Na15	N60L	Na60	N89S	N89L	N89CL	N89C	GN	Na89	NHI, (%)	GY Mg (ha^{-1})
Year (Y)	2017	23.3^{a}	10.2^{a}	161^{b}	33.4^{a}	39.0^{a}	8.92^{a}	13.2^{a}	109^{b}	204^{a}	53.5^{c}	7.84^{b}
	2018	28.3^{a}	11.2^{a}	227^{a}	9.0^{c}	6.41^{c}	3.81^{c}	12.4^{ab}	130^{a}	161^{b}	80.4^{a}	9.44^{a}
	2019	19.6^{b}	6.1^{b}	104^{c}	15.7^{b}	10.0^{b}	5.13^{b}	12.2^{b}	83^{c}	126^{c}	65.9^{b}	9.78^{ab}
F_c, p		135^{***}	135^{***}	159^{***}	91.4^{***}	629^{***}	941^{***}	98.6^{***}	3.29^{*}	104^{***}	256^{***}	481^{***}
Sulfur fertilizer (S)	S-S^{0}	25.0	9.3	165	19.6	18.7	6.42^{b}	12.8	111^{b}	169^{b}	65.9	9.17
	S-Ca	25.2	9.0	163	19.2	18.2	5.51^{a}	12.4	104^{a}	159^{a}	65.3	8.87
F_c, p		0.11	1.65	0.07	0.59	0.50	8.47^{**}	1.51	8.06^{**}	12.0^{**}	0.56	3.24
Sulfur dose (SD) (kg S ha^{-1})	0.0	25.0	9.2	158	19.7	19.4	5.82	12.6	105	163	65.6	8.78
	22.5	25.1	9.4	171	19.6	18.1	6.42	13.0	110	167	65.9	9.17
	45.0	25.1	9.2	159	20.0	18.3	5.73	12.3	110	166	66.2	9.19
	90.0	25.2	9.0	168	18.1	18.1	5.91	12.7	104	159	65.7	8.94
F_c, p		0.05	0.58	0.83	2.09	0.82	0.73	0.87	1.09	1.44	0.99	1.54
Source of variation of the studied interactions												
Y × S		***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Y × D		***	ns	*	ns	ns	ns	ns	**	**	ns	**
S × D		***	*	ns	**	***	ns	*	ns	ns	ns	ns
Y × S × D		***	ns	ns	***	***	ns	ns	ns	ns	ns	ns

Mean values within a column followed by the same letter indicate no significant difference between the treatments; ns – non-significant at $P \leq 0.05$; *, **, *** – significant at $P \leq 0.05$, 0.01, 0.001, respectively. Key: 15, 60, 89 – stages of maize growth, BBCH 15, 60 and 89, respectively; Na – total N bioaccumulation; S – stem; L – leaves; CL –maize cob cover leaves; CC – cob core; G – grain; NHI – nitrogen harvest index as those reported for Na60 (r = 0.88, $p \leq 0.001$). It should be clearly emphasized that there was no significant association between Na60 or N60L with GY (Table S1).

The total N accumulation in maize at the stage of physiological maturity (Na89) was highly variable in subsequent years of the research (Table 4). The influence of S carriers on Na89 was observed in each year of the study, being significant in favor of S^0 in 2017 and 2019 (2017: +7.2%; 2018: +2.6%; 2019: +9.7%) (Table S2). The variability of Na89 in years significantly depended on the Y × SD interaction (Table 4). The greatest accumulation of N was recorded in 2017, the year with the lowest yield (Figure 3). In this

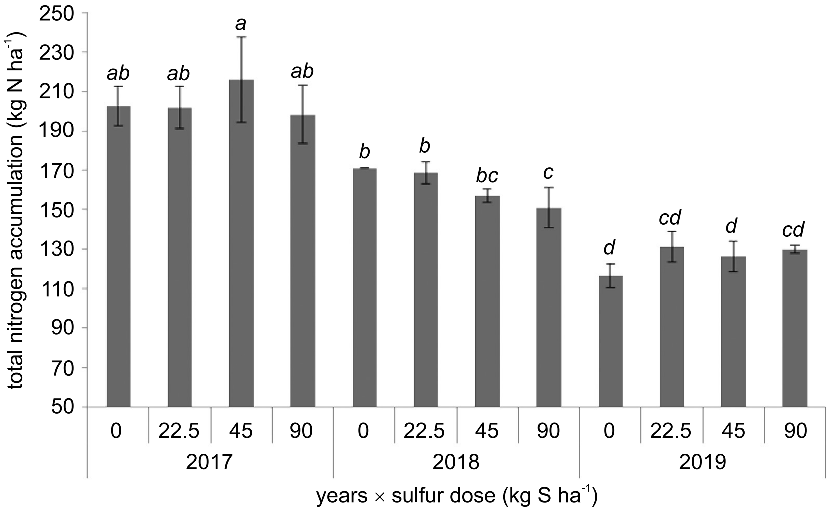

Fig. 3. Total nitrogen accumulation in maize at physiological maturity in response to the interaction between the years and sulfur dose. Labelling with the same letters suggests that there are no big differences between experimental treatments according to the Tukey's test. The standard error of the mean is expressed by the vertical bar in the column

particular year, Na89 on control S reached 202 kg N ha^{-1}. The application of 45 kg S ha^{-1} resulted in a significant, but slight increase in Na89 (+14 kg N ha^{-1}). In 2018, the N accumulation on control S was much lower compared to 2017, amounting to 161 kg N ha^{-1}. At the same time, it was reduced in response to increasing S doses above 22.5 kg S ha^{-1}. The lowest Na89 was noted in 2019, when it reached only 117 kg N ha^{-1}, i.e. 58% of that in 2017. Application of 22.5 kg S ha^{-1} raised the N accumulation by 12% (+14 kg N ha^{-1}). The relationship between Na89 and GY was bot negative and low, indirectly highlighting the decrease in GY in response to the increased amount of N in maize biomass at harvest (r = -0.50; Table S1). The strongest association with GY, among the studied maize parts, was obtained with almost the same probability for N in leaves (N89L), stems (N89S), and cob leaves (N89CL) (r = -0.82, -0.81, -0.77, respectively). The stepwise regression analysis clearly indicated N89Las the key GY predictor:

$$GY=10.01-0.055N89L \text{ for } n=24,\ R^2=0.68 \text{ and } p\leq 0.001 \quad (1)$$

The regression model obtained clearly shows that any increase in the N accumulation in leaves at the BBCH 89 stage of maize growth resulted in a reduction in GY. This maize trait was the lowest in 2018, reaching on average of 3.8 kg N ha^{-1}. In 2017, it was higher by 134%, and by 35% in 2019. In each year, higher N89L values were recorded for S^0, but a proven increase occurred only in 2019 (Table S2). N89L was strongly correlated with N89S (r = 0.98). At the same time, it was negatively associated with the nitrogen harvest index (NHI, r = -0.88). In contrast, NHI was strongly and positively correlated with GY (r = 0.83):

$$GY = 0.073NHI + 4.14 \text{ for } n=24,\ R^2=0.69 \text{ for } p\leq 0.001 \quad (2)$$

This regression model also demonstrates that any rise in the NHI results in the simultaneous GY increase. However, NHI showed only a significant response to the course of weather in subsequent growing seasons. The highest value of the index was recorded in 2018, when it exceeded 80%. In the other years, it was much lower (Table 4).

The variability of N accumulation in maize grain (GN) was significantly affected, similarly to GY, by the Y × SD interaction (Table 4). The greatest GN values were recorded in 2018 and the lowest, lower by $^1/_3$, in 2019. GN was significantly and positively correlated with Na15, N60L, Na60, but not with GY and N accumulation in maize vegetative parts at maturity (Table S1).

The main reason for the inefficiency of Na60 in the first two growing seasons was not the 'luxury N uptake' by maize, but the low capacity of the maize physiological sink, i.e. grain density (Menezes et al., 2017, Zawieja 2021). This type of crop demand for N during GFP is in agreement with the Körner's sink/source theory, pointing to the sink capacity of the seed crop as the yield driver (Körner, 2015). The key reason for the disruption of the yield formation process in 2017 can only be an abundant supply of N, resulting in excessive growth of the stem at the expense of the cob (Szczepaniak, 2016). In 2018, unfavorable conditions for the conversion of N into grain yield resulted from extremely high temperatures. The maximum temperatures, exceeding 30^o C, lasted for 13 days at the turn of July and August (Table 2). The theoretical yield loss of 43% (20.4 vs. 11.7 t ha^{-1}) resulted from the fact that the stress occurred at the beginning of the grain growth, a period which is considered as the most critical phase of maize yield formation (Sah et al. 2020). Such weather conditions may lead to a complete inhibition of photosynthesis, and the only source of assimilates is the remobilization of dry matter from the vegetative parts of the plant.

Significant relationships between GY and the amount of N in individual parts of maize were first noted at physiological maturity. The accumulation of N accumulated in all vegetative organs of maize was significantly, but at the same time negatively, associated with GY. The lack of a significant relationship between these N traits with GY indicates a serious disturbance in the formation of the yield in the grain filling period (GFP) of maize

(Ciampitti, Vyn 2013). This type of dependence indicates that the N accumulated in the vegetative parts of maize was not effectively converted into GY during the GFP. This conclusion is indirectly supported by a significant and at the same time positive relationship between NHI and GY. It is well known that NHI is a conservative trait of seed plants (Asibi et al. 2019). Hence, the dependence obtained indirectly informs that the key factor, impacting negatively the accumulation of N in grains, was the low sink capacity of maize (Körner 2015). In the presented case, it refers to the grains number per plant or unit area (i.e. grain density) – Zawieja (2021).

Indices of nitrogen management by maize during the grain filling period (GFP)

Five indices describing the economy of N in maize during the GFP were analyzed (Table 5). All of them were significantly associated with GY (Table S1). The most definite and positive relationship with Na60 was noted

Table 5

Indices of nitrogen balance/economy by maize during the grain filling period (GFP)

Factor	Level of factor	Nav89	NRQ	CRN-G	GFP-N	CGFPN-G
		(kg ha^{-1})		(%)	(kg ha^{-1})	(%)
Year (Y)	2017	94.6^{a}	66.9^{b}	62.1^{b}	43.0^{a}	37.9^{a}
	2018	31.6^{c}	195^{a}	149.3^{a}	-65.5^{b}	-50.7^{b}
	2019	43.0^{b}	61.4^{c}	74.6^{b}	21.7^{a}	25.4^{a}
F_c, p		1226***	134***	51.5***	68.5***	65.7***
Sulfur fertilizer (S) (kg ha^{-1})	S-S^0	57.5	107	93.9	3.61	6.12
	S-Ca	55.3	108	97.7	-4.22	2.32
F_c, p		0.84	0.02	0.27	0.73	0.27
Sulfur dose (SD) (kg ha^{-1})	0	57.4	100	87.2	5.22	12.8
	22.5	57.1	114	98.0	-4.73	2.01
	45	56.3	103	92.8	7.03	7.20
	90	54.8	113	105.2	-8.82	-5.22
F_c, p		1.43	1.08	1.27	1.00	1.27
Source of variation of the studied interactions						
Y × S		ns	ns	ns	ns	ns
Y × SD		ns	*	*	*	*
S × SD		***	ns	ns	ns	ns
Y × S × SD		ns	ns	ns	ns	ns

Mean values within a column followed by the same letter indicate no significant difference between the treatments; *, **, *** significant at $P \le 0.05$, 0.01, 0.001, respectively; ns – non significant. Legend: Nav89 – N accumulation in vegetative parts of maize at BBCH 89; NRQ – Nitrogen remobilization quota; CRN-G - contribution of CGFPN-G – contribution of nitrogen uptake during GFP in total N accumulated in grain for the Nitrogen Remobilization Quota (NRQ, r = 0.91). This simply means that the NRQ value was in 83% determined by the N accumulation in maize at the onset of flowering. However, its impact on GY was positive but low, explaining only 28% of GY variability. The impact of the S carrier on the NRQ was revealed the most in 2018 and 2019, but the effect was opposite. In 2018, Ca-S had a greater but insignificant impact on this maize trait (+5.8%). In 2019, S^0 showed a positive and significant effect (+16%) – Table S3. The variability of the NRQ in the subsequent years of study was significantly driven by the Y × SD interaction (Figure 4). In 2017, the NRQ increa-

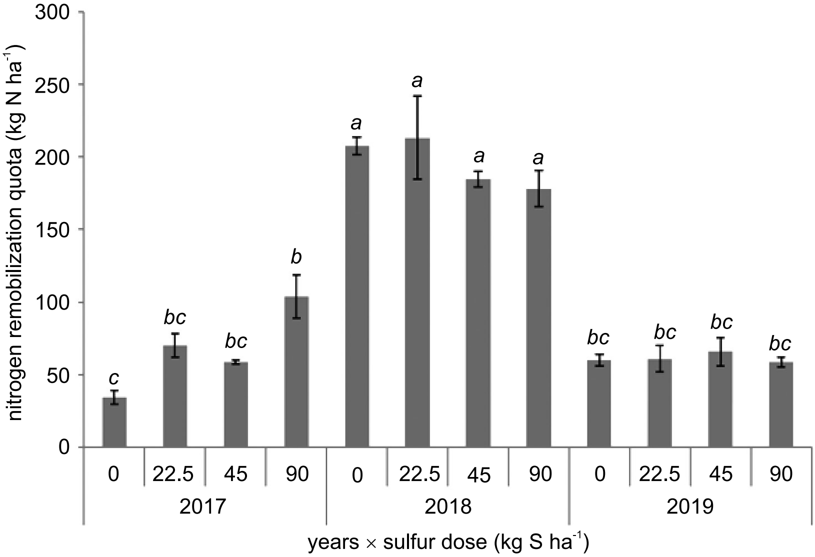

Fig. 4. The response of the Nitrogen Remobilization Quota to the interaction of the years and sulfur dose. Labelling with the same letters suggests that there are no big differences between experimental treatments according to the Tukey's test. The standard error of the mean is expressed by the vertical bar in the column

sed 3-fold, from 34 kg N ha^{-1} on control S to 104 kg N ha^{-1} on the plot applied with 90 kg S ha^{-1}. In 2018, the NRQ on the control S plot was 207 kg S ha^{-1}, stabilizing on the plot with 22.5 kg S ha^{-1}, and then gradually decreasing with increasing doses of S. In 2019, the index values were stable against doses of applied S. The NRQ was also positively and strongly associated with NHI, GN and N60L, and at the same time negatively with the N in the vegetative parts of maize at physiological maturity. The positive relationship of the NRQ with NHI highlights the significance of N remobilization on its contribution into grain. This conclusion is supported by the positive relationship between NHI and GY. It is well known that NHI is a conservative trait of seed plants (Asibi et al. 2019). The dependence obtained indirectly informs that the key factor, impacting negatively the accumulation of N in grains, was the low sink capacity of maize. It thus confirms the conclusions drown by Zhang et al. (2022) resulting from the use of excessive N doses.

The contribution of N from the vegetative parts of maize, called the N reutilization coefficient, ranges from 45% to 65%. The remaining N is taken up by the plant from the soil after the flowering of maize (Nasielski et al. 2019). The developed GFP-N index characterizes just the amount of N taken up by maize during the GFP from the soil (Table 5). This index was moderately but negatively correlated with GY:

$$GY = -0.011GFPN + 9.02 \text{ for } n=24,\ R^2=0.32 \text{ for } p\leq0.05 \qquad (3)$$

The regression model obtained clearly shows that the net soil uptake of N by maize during GFP resulted in a decrease in GY. This apparent contradiction results from the fact that the highest grain yield was obtained in 2018. In addition, GFP-N was negatively correlated with both Na60 and NHI, but positively with Ncv89 (Table S1). In fact, the actual N uptake by maize during the GFP took place only in the 2017 and 2019 seasons (Figure 5). In the first season, the uptake of N was high but varied, depen-

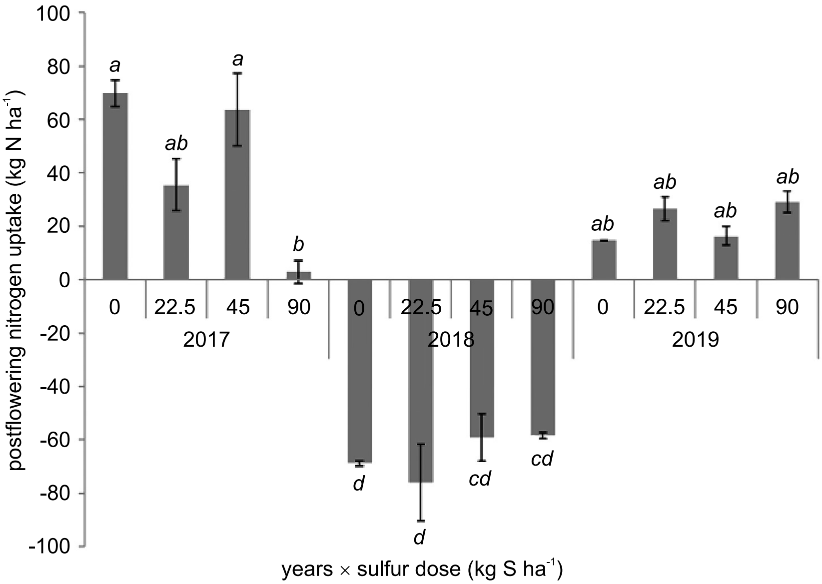

Fig. 5. The response of nitrogen post-flowering uptake to the interaction of the years and sulfur dose. Labelling with the same letters suggests that there are no big differences between experimental treatments according to the Tukey's test. The standard error of the mean is expressed by the vertical bar in the column

ding on the dose of S. On the control S plot, it reached 70 kg N ha^{-1}, while on the plot supplied with 90 kg S ha^{-1} it fell to just 3.0 kg N ha^{-1}. In 2019, a slightly opposite trend was observed, despite a 2-fold lower N uptake. On the control S plots, GFP-N amounted to 15 kg N ha^{-1} and doubled at 90 kg S ha^{-1}.

The second group of indices describing the post-flowering N balance concerns the share of remobilized N (CRN-G) and post-flowering N uptake

(CGFPN-G) in grain N. The CRN-G showed almost the same level of GY predictability as the NRQ (Table S1). At the same time, it was negatively correlated with Ncv89. The CRN-G index responded significantly to the Y × SD interaction (Figure S1). In 2017, the index values increased with the increase in S doses, reaching 100% on the plot fertilized with 90 kg S ha^{-1}. The most spectacular result was noted in 2018, in which all index values, regardless of the S dose, reached about 150%. In 2019, CRN-G indices, despite a 15-percentage range (66.5 → 81.2%) fluctuated around 75%. The second index, i.e. CGFPN-G, was also significantly affected by the Y × SD interaction (Figure S2). The post-flowering N uptake contributed to the grain N grain in the 2017 and 2019 seasons. In 2017, the highest CGFPN-G value of almost 68% was recorded for control S, while not exceeding 1% on the plot fertilized with 90 kg S ha^{-1}. In 2019, CGFPN-G increased from about 20% on the control S plot to 34% on the plot fertilized with 90 kg S ha^{-1}.

The relationships discussed above are fully reflected in the structure of N sources to the developing grains in the subsequent years of study (Figure 6). The observed regularity indicates that in a situation of low N

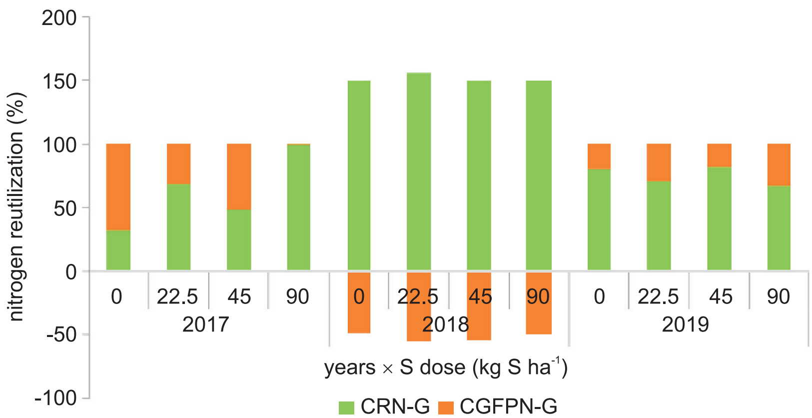

Fig. 6. Structure of nitrogen resources contribution into grain nitrogen during the grain filling period. Key: CRN-G – contribution of remobilized N into grain N; CGFPN-G – contribution of nitrogen uptake during GFP in total N accumulated in grain

resources in maize at the beginning of flowering, the grain yield is determined by the potential of the plant to uptake N from the soil (Figure 6). In 2017 and 2019, an important factor supporting the photosynthetic activity of maize during GFP was post-flowering N uptake. The type of N utilization by maize plants during GFP observed in both years is considered as typical for stay-green varieties (Ciampitti and Vyn 2013).

In this particular case, the role of S as a factor supporting the action of N remains to be clarified. Cooper and Hogg (1965) found that S^0 used as fertilizer needs at least six months to be completely converted to sulfate.

The effect of S added to cattle slurry, as indicated by the tested N indices, was mainly revealed during GFP. It is assumed that the N demand of the growing kernels under optimal moisture conditions is covered either by N resources in the plant or by its uptake from the soil (Gallais et al. 2007). These conditions prevailed in 2017, but the efficiency of remobilized N was extremely low (35%), despite the high N accumulation in the crop at the onset of flowering. Poor N remobilization was the main driver of the post-flowering N uptake. Moreover, it showed a specific response of the plant to the amount of applied S. The post-flowering soil N uptake ranged from 70 kg ha^{-1} on the S control to only 3 kg ha^{-1} on the plot fertilized with 90 kg S ha^{-1}. The discrepancy resulted from the fact that at the onset of flowering, the N accumulation in maize on control S achieved 133 kg N ha^{-1}, while reaching 194 kg N ha^{-1} on the plot supplied with 90 kg S ha^{-1}. The effect of the S dose observed in this experiment indicates a positive impact of the S supply on N uptake and accumulation during the vegetative growth of maize, but not on its post-flowering uptake. The increased remobilization of N from well-nourished S plants indicates its significant role in these processes (Carciochi et al. 2020). On the other hand, the results suggest that the post-flowering N uptake by maize was not dependent on its N nutritional status. The main factor inducing N uptake during GFP was grain density, as indirectly indicated by the positive association of NHI with GY.

The main cause for such remarkably high yields in 2019 was the extremely good productivity of N accumulated by the crop. This index on control S reached 85 kg grain kg^{-1} N in 2017 and 2018, but was by 60% higher in 2019, reaching 139 kg of grain kg^{-1} N. The results obtained in 2019 clearly indicate that maize is a crop with a high production potential, able to effectively use both plant and soil resources during GFP. The share of remobilized N in grain N showed a definite trend. It decreased from 80% on the S control to 66% on the plot with 90 kg S ha^{-1}. Grain yields on both plots were the same, which clearly confirms that the factor determining the yield of maize was the number of kernels per area unit. The N demand of the growing grains for N was partly covered by its uptake from the soil. This type of maize response during GFP to N is typical for stay-green varieties (Gallais et al. 2007).

CONCLUSIONS

The effect of S addition to cattle slurry, even if it affected the N uptake by maize plants in the pre-flowering growth, exerted no impact on grain yield. The yield-forming effect of S, regardless of the form applied (S carrier), resulted from the impact on the N economy during the grain filling period. Thus, a strong conclusion emerges that the yield-forming function of S in maize well-fed with N is not directly related to the plant N nutritional status.

The effect of S was manifested in two out of the three years of the study, with contrasting weather conditions, which must be emphasized. This study manifested that the influence of S on the grain yield of maize can be considered through its impact on N partitioning between the grain and vegetative parts of maize during the period of grain filling. The surge in grain yield in response to added S showed two opposite patterns. The first resulted from impaired N management, caused by excessive N accumulation by plants before flowering. In 2017, weather conditions were optimal for maize yield, but the yields were the lowest. The N resources accumulated by the crop at the beginning of flowering were not efficiently converted into grain yield, as shown by NHI of 52%. The action of S in the presence of a disturbed N partitioning process may focus on the increased remobilization of N from vegetative plant parts and the accompanying uptake from the soil. Quite a different pattern of maize yield's dependence on N balance during GFP emerged in 2019, a year with a prolonged drought. The addition of sulfur significantly affected the N utilization efficiency manifested by an increase of both NHI and unit nitrogen productivity.

The study distinctly indicates that the application of cattle slurry enriched with inorganic carriers of sulfur is an agronomic solution able to increase yields of maize, especially when the N productivity is disturbed. Therefore, this way of using slurry should be seen as an anti-stress preventive measure.

Author contributions

K.P-C.: conceptualization, writing of original draft. A.Z-R. and K.P-C: methodology, investigation. A.Z-G. and K.P-C.: data curation. A.Z and K.P-C: writing – review& editing. A.Z-R.: formal analysis. All authors have read and agreed to the submitted version of the manuscript.

ACKNOWLEDGMENTS

I thank my Parents for their help and funding part of the field research. Agnieszka Zawieja-Roszak.

Conflicts of interest

The authors declare no conflict of interest.

Supplement

This manuscript comes with supplementary material available on: https://jsite.uwm.edu.pl/issue/view/2-2024/

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