



OPEN

Increasing silage maize yield and nitrogen use efficiency as a result of combined rabbit manure and mineral nitrogen fertilization

Andrzej Wysokinski[✉] & Monika Kożuchowska

Combined application of organic and mineral fertilizers is crucial to obtaining high crop yields, increasing the utilization of nutrients by plants, and limiting their dispersion, thus protecting the environment, which underscores the importance of sustainable and minimally invasive agriculture. The aim of the field experiment was to determine the effect of application of rabbit manure (RM) and mineral nitrogen (N_{\min}) on the dry matter (DM) yield of maize and on nitrogen content, uptake, and use efficiency (NUE). RM application was tested at levels of 0, 20, 40 and 60 t·ha⁻¹, and N_{\min} application at 0, 50, 100 and 150 kg·ha⁻¹. Significant differences were noted in yield and in the content and uptake of nitrogen depending on both experimental factors. Increasing the application of RM and N_{\min} led to an increase in the yield of harvested maize and in the content and uptake of nitrogen. In terms of DM yield and nitrogen uptake (yield of crude protein), the most beneficial fertilizer variant was 60 t·ha⁻¹ RM applied together with 100 kg·ha⁻¹ N_{\min} . The highest NUE value was obtained following application of 20 t·ha⁻¹ RM together with 150 kg·ha⁻¹ N_{\min} .

To meet the high and continually growing demand for food, agriculture is required to continually increase its production power. High crop yields can be obtained by providing them with access to a large amount of nutrients. This has led to the appearance of areas that have been excessively fertilized with mineral fertilizers, resulting in environmental pollution from agricultural sources. Therefore, there is a need for more efficient plant utilization of fertilizer nutrients in order to protect the environment and at the same time ensure food security^{1,2}. The 4R principle should be applied: (1) the right nutrient source, (2) the right application rate, (3) the right application time, and (4) the right place of application. This will ensure adequate utilization of soil-applied nutrients by optimizing productivity.

The use of organic fertilizers for crops reduces the amount of nutrients introduced to the soil with inorganic fertilizers. Closing the nutrient cycle on a farm is important for protection of the natural environment and also can reduce fertilizer costs.

Manure contains macro and micronutrients for plants, and land application in crop production recycles those manure nutrients. The use of nutrients in animal manure can to a large extent (for example 37.3% N, 87.6% P₂O₅ and 65.9% K₂O in China) replace chemical fertilizers³.

Some countries (France, Spain, China, and Argentina) have large rabbit (*Oryctolagus cuniculus*) breeding industries^{4,5}. In some parts of Poland the development of large-scale rabbit farming is observed as well. According to the Statistical Yearbook of Agriculture⁶, between 2010 and 2020 the number of rabbits sent to industrial slaughter in Poland increased from 290,000 to 370,000. One fattening unit rabbit produces on average 10–12 kg of manure in one cycle (about 150 g per day as the sum of faeces and urine). The population of 370,000 slaughter rabbits will therefore produce approximately 4000 tons of manure, which must be managed in an environmentally-safe manner. Rabbit manure is a fertilizer rich in organic matter and nutrients for plants and has a low or medium C/N ratio^{7–10}. The average efficiency of ingested nitrogen in rabbit fattening is about 40% and drops sharply with age¹¹. Thus about 60% of ingested nitrogen ends up in faeces. Calvet et al.¹¹ report that urine and faeces contributed to overall nitrogen excretion in approximately the same proportions.

University of Siedlce, Faculty of Agricultural Sciences, Konarskiego 2 Str., 08110 Siedlce, Poland. ✉email: andrzej.wysokinski@uws.edu.pl

Rabbit manure can be used directly to fertilize crops^{10,12,13}, as an ingredient in plant growth substrates^{14,15}, as an adsorbent¹⁶, for biochar production^{17,18}, and even in anaerobic fermentation to produce methane¹⁹.

Organic fertilizers are known to improve the physical, chemical, and biological properties of soils. These fertilizers, including rabbit manure, increase the content of organic matter¹⁵, pH, cation exchange capacity²⁰, organic carbon content in soil, total fungi, and bacteria²¹. The use of manure as fertilizer increases the yield and quality of crops, e.g. the content of protein and minerals^{15,21,22}.

Rabbit manure used in combination with chemical fertilizers has a particularly beneficial effect on crop yield and quality^{10,22,23}. Combined application of nitrogen from RM (organic) and from mineral/inorganic nitrogen (from chemical fertilizers) increases the efficiency of nitrogen utilization from these fertilizers. In a pot experiment, combined application of rabbit manure and mineral nitrogen compared to the only nitrogen mineral treatment was shown to reduce the percentage of nitrogen lost from sandy and clayey soils while increasing the amount of mineral nitrogen and nitrogen present in the form of microbial biomass²⁴. According to Wu and coauthors²⁴, the data cannot be extrapolated to field conditions, and future research should verify the results in field conditions.

In Poland, in the years preceding the experiment, the production of silage maize systematically increased: 2010 year—395,000 ha; 2013 year—462,000 ha; 2015 year—555,000 ha; 2017 year—596,000 ha⁶. Including manures in the fertilization of silage maize can have a major impact on the success of its cultivation, affecting not only the yield, but also reducing the costs of cultivation.

The aim of the field experiment was to determine the effect of rabbit manure and supplementary application of nitrogen on the yield of silage maize, the content and uptake of nitrogen, and nitrogen use efficiency by this plant.

Methods

Experimental design

The field experiment was carried out in the years 2018–2019 at the Experimental Agricultural Station of the University of Siedlce (GPS, N: 52° 03′ 42.01″, E:22° 33′ 09.01″, 168 m above sea level, eastern Poland). Before setting up the experiment, 10 soil cores were taken from a 24 × 60 m area, from a 0.30 m layer, from which one soil sample was made. The soil consisted of the following fractions: 2.0–0.05 mm = 72.45%; 0.05–0.02 mm = 15.86%; 0.02–0.002 mm = 10.11%; < 0.002 mm = 1.56%. The soil pH measured in KCl 1 mol·dm⁻¹ was 5.5. The content of total nitrogen (N_{tot}) and organic carbon (C_{org}) amounted 0.79 and 8.40 g·kg⁻¹, respectively. The content of plant available phosphorus (P_{av}), potassium (K_{av}) and magnesium (Mg_{av}) were 66, 87 and 53 mg·kg⁻¹ of soil, respectively. Rainfall and air temperature recordings were obtained from the University's own weather station (Table 1).

The experiment was two-factorial, set up in a randomized block design in triplicate. The first factor was varied application rates of rabbit manure (RM): 0, 20, 40 and 60 t·ha⁻¹. The second factor was varied application rates of mineral nitrogen (N_{min}): 0, 50, 100 and 150 kg·ha⁻¹. The area of each plot was 9 m² (3 × 3 m). Supplementary fertilization with phosphorus and potassium was used in the treatments to obtain a nitrogen:phosphorus:potassium ratio of no less than 1:0.35:1. In the treatment without nitrogen application, phosphorus and potassium were applied in amounts corresponding to the average application rate in all fertilizer treatments. Mineral nitrogen was applied in the form of ammonium nitrate (34.0% N), phosphorus in the form of triple superphosphate (19.4% P), and potassium in the form of potassium chloride (49.8% K). All fertilizers were incorporated to the soil once in the first year (2018), prior to the experiment, 10 days before sowing the test plant. The effect on the succeeding crop was examined the following year. No fertilizer was applied for crop in 2019, and the experiment took place on the exact same plot and plot layout in both years. In both growing seasons the test plant was the 'Tapas' cultivar of maize (*Zea mays* L.), harvested for silage. Seeds were sown in the last 10 days of April at the rate of 100,000 per hectare, and the above-ground parts were harvested at an average height of 20 cm in the first 10 days of September. Winter triticale (*Triticosecale Wittm. ex A. Camus*) was grown in the field as a forecrop for maize. Its grain and straw were harvested in the summer of 2017. Rabbit manure contained 28.4% dry matter, total carbon 370 g·kg⁻¹ DM, total nitrogen 20.6 g·kg⁻¹ DM, carbon/nitrogen ratio 18.0, N-NH₄⁺ 4.22 g·kg⁻¹

Month	Average monthly temperatures in experimental years, °C		Total monthly rainfall in experimental years, mm	
	2018	2019	2018	2019
April	13.1	9.8	34.5	5.9
May	17.0	13.3	27.3	59.8
June	18.3	17.9	31.5	35.9
July, VII	20.4	18.5	22.4	29.7
August	20.6	19.9	24.5	43.9
September	15.9	14.2	27.4	17.4
Average/sum April–September	17.6	15.6	167.6	192.6
Average/sum May–August	19.1	17.4	105.7	169.3

Table 1. Rainfall and air temperatures in 2018 and 2019 (weather station of the University in Siedlce).

DM, $N\text{-NO}_3^-$ 0.07 g·kg⁻¹ DM, total phosphorus 7.06 gP·kg⁻¹ DM, and total potassium 13.4 gK·kg⁻¹ DM. The total amounts of nitrogen introduced to the soil and the plant available nitrogen in the treatments are shown in Table 2. According to legal regulations in Poland²⁵, the total amount of nitrogen introduced to the soil with manure is converted to nitrogen available for plants (nitrogen which can actually be taken up by the plant). The amount of this form of nitrogen is calculated by multiplying the total amount of nitrogen applied by appropriate factors (multipliers), established for the next 2 years after manure application. For rabbit manure applied in spring they are 0.35 in the first year and 0.15 in the second year. Nitrogen from mineral fertilizers is assumed to be available for plants only in the first year after their application.

Crop samplings, measurements and calculations

Maize plants were harvested from entire plots and then weighed. Four representative plants were collected from each plot for laboratory analyses. They were first chopped into pieces 1–2 cm in length and dried to obtain a constant mass at 105 °C for determination of dry matter (DM), and then they were ground in laboratory mills. Nitrogen content was measured by a standard Kjeldahl method following wet mineralization in concentrated H₂SO₄²⁶.

Nitrogen uptake by maize (N_{up}):

$$N_{up} = Y \cdot N_c,$$

where Y is the dry mass of maize, N_c is the total nitrogen content (concentration) in maize's dry mass.

Nitrogen use efficiency from total nitrogen doses (NUE_{tot}):

$$NUE_{tot} = (N_{up_N} - N_{up_N0}) / N_{applied} \times 100\%,$$

where N_{up_N} is the nitrogen uptake by maize fertilized with nitrogen, N_{up_N0} is the nitrogen uptake by maize nonfertilized with nitrogen, $N_{applied}$ is the total amount of nitrogen applied into soil with RM and N_{min} .

Nitrogen use efficiency from total nitrogen doses as the sum from 2 years (NUE_{tot_2}):

$$NUE_{tot_2} = (N_{up_N_2} - N_{up_N0_2}) / N_{applied} \times 100\%,$$

where $N_{up_N_2}$ is the sum for 2 years nitrogen uptake by maize fertilized with nitrogen, $N_{up_N0_2}$ is the sum for 2 years nitrogen uptake by maize nonfertilized with nitrogen, $N_{applied}$ is the total amount of nitrogen applied into soil with RM and N_{min} .

Nitrogen use efficiency from available nitrogen doses (NUE_{av}):

$$NUE_{av} = (N_{up_N} - N_{up_N0}) / N_{av} \times 100\%,$$

where N_{up_N} is the nitrogen uptake by maize fertilized with different doses of nitrogen, (in 1st, in 2nd, and as sum for 2 years, respectively), N_{up_N0} is the nitrogen uptake by maize nonfertilized with nitrogen. (in 1st, in 2nd, and as sum for 2 years, respectively), N_{av} is the amount of theoretically available pool of nitrogen, (from RM and N_{min} in 1st year, only from RM in 2nd year, and as the sum of nitrogen available in 1st and 2nd years, respectively).

Yield increase after application of RM (Y_{i_RM}) and N_{min} (Y_{i_Nmin}) separately:

$$Y_{i_RM} = Y_{RM} - Y_0,$$

$$Y_{i_Nmin} = Y_{Nmin} - Y_0,$$

Treatments	Nitrogen forms	N_{min} kg·ha ⁻¹			
		0	50	100	150
RM (t·ha ⁻¹)					
0	N_{tot}	0	50	100	150
	N_{av} in 2018	0	50	100	150
	N_{av} in 2019	0	0	0	0
20	N_{tot}	117	167	217	267
	N_{av} in 2018	41	91	141	191
	N_{av} in 2019	17	17	17	17
40	N_{tot}	233	283	333	383
	N_{av} in 2018	82	132	182	232
	N_{av} in 2019	35	35	35	35
60	N_{tot}	350	400	450	500
	N_{av} in 2018	123	173	223	273
	N_{av} in 2019	53	53	53	53

Table 2. Amounts of total nitrogen (N_{tot}) and nitrogen theoretically available for plants (N_{av}) in experimental treatments, kg·ha⁻¹.

where Y_{RM} is the yield of maize after only RM application, $Y_{N_{min}}$ is the yield of maize after only N_{min} application, Y_0 is the yield of maize without fertilization (control object).

Percent of yield increase after application of RM ($Y_{i_{RM}}$) and N_{min} ($Y_{i_{N_{min}}}$) separately:

$$\%Y_{i_{RM}} = (Y_{i_{RM}}/Y_0) \times 100\%,$$

$$\%Y_{i_{N_{min}}} = (Y_{i_{N_{min}}}/Y_0) \times 100\%,$$

where $Y_{i_{RM}}$ is the yield increase of maize after only RM application, $Y_{i_{N_{min}}}$ is the yield increase of maize after only N_{min} application, Y_0 is the yield of maize without fertilization (control object).

Yield increase after combined application of RM and N_{min} (interaction effect, $Y_{i_{RM_{N_{min}}}}$):

$$Y_{i_{RM_{N_{min}}}} = Y_{RM_{N_{min}}} - Y_0 - Y_{i_{RM}} - Y_{i_{N_{min}}},$$

where $Y_{RM_{N_{min}}}$ is the yield of maize after combined application of RM and N_{min} , Y_0 is the yield of maize without fertilization (control object), $Y_{i_{RM}}$ is the increase of maize yield after only RM application, $Y_{i_{N_{min}}}$ is the increase of maize yield after only N_{min} application.

Percent of yield increase after combined application of RM and N_{min} (interaction effect, $\%Y_{i_{RM_{N_{min}}}}$):

$$\%Y_{i_{RM_{N_{min}}}} = Y_{i_{RM_{N_{min}}}} / (Y_{i_{RM}} + Y_{i_{N_{min}}}) \times 100\%,$$

where $Y_{i_{RM_{N_{min}}}}$ is the yield increase after combined application of RM and N_{min} , $Y_{i_{RM}}$ is the increase of maize yield after only RM application, $Y_{i_{N_{min}}}$ is the increase of maize yield after only N_{min} application.

Statistical analysis

The results were subjected to analysis of variance (ANOVA) with the Fisher–Snedecor distribution at a significance level of $\alpha = 0.05$. Least significant difference (LSD) values at a significance level of $\alpha = 0.05$ were calculated by the Tukey test. The Statistica 13.1.336.0 PL statistics package (StatSoft Inc., Tulsa, OK, USA) was used for the calculations.

Three-factor analysis of variance for data of yield, nitrogen content, uptake and NUE_{tot} were performed according to the following model:

$$y_{ijlp} = m + a_i + b_j + c_l + ab_{ij} + ac_{il} + bc_{jl} + abc_{ijl} + e_{ijl},$$

where y_{ijlp} is the value of the examined characteristic, m is the population average, a_i is the effect of differentiated fertilization with RM, b_j is the effect of differentiated fertilization with N_{min} , c_l is the effect of year (Y), ab_{ij} is the effect of the interaction of RM \times N_{min} , ac_{il} is the effect of the interaction of RM \times Y , bc_{jl} is the effect of the interaction of $N_{min} \times Y$, abc_{ijl} is the effect of the interaction of RM \times $N_{min} \times Y$, e_{ijl} is the random error (numbers).

Two-factor analysis of variance for data of total NUE_{tot} as sum for 2 years and NUE_{av} calculated for nitrogen theoretically available in subsequent years of study were performed according to the following model:

$$y_{ijp} = m + a_i + b_j + ab_{ij} + e_{ij},$$

where y_{ijp} is the value of the examined characteristic, m is the population average, a_i is the effect of differentiated fertilization with RM, b_j is the effect of differentiated fertilization with N_{min} , ab_{ij} is the effect of the interaction of RM \times N_{min} , e_{ij} is the random error (numbers).

Relationships between selected traits were evaluated by simple correlation analysis ($\alpha = 0.05$) by the Statistica software.

Ethical approval

The authors hereby declare that all methods were carried out in accordance with relevant guidelines.

Results

The dry matter yield of silage maize differed between RM application rates, mineral nitrogen application rates, and years of the study (Table 3). On average for the 2 years of the study, application of increasing amounts of RM caused an increase in the dry weight of harvested maize. In the first year, the difference in yield following application of 40 and 60 t·ha⁻¹ RM was not significant. In the second year, a significant increase in the amount of silage maize was obtained following application of 40 and 60 t·ha⁻¹ RM, while the dose of 20 t·ha⁻¹ RM did not significantly affect the yield. As in the first year, the differences between these rates were also not significant.

On average for the 2 years of the study, increasing application of mineral nitrogen (N_{min}) by 50 kg·ha⁻¹ did not result in an increase in the dry weight of harvested maize between adjacent application rates (Table 3). In comparison with the control without N_{min} application, the yield was higher following application of 100 and 150 kg N_{min} ·ha⁻¹, and in comparison with application of 50 kg N_{min} ·ha⁻¹, the yield was higher following application of 150 kg N_{min} ·ha⁻¹. Analysis of interactions indicates that in the first year the yield increased significantly at application of up to 100 kg N_{min} ·ha⁻¹, while application of 150 kg N_{min} ·ha⁻¹ did not result in a significant increase in yield. In the second year, maize yield was not significantly dependent on the amount of N_{min} applied in the first year. On average for the 2 years of the study, the yield in the second year was lower than in the first year.

The actual effect of the interaction of RM and N_{min} in determining maize yield, in comparison with the sum of the effects of RM and N_{min} applied separately, is shown in Table 4. Based on the data the effect of the interaction of RM and N_{min} can be ranked in the following ranges of percentages: 1 (> 30.0%) RM 40 t + N_{min} 150 kg; 2

Treatments	Years		LSD _{0.05} , $p_{RM \times Years, N_{min} \times Years}$	Averages	LSD _{0.05} , $p_{RM, N_{min}}$
	2018	2019			
RM t·ha ⁻¹					
0	11.3 ± 1.6 A	12.4 ± 1.4 A	1.7 $p < 0.0001$	11.8 ± 1.6 a	1.2 $p < 0.0001$
20	14.0 ± 1.7 B	13.9 ± 0.6 AB		14.0 ± 1.3 b	
40	16.9 ± 3.6 C	14.1 ± 1.1 BC		15.5 ± 3.0 c	
60	18.3 ± 3.1 C	15.7 ± 1.7 C		17.0 ± 2.8 d	
N _{min} kg·ha ⁻¹					
0	12.5 ± 2.5 A	13.6 ± 1.7 A	1.7 $p = 0.0002$	13.1 ± 2.2 a	1.2 $p < 0.0001$
50	14.3 ± 2.8 B	13.9 ± 1.8 A		14.1 ± 2.3 ab	
100	16.1 ± 4.1 C	14.1 ± 1.8 A		15.1 ± 3.2 bc	
150	17.5 ± 3.8 C	14.5 ± 1.7 A		16.0 ± 3.3 c	
Averages	15.1 ± 3.8 b	14.0 ± 1.7 a			
LSD _{0.05} , p for years	0.7, $p = 0.0016$				

Table 3. Maize yield [DM t·ha⁻¹] as a result of different RM and N_{min} application rates and years, mean values ± standard deviation (SD). a,b,c,d—means for investigated factors with different lowercase letters are significantly different; A,B,C—means for interactions with different capital letters in the columns are significantly different.

Treatments	N _{min} kg·ha ⁻¹								Averages	
	0		50		100		150		t·ha ⁻¹	%
	t·ha ⁻¹	%	t·ha ⁻¹	%	t·ha ⁻¹	%	t·ha ⁻¹	%		
RM t·ha ⁻¹										
0	–	–	0.9 ²	8.5 ⁴	1.7 ²	16.0 ⁴	2.4 ²	22.6 ⁴	1.7 ²	15.7 ⁴
20	2.1 ¹	19.8 ³	0.1 ⁵	3.3 ⁶	–0.3 ⁵	–7.9 ⁶	0.3 ⁵	7.9 ⁶	<0.1 ⁵	1.1 ⁶
40	3.1 ¹	29.2 ³	0.4 ⁵	10.0 ⁶	0.3 ⁵	6.3 ⁶	1.7 ⁵	30.9 ⁶	0.8 ⁵	15.7 ⁶
60	4.7 ¹	44.3 ³	0.1 ⁵	1.8 ⁶	1.4 ⁵	21.9 ⁶	0.2 ⁵	2.8 ⁶	0.6 ⁵	8.8 ⁶
Averages	3.3 ¹	31.1 ³	0.2 ⁵	5.0 ⁶	0.5 ⁵	6.8 ⁶	0.7 ⁵	13.9 ⁶	0.5 ⁵	8.5 ⁶

Table 4. Effect of the interaction of RM and N_{min} in determining maize yield (DM t·ha⁻¹) and % increase in yield. ¹Y_{i, RM}, effect of using only RM. ²Y_{i, N_{min}}, effect of using only N_{min}. ³Y_{i, RM}, effect of using only RM. ⁴Y_{i, N_{min}}, effect of using only N_{min}. ⁵Y_{i, RM, N_{min}}, effect of using combined application of RM and N_{min} (interaction effect). ⁶Y_{i, RM, N_{min}}, % effect of using combined application of RM and N_{min} (interaction effect).

(20.1–30.0%) RM 60 t + N_{min} 100 kg; 3 (10.1–20.0%) none; 4 (0.1–10.0%) RM 20 t, 40 t and 60 t, all + N_{min} 50 kg, RM 40 t + N_{min} 100 kg, RM 20 t and 60 t, both + N_{min} 150 kg; 5 (≤ 0.0%) RM 20 t + N_{min} 100 kg. The positive effects of the interaction may be due on the one hand to the increase in the mineralization rate of organic fertilizers induced by chemical nitrogen fertilizers, and on the other hand to the reduction in the dissipation of mineral nitrogen in the environment caused by organic fertilizer.

The content of nitrogen in the entire dry weight of maize was significantly influenced by the RM application rates, mineral nitrogen application rates, and years of the study (Table 5). On average for the 2 years and in the second year of the study, the nitrogen content in maize increased following the application of 20 and 40 t·ha⁻¹ RM, while the application of 60 t·ha⁻¹ RM did not cause a significant increase in this parameter. Analysis of interactions shows that in the first year following the application of RM significantly more nitrogen was obtained in maize following the application of 40 and 60 t·ha⁻¹ RM than when RM was not applied.

On average for the 2 years of the study, increasing application of mineral nitrogen (N_{min}) by 50 kg·ha⁻¹ did not significantly increase the content of nitrogen in the maize between adjacent rates of application. In comparison to the control without N_{min} application, maize contained more nitrogen following application of 100 and 150 kg N_{min}·ha⁻¹. In comparison to the application of 50 kg N_{min}·ha⁻¹, more nitrogen was noted in maize following application of 150 kg N_{min}·ha⁻¹. On average for the years of the study, the nitrogen content in maize harvested in the first year was higher than in the second year.

Nitrogen uptake by maize was significantly dependent on RM application rates, mineral nitrogen application rates, and the years of the study (Table 6). On average for the 2 years of the study, nitrogen uptake by maize increased with the RM application rate. Following application of 20, 40 and 60 t·ha⁻¹ RM, nitrogen uptake was 38.2%, 72.2%, and 94.3% higher, respectively, than without RM application.

On average for the 2 years of the study, each increase in the application of mineral nitrogen (N_{min}) by 50 kg·ha⁻¹ caused a significant increase in nitrogen uptake by maize. Following application of 50, 100 and 150 kg N_{min}·ha⁻¹, nitrogen uptake was 15.7%, 30.7%, and 44.2% higher, respectively, than without application

Treatments	Years		LSD _{0.05} , p RM \times Years, N _{min} \times Years	Averages	LSD _{0.05} RM, N _{min}
	2018	2019			
RM t·ha ⁻¹					
0	10.87 \pm 1.22 A	6.72 \pm 0.48 A	1.15 $p=0.0015$	8.79 \pm 2.31 a	0.81 $p<0.0001$
20	11.77 \pm 1.59 AB	8.61 \pm 1.70 B		10.19 \pm 2.28 b	
40	12.70 \pm 0.69 B	9.99 \pm 0.91 C		11.35 \pm 1.60 c	
60	12.63 \pm 0.69 B	11.00 \pm 1.71 C		11.81 \pm 1.52 c	
N _{min} kg·ha ⁻¹					
0	11.04 \pm 1.61	8.35 \pm 1.59	$p=0.3941$	9.69 \pm 2.08 a	0.81 $p<0.0001$
50	11.81 \pm 1.21	8.90 \pm 1.97		10.35 \pm 2.18 ab	
100	12.56 \pm 0.92	9.03 \pm 2.19		10.80 \pm 2.44 bc	
150	12.57 \pm 0.88	10.04 \pm 2.31		11.30 \pm 2.15 c	
Averages	11.99 \pm 1.32 b	9.08 \pm 2.06 a			
LSD _{0.05} , p for years	0.43, $p<0.0001$				

Table 5. Nitrogen content [g N·kg⁻¹ DM] in maize as a result of different RM and N_{min} application rates and years, mean values \pm SD. a,b,c—means for investigated factors with different lowercase letters are significantly different; A,B,C—means for interactions with different capital letters in the columns are significantly different.

Treatments	Years		LSD _{0.05} , p RM \times Years, N _{min} \times Years	Averages	LSD _{0.05} , p RM, N _{min}
	2018	2019			
RM t·ha ⁻¹					
0	123.9 \pm 29.9	83.3 \pm 11.2	$p=0.0506$	103.6 \pm 30.3 a	17.0 $p<0.0001$
20	166.8 \pm 39.5	119.6 \pm 24.3		143.2 \pm 40.1 b	
40	215.9 \pm 50.0	140.9 \pm 14.9		178.4 \pm 52.6 c	
60	230.5 \pm 41.0	172.1 \pm 27.5		201.3 \pm 45.3 d	
N _{min} kg·ha ⁻¹					
0	141.1 \pm 44.6 A	114.4 \pm 29.8 A	24.0 $p=0.0005$	127.7 \pm 39.5 a	17.0 $p<0.0001$
50	170.1 \pm 43.2 B	125.4 \pm 36.2 AB		147.7 \pm 45.2 b	
100	204.6 \pm 60.1 C	129.2 \pm 40.8 AB		166.9 \pm 63.3 c	
150	221.4 \pm 51.9 C	146.9 \pm 42.2 B		184.1 \pm 59.9 d	
Averages	184.3 \pm 58.0 b	129.0 \pm 38.2 a			
LSD _{0.05} , p for years	9.1, $p<0.0001$				

Table 6. Nitrogen uptake [kg N·ha⁻¹] by maize as a result of different RM application rates and N_{min} and years, mean values \pm SD. a,b,c,d—means for investigated factors with different lowercase letters are significantly different; A,B,C—means for interactions with different capital letters in the columns are significantly different.

of N_{min}. On average for the years of the study, nitrogen uptake by maize in the first year was 42.9% greater than in the second year.

The total nitrogen uptake as a sum from 2 years of research was significantly dependent on RM application rates or mineral nitrogen application rates (Table 7). This parameter increased with the RM application rate. The total nitrogen uptake from 2 years did not increase significantly after each increase in the mineral nitrogen dose by 50 kg·ha⁻¹. Significant differences in this parameter were obtained between the objects differing in the application of 100 kg N_{min}·ha⁻¹ into the soil.

The nitrogen use efficiency of maize was not significantly dependent on RM application rates or mineral nitrogen application rates (Table 8). The value of this parameter was significantly dependent on the year of the study. The nitrogen use efficiency of maize obtained in the first year following application of fertilizer was twice as high as in the second year.

Table 9 shows the effect of application of mineral nitrogen on NUE from RM as the sum from 2 years. NUE was shown to increase with each successive increase in application of mineral nitrogen, but the differences were not confirmed statistically. The total NUE from the 2 years of the study following application of 20 and 40 t·ha⁻¹ RM was nearly identical, while following application of 60 t·ha⁻¹ RM it was somewhat lower, but the significance of the differences was not confirmed statistically in this case as well. All NUE values were above 50%. An NUE value above 70% was obtained only after the application of 20 t·ha⁻¹ RM and 150 kg·ha⁻¹ N_{min}. NUE values in the range of 60.1–70% were obtained following application of 20 t·ha⁻¹ RM with supplementary application of N_{min} at 50 and 100 kg·ha⁻¹; 40 t·ha⁻¹ RM with supplementary application of N_{min} at all rates; and 60 t·ha⁻¹ RM with supplementary application of N_{min} at 100 kg·ha⁻¹. NUE values in the range of 50–60% were

Treatments	N_{\min} kg·ha ⁻¹				Averages	LSD _{0.05} , p for RM doses
	0	50	100	150		
RM t·ha ⁻¹						
20	221.4 ± 7.6	263.1 ± 7.9	296.2 ± 26.9	365.2 ± 30.6	286.4 ± 57.8 a	39.9 $p < 0.0001$
40	292.8 ± 27.7	334.9 ± 21.2	372.6 ± 37.5	426.9 ± 62.9	356.8 ± 62.1b	
60	345.1 ± 5.7	385.7 ± 39.8	444.6 ± 87.4	434.9 ± 28.1	402.6 ± 59.8 c	
Averages	286.4 ± 55.8 a	327.9 ± 58.1 ab	371.1 ± 81.1bc	409.0 ± 50.1c	Interaction RM/ N_{\min} $p = 0.7204$	
LSD _{0.05} , p for N_{\min} doses	50.9 $p < 0.0001$					

Table 7. Total nitrogen uptake (sum from 2018 and 2019) [kg N·ha⁻¹] as a result of different RM and N_{\min} application rates, mean values ± SD. a,b,c—means for investigated factors with different lowercase letters are significantly different.

Treatments	Years		p RM × Years, N_{\min} × Years	Averages	p RM, N_{\min}
	2018	2019			
RM t·ha ⁻¹					
20	43.8 ± 7.8	22.1 ± 9.9	$p = 0.1095$	33.0 ± 14.1	$p = 0.2706$
40	43.9 ± 9.7	20.3 ± 3.8		32.1 ± 14.0	
60	35.1 ± 8.6	22.5 ± 5.8		28.8 ± 9.7	
N_{\min} kg·ha ⁻¹					
50	36.4 ± 6.1	22.5 ± 7.2	$p = 0.1933$	29.5 ± 9.7	$p = 0.3826$
100	43.1 ± 9.2	19.3 ± 6.6		31.2 ± 14.5	
150	43.3 ± 11.4	23.1 ± 6.7		33.2 ± 13.8	
Averages	40.9 ± 9.4 b	21.6 ± 6.8 a			
LSD _{0.05} , p for years	4.4, $p < 0.0001$				

Table 8. Nitrogen use efficiency [%] from total nitrogen application (N_{tot}) as a result of different RM application rates and years, mean values ± SD. a,b—means for investigated factors with different lowercase letters are significantly different.

Treatments	N_{\min} kg·ha ⁻¹				Averages	p for RM doses
	0	50	100	150		
RM t·ha ⁻¹						
20	50.3 ± 6.5	60.2 ± 4.7	61.6 ± 12.4	75.9 ± 11.5	62.0 ± 12.4	$p = 0.3396$
40	55.7 ± 11.9	60.8 ± 7.5	62.9 ± 11.2	68.9 ± 16.4	62.1 ± 11.5	
60	52.1 ± 1.6	55.7 ± 9.9	62.6 ± 19.4	54.4 ± 5.6	56.2 ± 10.5	
Averages	52.7 ± 7.2	58.9 ± 7.1	62.4 ± 12.8	66.4 ± 14.1	Interaction RM/ N_{\min} $p = 0.6158$	
p for N_{\min} doses	$p = 0.0829$					

Table 9. Total nitrogen use efficiency (sum from 2 years) [%] from total nitrogen application (N_{tot_2}) as a result of different RM and N_{\min} application rates, mean values ± SD.

obtained following application of 60 t·ha⁻¹ RM and N_{\min} at 50 and 150 kg·ha⁻¹ and following application of all levels of RM without N_{\min} .

No significant interactions were found between different RM and N_{\min} rates in the case of yield, content and nitrogen uptake (as an average and sum for 2-years) by maize.

The mean values of the examined property for the two-way interactions RM × N_{\min} presented in Table 10 were not significant—all p -values > 0.05.

NUE_{av} values calculated separately for each year for nitrogen theoretically available for plants were not significantly dependent on the level of RM application in either year or on the amount of N_{\min} applied in the first year (Table 11). In the second year of the study, application of 0, 50 and 100 kg·ha⁻¹ N_{\min} together with RM did not significantly influence NUE_{av} from the pool of theoretically available nitrogen applied in the form of RM. The highest value for this parameter was obtained following the application of N_{\min} at 150 kg·ha⁻¹ together with RM. NUE_{av} values calculated for N theoretically available for plants in total for the 2 years of the study were not significantly dependent on either the RM or N_{\min} application rate.

Treatments	N_{min} kg-ha ⁻¹			
	0	50	100	150
RM t-ha ⁻¹				
0				
Yield	10.6 ± 1.9	11.5 ± 1.2	12.3 ± 1.0	13.0 ± 1.4
N content	7.88 ± 1.59	8.77 ± 2.52	9.09 ± 2.50	9.43 ± 2.79
N uptake	81.3 ± 7.7	99.2 ± 23.8	111.0 ± 28.9	123.1 ± 40.1
20				
Yield	12.7 ± 1.0	13.7 ± 0.4	14.1 ± 0.6	15.4 ± 1.3
N content	8.84 ± 1.56	9.65 ± 1.87	10.49 ± 2.64	11.78 ± 2.31
N uptake	110.7 ± 11.8	131.5 ± 22.8	148.1 ± 38.2	182.6 ± 44.3
NUE _{tot}	–	30.1 ± 12.1	30.8 ± 15.9	38.0 ± 15.3
40				
Yield	13.7 ± 0.7	15.0 ± 1.3	15.7 ± 2.3	17.8 ± 4.7
N content	10.72 ± 1.74	11.14 ± 1.64	11.67 ± 2.03	11.85 ± 0.96
N uptake	146.4 ± 24.6	167.4 ± 33.1	186.3 ± 56.2	213.5 ± 70.4
NUE _{tot}	–	30.4 ± 10.4	31.5 ± 15.8	34.5 ± 17.4
60				
Yield	15.3 ± 1.6	16.3 ± 2.4	18.4 ± 4.0	17.9 ± 2.0
N content	11.33 ± 1.62	11.84 ± 1.52	11.93 ± 1.97	12.16 ± 1.19
N uptake	172.6 ± 24.8	192.6 ± 34.0	222.3 ± 68.2	217.4 ± 33.3
NUE _{tot}	–	27.9 ± 7.7	31.3 ± 14.5	27.2 ± 6.0
<i>p</i> value for interaction RM × N_{min} , and RM × N_{min} × Year				
Yield	RM × N_{min} = 0.7483, RM × N_{min} × Year = 0.3647			
N content	RM × N_{min} = 0.4938, RM × N_{min} × Year = 0.3799			
N uptake	RM × N_{min} = 0.5563, RM × N_{min} × Year = 0.7202			
NUE _{tot}	RM × N_{min} = 0.5214, RM × N_{min} × Year = 0.7663			

Table 10. Level of RM × N_{min} interaction for yield data, nitrogen content and uptake, and NUE_{tot} value, mean values ± SD.

Years of study	Treatments	RM, t-ha ⁻¹			Averages	LSD _{0.05} <i>p</i> for N_{min} doses
		20	40	60		
2018	N_{min} kg-ha ⁻¹					<i>p</i> = 0.0552
	0	87.3 ± 4.6	97.6 ± 8.8	88.2 ± 3.2	91.0 ± 5.5	
	50	67.4 ± 8.9	83.9 ± 3.4	77.4 ± 5.9	76.2 ± 6.1	
	100	68.2 ± 5.4	81.0 ± 11.3	82.1 ± 12.9	77.1 ± 9.2	
	150	70.2 ± 0.7	79.9 ± 13.0	57.4 ± 5.0	69.1 ± 11.4	
	Averages	73.3 ± 9.2	85.6 ± 10.0	76.3 ± 7.7		
	<i>p</i> for RM doses	<i>p</i> = 0.1688			Interaction RM/ N_{min} <i>p</i> = 0.8180	
2019	N_{min} kg-ha ⁻¹					99.3 <i>p</i> = 0.0055
	0	135.2 ± 21.1	143.3 ± 20.9	139.8 ± 19.6	139.4 ± 18.1a	
	50	230.4 ± 121.8	175.7 ± 43.9	168.3 ± 32.5	191.5 ± 72.9ab	
	100	220.0 ± 90.1	178.7 ± 26.6	186.7 ± 84.3	195.1 ± 65.9ab	
	150	403.1 ± 178.6	225.6 ± 40.8	218.3 ± 37.4	282.3 ± 130.2b	
	Averages	247.2 ± 142.8	180.8 ± 42.4	178.3 ± 51.9		
	<i>p</i> for RM doses	<i>p</i> = 0.0614			Interaction RM/ N_{min} <i>p</i> = 0.3841	
For N available in both years	N_{min} kg-ha ⁻¹					<i>p</i> = 0.4688
	0	101.4 ± 13.1	111.3 ± 23.7	103.7 ± 3.2	105.4 ± 14.4	
	50	93.0 ± 7.3	103.2 ± 12.7	98.7 ± 17.6	98.3 ± 12.3	
	100	84.5 ± 17.0	96.8 ± 17.3	102.2 ± 21.7	94.5 ± 21.4	
	150	97.4 ± 14.7	99.0 ± 23.6	83.5 ± 8.6	93.3 ± 16.3	
	Averages	94.1 ± 13.2	102.6 ± 17.9	97.0 ± 18.0		
	<i>p</i> for RM doses	<i>p</i> = 0.4978			Interaction RM/ N_{min} <i>p</i> = 0.8513	

Table 11. Nitrogen use efficiency [%] from nitrogen theoretically available for plants (presented in Table 2) in each year of the study as a result of different RM and N_{min} application rates, mean values ± SD. a,b—means for investigated factors with different lowercase letters are significantly different.

Correlation analysis revealed significant relationships between total and available nitrogen doses, and: silage maize yield, nitrogen content, nitrogen uptake (Table 12). Nitrogen use efficiency in 2019 and NUE as sum from 2018 and 2019 were significantly correlated with total nitrogen doses.

Discussion

Animal excrements (faeces and urine) are traditionally used to fertilize soil and plants. They owe their value as fertilizer to high content of organic matter and nutrients for plants. One of the most important nutrients for plants contained in these fertilizers is nitrogen^{8,27}. Organic forms of nitrogen applied to the soil gradually become available for plants²⁸. Mineralization of organic compounds from these fertilizers, spread out over time, causes the gradual release of nitrogen in available forms, which enables a regular supply of this macronutrient to plants. In the case of chemical fertilizers, the entire pool of nitrogen is usually available for plants immediately after their application, but decreases significantly in the later stage of their growth²⁹. This is the result of nitrogen being taken up by plants and dissipated in the environment. The combined application of these fertilizers limits the dissipation of nitrogen from mineral fertilizers in the environment³⁰. This may be the result of biological sorption of mineral nitrogen (its immobilization process). Fertilization based on the combined application of organic fertilizers and nitrogen mineral fertilizers can ensure an optimal supply of nitrogen to plants³¹ and may result in higher crop yields^{30,32}. The present study showed that the biomass yield of maize increased in the first year after application of RM up to 40 t·ha⁻¹ and N_{min} up to 100 kg·ha⁻¹. Further increases to 60 t·ha⁻¹ RM and 150 kg·ha⁻¹ N_{min} did not lead to a significant increase in the yield of maize. In that second year of the study, an increase in yield in comparison to the treatment without RM application was obtained following application at the two highest rates (40 and 60 t·ha⁻¹). In that year, varied application of N_{min} did not significantly differentiate the yields of the test plant, which is consistent with the idea of the rapid but short-term fertilizing effect of chemical nitrogen fertilizers. The dry matter yield per kg of applied nitrogen decreases as the rate of application increases. Nitrogen applied in small amounts is then the major growth-limiting factor. Increasing the supply of nitrogen reduces productivity of applied N, because the yield is determined by factors other than the limiting nutrient^{33,34}.

An important element of the experiment was the determination of the most beneficial proportion of rabbit manure and mineral nitrogen application for the yield of the test plant, nitrogen content and uptake, and NUE. The study showed that the most favourable proportion in terms of the biomass yield of maize was 60 t·ha⁻¹ RM and 100 kg·ha⁻¹ N_{min}, which introduced a total of 450 kg·ha⁻¹ nitrogen into the soil, while the amount of nitrogen theoretically available for plants in the first and second year was 223 and 53 kg·ha⁻¹, respectively. Other researchers reported that for cultivation of forage maize in semi-arid areas, the optimal application rate of nitrogen in chemical fertilizer, and thus nitrogen available for plants, was 210 kg·ha⁻¹, but that was the highest rate tested in the study³⁴. Correlation analysis ($p < 0.05$) shows that the silage maize yields were significantly dependent on the application rates of nitrogen: total ($r = +0.862$) and theoretically available for plants ($r = +0.849$). Increasing maize yields caused by increasing nitrogen application rates is a common phenomenon and consistent with researchers' expectations^{35,36}. Good supply of nitrogen to plants promotes faster photosynthesis by increasing capture of sunlight by crops and the efficiency of conversion to biomass, thus improving biomass production³⁷. Photosynthesis activity is associated with nitrogen content in leaves³⁸. The amount of dry matter of silage maize increases with the level of nitrogen in the leaves³³. Combined application of organic and chemical nitrogen fertilizers has proven very effective in crops of other plants. In potato, the ideal combination for optimizing yield has been shown to be, on average, 31 t·ha⁻¹ of organic manure and 187.5 kg·ha⁻¹ of nitrogen fertilizer³⁹. Similar fertilization systems based on partial replacement of mineral nutrients for plants with nutrients applied in the form of organic fertilizers, including rabbit manure, have resulted in similar or even higher maize yields than inorganic fertilizer applied at the same nitrogen:phosphorus:potassium rate⁴⁰.

Increasing the amount of nitrogen introduced to the soil caused an increase in its content in the biomass of maize as well as its uptake by the plant. Converting nitrogen content to crude protein content (CP) ($\times 6.25$) and nitrogen uptake to crude protein yield (CPY) shows that application of RM at 20–60 t·ha⁻¹ resulted in CP from 7.4 to 7.9% in the biomass of maize grown in the first year after fertilizer application, and CPY from 1042 to

Specification	N _{tot} dose	N _{av} dose
Yield in 2018	0.875*	0.824*
Yield in 2019	0.688*	0.568*
Yield, as the sum 2018 + 2019	0.862*	0.849*
N content in 2018	0.671*	0.696*
N content in 2019	0.829*	0.715*
N uptake in 2018	0.872*	0.870*
N uptake in 2019	0.899*	0.770*
N uptake, as the sum 2018 + 2019	0.927*	0.872*
NUE _{tot} in 2018	0.027	–
NUE _{tot} in 2019	0.570*	–
NUE _{tot} as the sum 2018 + 2019	0.308*	–

Table 12. Linear correlation coefficients between N_{tot} and N_{av} rates and selected properties of silage maize, values marked with * are important ($\alpha < 0.05$).

1441 kg·ha⁻¹. In the second year following application of RM at 20–60 t·ha⁻¹, CP ranged from 5.4 to 6.9%, and CPY from 748 to 1076 kg·ha⁻¹. The values obtained in the first year after fertilizer application correspond to the typical protein content in maize silage^{41,42}. Analysis of the interaction of RM and N_{min} application shows that the highest average CP content from the 2 years (7.6%) was obtained following the combined application of RM and N_{min} at the highest rates, i.e. 60 t·ha⁻¹ RM and 150 kg·ha⁻¹ N_{min}, which introduced a total of 500 kg·ha⁻¹ N to the soil, including 273 and 53 kg·ha⁻¹ of nitrogen theoretically available for plants in the first and second year, respectively. However, the highest average crude protein yield from the 2 years (1389 kg·ha⁻¹) was obtained following the combined application of 60 t·ha⁻¹ RM and 100 kg·ha⁻¹ N_{min}. On this treatment into soil was applied 50 kg less total and theoretically available nitrogen than in the case of the highest nitrogen application. Ma and coauthors³⁴ tested nitrogen application rates from 70 to 210 kg·ha⁻¹ in chemical fertilizer and obtained 5.2% to 7.0% protein content in forage maize. In the present study, the average crude protein content in the maize biomass was slightly higher. Literature data indicate that it is possible to obtain even higher CP content in forage maize⁴³. Following application of N_{min} alone at 50–150 kg·ha⁻¹, CP content ranged from 5.5 to 5.9%, while following application of RM alone at 20–60 t·ha⁻¹, CP content ranged from 5.5 to 7.1%. After combined application of RM and N_{min} in all combinations tested, CP ranged from 6.0 to 7.6%. Correlation analysis ($p < 0.05$) indicates that the nitrogen content and, consequently, the crude protein content in the first and second year after fertilizer application was significantly correlated with the total amount of nitrogen applied ($r = +0.671$ and $r = +0.829$, respectively), as well as with the amount of nitrogen theoretically available for plants ($r = +0.696$ and $r = +0.715$, respectively). Nitrogen uptake and, consequently, crude protein yield in the first and second year after fertilization was also significantly correlated with the total nitrogen applied ($r = +0.872$ and $r = +0.899$, respectively), as well as with the amount of nitrogen theoretically available for plants ($r = +0.870$ and $r = +0.770$, respectively). This means that as the level of nitrogen application increased (total and theoretically available for plants), uptake of nitrogen and its accumulation in the maize biomass increased. This translates directly to the NUE values, which were generally somewhat smaller following application of higher levels of nitrogen, but did not differ significantly depending on the levels of RM and N_{min} application. However, literature data indicate that the NUE value significantly decreases as N application increases^{34,44}. NUE values ranged from 35.1 to 43.9% in the first year following application of RM and N_{min}, and from 19.3 to 23.1% in the second year. These are higher values than in other studies, in which NUE from urea applied at 70–210 kg·ha⁻¹ nitrogen was within the range of 24.4–31.7%³⁴. Other researchers obtained a significant decrease in nitrogen recovery efficiency from various levels of nitrogen application after replacing inorganic nitrogen with organic nitrogen (applied with manure) at a level of at least 50%⁴⁵. In that study, the highest value of this parameter was obtained following application of inorganic and organic nitrogen in a 0.75/0.25 ratio (about 40–50% in maize cultivation). Another study found that the optimal level of replacement of synthetic nitrogen with organic nitrogen, leading to an increase in NUE, was 44%⁴⁶. The NUE values above 60% obtained in the present study following application of RM at 20 t·ha⁻¹ (117 kg·ha⁻¹ total nitrogen, 41 kg·ha⁻¹ theoretically available nitrogen) and 40 t·ha⁻¹ (233 kg·ha⁻¹ total N, 82 kg·ha⁻¹ theoretically available nitrogen) with supplementary application of N_{min} at 50–150 kg·ha⁻¹ and following application of 60 t·ha⁻¹ RM (350 kg·ha⁻¹ total nitrogen, 123 kg·ha⁻¹ theoretically available nitrogen) with 100 kg·ha⁻¹ N_{min} indicate an appropriate level of nitrogen use by the plants and are in line with current sustainable development strategies for the agricultural sector. Fertilization regimes based on balanced synthetic nitrogen and manure are ideal for sustainable nitrogen management⁴⁷, increasing NUE⁴⁸. Calculation of NUE for the pool of nitrogen theoretically available for plants (NUE_{av}) sheds new light on NUE values. The NUE_{av} values obtained in the first year, most often in the range of 60–80%, are indicative of incomplete use of the pool of nitrogen theoretically available for plants. This may have been due to unfavourable moisture conditions during the growing season. The NUE_{av} values for the pool of nitrogen theoretically available for plants in the second year, exceeding 100% in each case, and in some cases even 200%, are indicative of greater nitrogen availability for plants than was predicted. They may also indicate that the pool of available nitrogen unused in the first year was used in the second year. NUE_{av} values for the pool of nitrogen theoretically available for plants in total for both years of the study was most often over 90%, and in five cases exceeded 100% (on average 98%), indicating total or nearly total utilization of this pool of nitrogen. It also indicates that the factors chosen to convert the total amount of nitrogen introduced to the soil with manure to nitrogen available for plants were well-selected²⁵.

Conclusion

Given the dry matter yield of maize, nitrogen uptake (crude protein yield), nitrogen use efficiency from applied nitrogen, and the effect of the interaction of organic and mineral fertilizer, fertilization of maize for silage with rabbit manure at up to 40 t·ha⁻¹ and supplementary application of mineral nitrogen in amounts up to 150 kg·ha⁻¹ is justified. When application of rabbit manure is increased to 60 t·ha⁻¹, application of N_{min} should be decreased so as not to exceed 100 kg·ha⁻¹. These fertilizer combinations introduce up to 232 kg·ha⁻¹ of nitrogen theoretically available for plants to the soil in the first growing season, and the NUE_{av} from this pool of nitrogen is about 80%. The values of all parameters tested (yield, nitrogen content and uptake, NUE_{tot} and NUE_{av}) indicate the need for additional application of nitrogen for crops grown in the second year following application of RM, but after taking into account the small amount of available nitrogen remaining after application of RM in the first year.

Data availability

The datasets generated and analysed during the current study are not publicly available due to they are the authors' own data, but are available from the corresponding author on reasonable request.

Received: 14 December 2023; Accepted: 8 March 2024

Published online: 11 March 2024

References

- Penuelas, J., Coello, F. & Sardans, J. A better use of fertilizers is needed for global food security and environmental sustainability. *Agric. Food Secur.* **12**, 5. <https://doi.org/10.1186/s40066-023-00409-5> (2023).
- You, L. *et al.* Spatial variation in actual and required nitrogen use efficiency and the potential to close the gap by management practices. *Sci. Total Environ.* **903**, 166657. <https://doi.org/10.1016/j.scitotenv.2023.166657> (2023).
- Song, D.-L., Hou, S.-P., Wang, X.-B., Liang, G.-Q. & Zhou, W. Nutrient resource quantity of animal manure and its utilization potential in China. *J. Plant Nutr. Fertil.* **24**(5), 1131–1148. <https://doi.org/10.11674/zwyf.17415> (2018).
- Dinuccio, E., Biagini, D., Rosato, R., Balsari, P. & Lazzaroni, C. Organic matter and nitrogen balance in rabbit fattening and gaseous emissions during manure storage and simulated land application. *Agric. Ecosyst. Environ.* **269**, 30–38. <https://doi.org/10.1016/j.agee.2018.09.018> (2019).
- Li, D. Y. & Wu, L. P. Analysis of the competitiveness of Chinese rabbit industry. In *Proc. 12th World Rabbit Congress, Nantes, France, Communication, F-08 4* (2021).
- Statistical Yearbook of Agriculture* (Central Statistical Office, 2022).
- Moral, R. *et al.* Characterisation of the organic matter pool in manures. *Bioresour. Technol.* **96**, 153–158. <https://doi.org/10.1016/j.biortech.2004.05.003> (2005).
- Moula, M., Gad-El Moula, H., Mohamed, A. A. A., Elmorsy, A. T. & Farag, A. A. The action and interaction of different planting dates and organic fertilizers on the growth and yield of Okra plants. *J. Agric. Ecol. Res. Int.* **14**(1), 1–14. <https://doi.org/10.9734/JAERI/2018/38255> (2018).
- Li, R., Hao, H., Sun, H., Wang, L. & Wang, H. Composted rabbit manure as organic matrix for manufacturing horticultural growing media: Composting process and seedling effects. *Sustainability* **14**, 5146. <https://doi.org/10.3390/su14095146> (2022).
- El-Beltagi, H. S. *et al.* Effect of organic and bio-fertilization on fruit yield, bioactive constituents, and estragole content in fennel fruits. *Agronomy* **13**, 1189. <https://doi.org/10.3390/agronomy13051189> (2023).
- Calvet, S., Estellés, F., Hermida, B., Blumetto, O. & Torres, A. G. Experimental balance to estimate efficiency in the use of nitrogen in rabbit breeding. *World Rabbit Sci.* **16**(4), 205–211. <https://doi.org/10.4995/wrs.2008.615> (2008).
- Adekiya, A. O. *et al.* Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of okra. *Sci. Rep.* **10**, 16083. <https://doi.org/10.1038/s41598-020-73291-x> (2020).
- Zhang, X. *et al.* Effects of organic fertilizers on yield, soil physico-chemical property, soil microbial community diversity and structure of *Brassica rapa* var. *Chinensis*. *Front. Microbiol.* **14**, 1132853. <https://doi.org/10.3389/fmicb.2023.1132853> (2023).
- Li, R., Wang, H., Duan, E., Fan, J. & Wang, L. Rabbit manure compost for seedling nursery cultures: Suitability and optimization of the manufacturing production process. *Agriculture* **12**(12), 2156. <https://doi.org/10.3390/agriculture12122156> (2022).
- Li, R., Hao, H., Yang, C., Wang, L. & Wang, H. Rabbit manure compost as a peat substitute for compound growing media: Proportioning optimization according to physicochemical characteristics and seedling effects. *Front. Plant Sci.* **13**, 1008089. <https://doi.org/10.3389/fpls.2022.1008089> (2022).
- González, R. C., García, B. G. G., del González-Chávez, M. C. A. & Solís-Domínguez, F. A. Trace elements adsorption from solutions and acid mine drainage using agricultural by-products. *Soil Sediment Contam.* **1**(4), 1–19. <https://doi.org/10.1080/15320383.2021.1942430> (2021).
- Huang, W., Chen, J. & Zhang, J. Adsorption characteristics of methylene blue by biochar prepared using sheep, rabbit and pig manure. *Environ. Sci. Pollut. Res.* **25**, 29256–29266. <https://doi.org/10.1007/s11356-018-2906-1> (2018).
- Medyńska-Juraszek, A., Marcinkowska, K., Gruszka, D. & Kluczek, K. The effects of rabbit-manure-derived biochar co-application with compost on the availability and heavy metal uptake by green leafy vegetables. *Agronomy* **12**(10), 2552. <https://doi.org/10.3390/agronomy12102552> (2022).
- Li, K., Liu, R. & Sun, Ch. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. *Bioresour. Technol.* **198**, 133–140. <https://doi.org/10.1016/j.biortech.2015.08.151> (2015).
- Ikrarwati, *et al.* Quality of growth media and yields of *Allium ascalonicum* L. on ultisol soil combined with rabbit manure. *IOP Conf. Ser. Earth Environ. Sci.* **715**(1), 012039. <https://doi.org/10.1088/1755-1315/715/1/012039> (2021).
- El-Mogy, M. M. *et al.* Comparative effects of different organic and inorganic fertilisers on soil fertility, plant growth, soil microbial community, and storage ability of lettuce. *Agriculture* **66**(3), 87–107. <https://doi.org/10.2478/agri-2020-0009> (2020).
- Nada, R. S., Ashmawi, A. E., Mady, E., Randhir, T. O. & Elateeq, A. A. Effect of organic manure and plant growth promoting microbes on yield, quality and essential oil constituents of fennel bulb (*Foeniculum vulgare* Mill.). *J. Ecol. Eng.* **23**(5), 149–164. <https://doi.org/10.12911/22998993/147252> (2022).
- Youssef, M. A. & Eissa, M. A. Comparison between organic and inorganic nutrition for tomato. *J. Plant Nutr.* **40**(13), 1900–1907. <https://doi.org/10.1080/01904167.2016.1270309> (2017).
- Wu, C.-L., Shen, Q.-R., Mao, J.-D. & Xu, Y.-C. Fate of 15N after combined application of rabbit manure and inorganic N fertilizers in a rice-wheat rotation system. *Biol. Fertil. Soils* **46**(2), 127–137. <https://doi.org/10.1007/s00374-009-0420-9> (2010).
- Regulation of the Council of Ministers of February 12, Journal of Laws Republic of Poland on February 14, 2020, Item 243 on the Adoption of the Programme of Actions Aimed at Reducing Water Pollution with Nitrates from Agricultural Sources and Preventing Further Pollution.*
- Nelson, D. W. & Sommers, L. E. Determination of total nitrogen in plant material. *Agron. J.* **65**, 109–112. <https://doi.org/10.2134/agronj1973.00021962006500010033x> (1973).
- Drózdź, D. *et al.* Management of poultry manure in Poland—Current state and future perspectives. *J. Environ. Manag.* **264**, 110327. <https://doi.org/10.1016/j.jenvman.2020.110327> (2020).
- Hartz, T. K., Mitchell, J. P. & Giannini, C. Nitrogen and carbon mineralization dynamics of manures and composts. *HortScience* **35**(2), 209–212. <https://doi.org/10.21273/HORTSCI.35.2.209> (2000).
- Lin, M. *et al.* Optimally combined application of organic and chemical fertilizers increases grain yield and improves rhizosphere microecological properties in rice ratooning. *Crop Sci.* **63**, 764–783. <https://doi.org/10.1002/csc.20891> (2023).
- Liu, B., Wang, X., Ma, L., Chadwick, D. & Chen, X. Combined applications of organic and synthetic nitrogen fertilizers for improving crop yield and reducing reactive nitrogen losses from China's vegetable systems: A meta-analysis. *Environ. Pollut.* **269**, 116143. <https://doi.org/10.1016/j.envpol.2020.116143> (2021).
- Zhai, L. *et al.* Combined application of organic fertilizer and chemical fertilizer alleviates the kernel position effect in summer maize by promoting post-silking nitrogen uptake and dry matter accumulation. *J. Integr. Agric.* **1**, 1–22. <https://doi.org/10.1016/j.jia.2023.05.003> (2023).
- Iqbal, A. *et al.* Combined application of manure and chemical fertilizers alters soil environmental variables and improves soil fungal community composition and rice grain yield. *Front. Microbiol.* **13**, 856355. <https://doi.org/10.3389/fmicb.2022.856355> (2022).
- Bernardi, A. C., Souza, G., Polidoro, J. C., Paiva, P. R. P. & Monte, M. B. Yield, quality components, and nitrogen levels of silage corn fertilized with urea and zeolite. *Commun. Soil Sci. Plant Anal.* **42**(11), 1266–1275. <https://doi.org/10.1080/00103624.2011.571980> (2011).
- Ma, R., Jiang, C., Shou, N., Gao, W. & Yang, X. An optimized nitrogen application rate and basal topdressing ratio improves yield, quality, and water- and N-use efficiencies for forage maize (*Zea mays* L.). *Agronomy* **13**, 181. <https://doi.org/10.3390/agronomy13010181> (2023).

35. Islam, M. R. & Garcia, S. C. Rates and timing of nitrogen fertilizer application on yield, nutritive value and nutrient-use efficiency of early- and late-sown forage maize. *Grass Forage Sci.* **67**, 24–33. <https://doi.org/10.1111/j.1365-2494.2011.00816.x> (2012).
36. Agnew, J. *et al.* The effect of nitrogen fertility rate and seeding rate on yield, nutritive value and economics of forage corn in a low corn heat unit region of Western Canada. *Field Crops Res.* **283**, 108520. <https://doi.org/10.1016/j.fcr.2022.108520> (2022).
37. Novoa, R. & Loomis, R. S. Nitrogen and plant production. *Plant Soil* **58**, 177–204. <https://doi.org/10.1007/BF02180053> (1981).
38. Plénet, D. & Lemaire, G. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical N concentration. *Plant Soil* **216**, 65–82. <https://doi.org/10.1023/A:1004783431055> (1999).
39. Gelaye, Y. Effect of combined application of organic manure and nitrogen fertilizer rates on yield and yield components of potato: A review. *Cogent Food Agric.* **9**, 2217603. <https://doi.org/10.1080/23311932.2023.2217603> (2023).
40. Lazzano, C., Revilla, P., Malvar, R. A. & Dominguez, J. Yield and fruit quality of four sweet corn hybrids (*Zea mays*) under conventional and integrated fertilization with vermicompost. *J. Sci. Food Agric.* **91**(7), 1244–1253. <https://doi.org/10.1002/jsfa.4306> (2011).
41. Carvalho, W. G. *et al.* Silage quality of corn and sorghum added with forage peanuts. *Rev. Caatinga* **29**(2), 465–472. <https://doi.org/10.1590/1983-21252016v29n224rc> (2016).
42. Szymańska, G., Sulewska, H., Faligowska, A. & Ratajczak, K. Evaluation of nutritive value and quality of maize (*Zea mays* L.) silage depending on cutting height and ensiling additive. *Fragm. Agron.* **35**(1), 94–105. <https://doi.org/10.26374/fa.2018.35.09> (2018).
43. Mandić, W., Bijelić, Z., Krnjaja, V., Ružić Muslić, D. & Petrović, V. C. Effect of different nitrogen fertilization levels on maize forage yield and quality. In *Proc./11th International Symposium Modern Trends in Livestock Production, 11–13 October 2017, Belgrade* 290–303 (2017).
44. Sung, J., Kim, W., Oh, T. K. & So, Y. S. Nitrogen (N) use efficiency and yield in rice under varying types and rates of N source: Chemical fertilizer, livestock manure compost and food waste-livestock manure compost. *Appl. Biol. Chem.* **66**, 4. <https://doi.org/10.1186/s13765-022-00766-y> (2023).
45. Tong, B., Hou, Y., Wang, S. & Ma, W. Partial substitution of urea fertilizers by manure increases crop yield and nitrogen use efficiency of a wheat–maize double cropping system. *Nutr. Cycl. Agroecosyst.* **127**, 11–21. <https://doi.org/10.1007/s10705-022-10219-z> (2023).
46. Ren, K. *et al.* Effect of replacing synthetic nitrogen fertilizer with animal manure on grain yield and nitrogen use efficiency in China: A meta-analysis. *Front. Plant Sci.* **14**, 1153235. <https://doi.org/10.3389/fpls.2023.1153235> (2023).
47. Tian, X., Zhang, Ch. & Ju, X. Crop responses to application of optimum nitrogen fertilizers on soils of various fertilities formed from long-term fertilization regimes. *Eur. J. Agron.* **148**, 126857. <https://doi.org/10.1016/j.eja.2023.126857> (2023).
48. Duan, Y. *et al.* Long-term evaluation of manure application on maize yield and nitrogen use efficiency in China. *Soil Sci. Soc. Am. J.* **75**(4), 1562–1573. <https://doi.org/10.2136/sssaj2010.0315> (2011).

Author contributions

Conceived the experiment: A.W. Project planning: A.W. Experimental work: M.K. and A.W. Resources: A.W. and M.K. Data analysis: A.W. Prepared tables: A.W. Wrote the main manuscript text: A.W. Both authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.W.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024