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RESEARCH

Variability of mineral nitrogen contents in soil as affected by meat and bone meal used as fertilizer

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In recent years, a number of alternative sources of organic matter have been discovered, such as products made of waste materials and recycled into composts or as meal of meat and bone. Meat and bone meal, a by-product of the meat industry, is rich in N and P and hence it can be a viable alternative to mineral fertilizers. This study determined the direct effect of different doses of meat and bone meal (MBM) used as fertilizer on the content of mineral N in soil. The effect of MBM fertilizer applied at rates of 1.0, 1.5, 2.0, and 2.5 t ha^{-1} was compared with no fertilization. The experiment was conducted in the years 2007-2009 at the research station in Bałcyny, Poland. MBM was applied every year for 3 yr, with the following crop sequence: 2007 winter wheat (*T. aestivum*), 2008 winter rape (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg.), and 2009 spring wheat. Determination of mineral N (NO_3^--N and NH_4^+-N) were taken from the 0-30 cm layer, each year, during the full plant vegetation. The study found that changes in the mineral N content in soil depended on the dose of MBM and the crop species in a sequence. Each 0.5 t of MBM above 1.0 t ha^{-1} increased the mineral N content by an average of 4 mg. MBM applied every year at 2.0 and 2.5 t ha^{-1} produced a 2.33- and 2.56-fold increase in the mineral N content compared to unfertilized soil. The rate of release of NO_3^--N was found to be the highest at those sites in all the years of study, while that of NH_4^+-N was highest during the first 2 yr of study. The levels of NO_3^--N lay within the range of very low fertility. A strong correlation was found between NO_3^--N and NH_4^+-N content in soil and the N content in winter and spring wheat (*Triticum aestivum* L.) grain and in winter rapeseed (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg.) The NO_3^--N and NH_4^+-N compounds released from MBM were a good source of N for the plants.

Key words: Ammonium nitrogen, *Brassica rapa*, mineral nitrogen, nitrate nitrogen, nitrogen dynamics, *Triticum aestivum*.

INTRODUCTION

Meat and bone meal (MBM) is a by-product in meat processing industry and an organic fertilizer which has been used increasingly often in Europe (Jeng et al., 2006; Tammeorg et al., 2012). Before 2000, MBM was used in fodder additives, but due to the risk of transmission of contagious diseases through various vectors, including the most dangerous vector of bovine spongiform encephalopathy (BSE), its use was banned in EU countries. The MBM is a natural source of elements and it contains mainly N (8%) and P (5%) (Tenuta and Lazarovits, 2003; Jeng et al., 2006; Garcia and Rosentrater, 2008; Ylivainio et al., 2008; Chen et al., 2011). Therefore, MBM has an indirect positive effect on the environment because it limits the demand for mineral fertilizers and provides a way of destroying huge amounts of meat processing industry waste (Jeng et al., 2004; 2006; Mondini et al., 2008; Ylivainio et al., 2008; Fernandes et al., 2010; Chen et al., 2011).

There have been papers published in recent years on the effect of organic fertilizers on the content of the main storage polymers (carbohydrates and proteins) and micro- and macronutrients in the grains of various crops (Ylivainio et al., 2008; Chen et al., 2011; Konopka et al., 2012; Stępień and Wojtkowiak, 2013). A generally positive effect of MBM on the yield and quality has been reported.

Mineralization of N and C are the main processes which regulate the availability of nutrients to plants and the release of toxic compounds (Abaye and Brookes, 2006; Mondini et al., 2010). A high content of organic matter in MBM (approx. 50% C) has a beneficial effect on the physical, chemical and microbiological properties of soil. A low C:N ratio in MBM favors a more rapid mineralization of N than many other organic fertilizers (Jeng et al., 2004; Mondini et al., 2008; Tammeorg et al., 2012). The final stage of the transformation of N, which is present in organic compounds in soil, is the amount of ammonium and N ions (Van Den Bossche et al., 2009). Many researchers (Jeng et al., 2004; Chaves et al., 2005; Jeng et al., 2006; Jeng and Vagstad, 2009) have reported

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that, through mineralization of MBM, N is released to the soil and it usually becomes available to plants during the first year when MBM is used. However, being affected by numerous factors, the process of mineralization of organic matter is highly unpredictable. Therefore, the amount and timing of the application of such waste should be considered carefully in order to avoid loss of NO_3^--N and to optimize N use. Jeng and Vagstad (2009) go so far as to recommend that the fertilizer should not be used in early spring or in late summer because of the possibility of N loss to the environment.

The aim of this study was to determine the effect of different doses of meat and bone meal used as fertilizer on the content of mineral N species in soil.

MATERIALS AND METHODS

Site and experimental set-up

The findings published in this paper were obtained in a field experiment set up in a randomized complete-block design in four replicates. The experiment with the use of MBM was conducted in the years 2007-2009 at the research station in Bałcyny (53º36' N, 19º51' E; 137 m a.s.l.), Poland. The experiment in which MBM was applied every year was conducted for 3 yr, with the following crop sequence: 2007 winter wheat (*T. aestivum*); 2008 winter rape (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg.), and 2009 spring wheat. The area of plots was 24.75 m^2.

Sowing, agricultural procedures, and harvesting were performed in accordance with agrotechnical requirements for those crops. Ploughing at a depth of 25 cm was combined with complementary cultivations. Weed control was performed only mechanically, while pest or disease control was not performed at all. Physical and chemical soil properties before the experiment started in 2007 are presented in Table 1. The plan of the experiment and doses of nutrients supplied with MBM are presented in Table 2.

Meal meat bone (MBM)

MBM contained small amounts of K, which is why it was classified as an N-P fertilizer (Table 3). The MBM used in the study was in the form of powder and classified as category 3, which comprises animal by-products derived from the manufacture of products intended for human consumption and it was purchased from the Animal By-Products Disposal Plant SARIA Poland. MBM contained, on average, 95.5% DM, 410.0 g C, 66.5 g N, 42.1 g P, 4.2 g K, 31.0 g Ca, and 2.0 g Mg kg^{-1} DM. Due to small K content of MBM (4.2 g), additional K at 83.1 kg K ha^{-1} as 49.8% potassium salt was applied in plots fertilized with it. MBM was applied before sowing at 1.0, 1.5, 2.0, and 2.5 t ha^{-1} (Table 3). The maximum amounts of MBM did not exceed those provided for in the Regulation of the Ministry of Agriculture and Rural Development of 7 December 2004 (as subsequently amended) regarding veterinary requirements applicable to soil-enriching additives.

Table 1. Physical and chemical soil properties before the experiment started in 2007.

Measured parameters	Corresponding values
Soil type (FAO, 2006)	Haplic Cambisol
Soil density, g cm^{-3}	1.36
Sample-time moisture, %	18.9
Soil temperature, ºC	17.2
pH in KCl	5.60
Coherence of soil, MPa	1.83
Total organic C, g kg^{-1} DM	10.01
Total N, g kg^{-1} DM	0.91
P, mg kg^{-1}	85.0
K, mg kg^{-1}	152.4
Mg, mg kg^{-1}	56.3

Table 2. Plan of the experiment and doses of nutrients supplied with meat and bone meal (MBM).

Treatments	N	P	K MBM	K K_{min}	Mg	Ca
	kg ha^{-1}					
Control without fertilization	0.0	0.0	0.0	0.0	0.0	0.0
1.0 t MBM ha^{-1}	66.5	39.8	4.1	83.1	2.0	19.0
1.5 t MBM ha^{-1}	99.8	59.7	6.2	83.1	3.0	28.5
2.0 t MBM ha^{-1}	133.0	79.6	8.2	83.1	4.0	38.0
2.5 t MBM ha^{-1}	166.3	99.5	10.3	83.1	5.0	47.5

MBM: Meat and bone meal applied with K mineral fertilizer; K_{min}: K of mineral.

Table 3. Chemical characteristics of the meat and bone meal used in the experiment (mean 2007-2009).

Parameter, unit	Mean value
Dry matter, %	95.5
Total N, g kg^{-1} DM	66.5
Total organic C, g kg^{-1} DM	410.0
C/N ratio	6.2
Total P, g kg^{-1} DM	42.1
Total K, g kg^{-1} DM	4.2
Total Ca, g kg^{-1} DM	31.0
Total Mg, g kg^{-1} DM	2.0

Sampling, measurements, and analyses

Before the experiment started in 2007 and during the vegetation period, soil samples from the arable layer 0-30 cm were taken each year during the period of 2007-2009 in order to determine the main parameters of soil fertility. The soil samples were joined to make combined samples, then dried and sieved through a 1 × 1 mm mesh sieve. The chemical analyses were conducted at the certified laboratory of the Regional Chemical and Agricultural Station in Olsztyn by the following methods: pH by potentiometric measurement in mixture of soil and 1 M KCl solution (1:5); organic C (C_{org}) by oxidation with $K_2Cr_2O_7$ + H_2SO_4 solution and measurement of the absorbance on a spectrophotometer; total N by mineralization of a sample with H_2SO_4 with an addition of a catalyst (Se mixture), distillation, followed by titration with sodium hydroxide solution against Tashiro indicator; available species of P and K were determined in the extract by the Egner-Riehm method; Mg by the Schachtschabel method; Ca by flame photometry in 0.03 N acetic acid extract; and NH_4^+-N was determined colorimetrically with Nessler's reagent and NO_3^--N colorimetrically with phenyldisulphophenolic acid.

Samples for determination of mineral N (NO_3^--N and NH_4^+-N) were taken from the 0-30 cm layer, each year during the period between 2007 and 2009, during the full plant vegetation: 2007 (winter wheat) blooming phase – BBCH 61-69; June 2007; 2008 (winter rape) blooming phase – BBCH 61-69, May 2008; 2009 (spring wheat) blooming phase – BBCH 61-69, June 2009 (Meier, 2001).

The protein content in grain/seeds was determined by the Kjeldahl method (Determination of crude protein ICC Standard nr 105/2, ICC. 1980). Protein yield was determined by multiplying the grain/seeds protein content by a grain/seeds yield.

Weather conditions

Compared to the multi-year period between 1961 and 2000, the temperature during the vegetation period (March-September) in the years 2007-2009 was higher by 0.6 to 1.3 °C on average (Table 4). The warmest vegetation period was recorded in 2007 (13.2 °C). This was caused mainly by the temperature in March, May, and June, which was higher than in the subsequent years. The vegetation periods in the 2007-2009 period differed in terms of rainfall, both compared to the multi-year period and between one another. The vegetation period in 2007 was much wetter (127% of the multi-year average), whereas that in 2008 was the driest (84% of the multi-year average), yet was still classified as average. Compared to the multi-year period, a considerable shortage of rainfall was recorded in April and excessive rainfall was recorded in May and June 2009.

Statistical analyses

The results were statistically processed with STATISTICA 10.0 software (StatSoft, Tulsa, Oklahoma, USA). The statistical calculations were performed with a one-way ANOVA. Apart from the basic parameters, standard deviation and statistically homogenous groups were determined with Duncan's test at $\alpha = 0.05$. Coefficients of linear correlation (r Pearson's) were calculated.

Table 4. Weather conditions in 2007-2009 and the multi-annual average of 1961-2000.

	Mean temperature				Precipitation			
Month	2007	2008	2009	1961-2000	2007	2008	2009	1961-2000
	°C				mm			
March	5.4	2.9	1.9	1.4	27.9	47.1	68.0	29.0
April	7.3	7.8	9.7	7.0	26.8	33.8	3.7	35.0
May	13.7	12.3	12.2	12.5	79.7	48.4	89.6	58.0
June	17.5	16.6	14.7	15.8	60.8	27.8	133.1	70.0
July	17.5	18.3	18.9	17.2	176.5	47.0	82.2	82.0
August	18.2	17.8	18.5	16.8	81.0	103.1	25.7	75.0
September	12.6	11.8	14.7	12.6	65.4	17.0	15.6	59.0
Mean	12.5	12.0	12.1	11.4				
Sum					567.0	428.8	476.4	462.0

RESULTS AND DISCUSSION

Applying MBM as fertilizer did not bring about any considerable changes in pH (5.47-5.62 in 1 mol KCl dm^{-3}) (Table 5). The findings of other studies (Valenzuela et al., 2001; Deydier et al., 2003; Jeng et al., 2006) of the effect of MBM on soil pH have been frequently inconclusive and contradictory. This may be caused by different soil composition, slaughter waste, different processing methods or by different weather conditions during the experiment. Similar to the study conducted by Jeng et al. (2006), MBM as a potential source of Ca did not raise soil pH, which may be an effect of the action of microorganisms, whose presence is stimulated by such organic waste.

A high content of available P was found in soil with no fertilization and in that fertilized with MBM at 1.0 and 1.5 (82.5 and 79.2 g kg^{-1}) and the content was very high when MBM was applied at 2.0 and 2.5 t ha^{-1} (94.0 and 91.5 g kg^{-1}) (Table 5). The soil under study contained similar amounts of available K (137.1-142.6 g kg^{-1}). The levels of MBM fertilization applied in the study did not bring about a change in the organic C content (9.03-9.38 g·kg^{-1}) or total N (0.870-0.943 g kg^{-1}) in soil. A positive effect of organic fertilizers on the total content of C and N was shown by Nett et al. (2010) and Zhengchao et al. (2013) and that of post-slaughter waste by Cayuela et al. (2009).

The C/N ratio in the MBM used in this study (Table 3) was higher (6.2) than that used in other European countries: 3.7 (Jeng et al., 2004) and 3.6-4.4 (Mondini et al., 2008). The C/N ratio is frequently used to estimate the quality of organic matter in soil, e.g. in terms of the decomposition and nitrification processes. Introduction of organic substances with a low C/N ratio (below 10) stabilizes the processes of mineralization of C and N (Dignac et al., 2002). No significant differences within the C/N ratio in soil have been found in this study.

The total content of available species of mineral N (NH_4^+-N and NO_3^--N) in soil ranged from 7.56 to 19.35 mg kg^{-1} DM, which accounted for 0.83% to 2.12% of the total N content (Table 5). Jeng et al. (2004) showed that although N is present in MBM in organic forms, it is available to plants at 80% during the first year. The content of mineral N (N_{min}) in this study increased significantly until the MBM dose was raised to 2.0 t ha^{-1}. After annual

Table 5. Physical and chemical parameters of soils (2007-2009 mean).

Parameters	Without fertilization	MBM 1.0 t ha^{-1}	MBM 1.5 t ha^{-1}	MBM 2.0 t ha^{-1}	MBM 2.5 t ha^{-1}
pH	5.62a	5.57a	5.47a	5.54a	5.47a
P, mg kg^{-1}	71.0b	82.5ab	79.2ab	94.0a	91.5ab
K, mg kg^{-1}	142.6a	141.6a	140.4a	137.3a	137.1a
C_{org}, g kg^{-1}	9.37a	9.32a	9.03a	9.38a	9.23a
N_{tot}, g kg^{-1}	0.920a	0.870a	0.943a	0.938a	0.912a
C_{org}/N_{tot}	10.19b	15.87a	9.57b	9.99b	10.12b
N_{min}, mg kg^{-1}	7.56d	9.99c	13.62b	17.67a	19.35a

Values with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$).
MBM: Meat and bone meal; C_{org}: organic C; N_{tot}: total N; N_{min}: mineral N.

application of MBM at 2.0 and 2.5 t ha^{-1}, a 2.33- and 2.56-fold increase in N_{min} content was recorded, respectively, compared to unfertilized soil. Each additional 0.5 t of MBM above 1.0 t ha^{-1} increased the content of N_{min} in soil by 4 mg. An increase in the content of mineral species of N in soil caused by mineralization of the organic materials, including MBMs, has been confirmed by studies conducted by Mondini et al. (2008), Cayuela et al. (2009), and Nogalska (2013).

The final issue of the transformation of N in soil present in organic matter is the amount of ammonium and N ions. Depending on the MBM dose and the crop, the domination of the nitrate (6.83-26.30) over the ammonium (4.94-21.26) form of mineral N was observed (Table 6). The domination of N ions can be attributed to the rate of mineralization of organic N, which favored accumulation of this N species. The nitrification process in this case was faster and ammonium ions were probably oxidized to nitrate ones. The rapid process of nitrification of organic substance and the resulting smaller amounts of NH_4^+-N were confirmed by laboratory tests conducted by Van Den Bossche et al. (2009). The content of nitrate N determined during the study in the arable layer lay within the standard range and was classified as very low. Jeng and Vagstad (2009) do not recommend the application of animal meals in early spring or late autumn due to possible leaching of nitrates. The mineralization of nitrates from the MBM-treated soil was extremely rapid: within 2 wk incubation, nitrate concentrations exceeded the maximum levels found in other fertilizer treatments at the end of the 133-d experiment (Tammeorg et al., 2012).

The highest rate of NO_3^--N release was observed after MBM was applied at 2.0 and 2.5 t ha^{-1} during all the years of study, while that of NH_4^+-N during the first 2 yr. The amount of nitrate N released in soil may have been affected by insufficient use by plants (with an excessive amount of this N species) released from organic matter. Nogalska (2013) confirmed a considerable increase in NO_3^--N content as a result of the annual application of MBM at 2.5 t ha^{-1} and even consecutive use of large doses of MBM (4.0 and 5.0 t ha^{-1}). The application of MBM at the lowest dose (1.0 t ha^{-1}) in this study did not result in a change in the content of NO_3^--N or NH_4^+-N compared to control (no fertilization).

The NO_3^--N/NH_4^+-N at most sites was higher than 1 and there was no significant dependence on the system of fertilization (Table 6). According to Pawluczuk and Stępień (2010) this is indicative of high biological activity of soils and favorable conditions for nitrification. NO_3^--N and NH_4^+-N released as a result of nitrification were a source of N for plants and they affected the crop yield when the largest dose of MBM was applied (2.5 t ha^{-1}) in all the years of study (Table 7).

Moreover, MBM applied at 1.0 to 2.5 t ha^{-1} had a particularly beneficial effect on the yield of grain of winter wheat (2007), which was attributable to the high content of mineral N in soil in that year of study. Such diverse content of this N species may have been caused by biological N supplied with faba bean grown as a forecrop, which was confirmed by Rodrigues et al. (2006). Therefore, as recommended by Adamiak et al. (2002), N fertilization should be balanced carefully in order to avoid loss of NO_3^--N and to optimally employ N from MBM.

This study has shown that the content of NO_3^--N and NH_4^+-N was not correlated with that of total N and, in most cases, with the other components responsible for soil fertility (Table 8). The NO^-_3-N content in the second year of the study was negatively correlated with

Table 6. Content of NO_3^--N and NH_4^+-N in soil.

Treatment	2007[1]			2008[1]			2009[1]		
	NO_3^--N	NH_4^+-N	NO_3^--N/ NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N/ NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N/ NH_4^+-N
	mg kg^{-1}								
Without fertilization	9.12c	4.94b	1.85a	6.83c	9.08c	1.20a	9.60a	9.60a	0.96a
MBM 1.0 t ha^{-1}	14.14bc	7.29b	2.05a	9.27bc	10.52c	0.98a	9.47a	9.47a	1.25a
MBM 1.5 t ha^{-1}	18.05abc	13.80a	1.31a	12.24b	15.08b	0.92a	9.18a	9.18a	1.68a
MBM 2.0 t ha^{-1}	24.73ab	16.26a	1.54a	18.32a	19.01a	1.14a	11.33a	11.33a	1.73a
MBM 2.5 t ha^{-1}	26.30a	16.43a	1.60a	17.84a	20.23a	0.85a	14.04a	14.04a	1.44a

[1]During the full plant vegetation: 2007 (winter wheat) blooming phase – BBCH 61-69; June 2007; 2008 (winter rape) blooming phase – BBCH 61-69, May 2008; 2009 (spring wheat) blooming phase – BBCH 61-69, June 2009.
Values with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$).
MBM: Meat and bone meal.

Table 7. The effect of meat and bone meal (MBM) on grain, seeds, and protein yield.

Treatment	2007 (winter wheat)		2008 (winter rape)		2009 (spring wheat)	
	Grain yield	Protein yield	Seed yield	Protein yield	Grain yield	Protein yield
	t ha^{-1}	kg ha^{-1}	t ha^{-1}	kg ha^{-1}	t ha^{-1}	kg ha^{-1}
Without fertilization	5.13c	587.0c	3.94b	747.6c	4.42b	720.9c
MBM 1.0 t ha^{-1}	5.77b	648.5bc	4.59ab	923.1bc	4.77ab	800.7bc
MBM 1.5 t ha^{-1}	6.17ab	774.0ab	4.95ab	973.9ab	4.83ab	799.2bc
MBM 2.0 t ha^{-1}	6.16ab	856.1a	4.77ab	989.2ab	4.86ab	859.7ab
MBM 2.5 t ha^{-1}	6.37a	931.1a	5.27a	1147.4a	4.99a	960.7a

Values with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$).
Protein yield was determined by multiplying the grain/seeds protein content by a grain/seeds yield.

Table 8. Coefficient of correlation between the content of NO_3^--N and NH_4^+-N in soil and the parameters pH, P, K, N_{tot}, C_{org}, C/N, N_{tot} in grain/seeds, and yield of grain/seeds.

Parameters		NO_3^--N			NH_4^+-N		
		2007	2008	2009	2007	2008	2009
Soil parameters	pH	ns	-0.650	ns	ns	-0.697	ns
	P	ns	ns	0.851	ns	ns	ns
	K	ns	-0.658	ns	ns	-0.678	ns
	N_{tot}	ns	ns	ns	ns	ns	ns
	C_{org}	ns	ns	ns	ns	-0.703	ns
	C/N	ns	ns	ns	ns	ns	ns
N_{tot} in grain/seeds		0.814	0.770	0.812	0.808	0.827	0.713
Grain yield/seeds		0.734	0.655	0.715	0.860	0.734	ns

ns: Nonsignificant differences ($P \leq 0.05$); N_{tot}: total N; C_{org}: organic C.

K content (r = -0.658) and with pH (r = -0.650), while NH_4^+-N with the content of K (r = -0.678), organic C (r = 0.703) and pH (r = -0.697) of the soil. Furthermore, positive correlation of NO_3^--N content with the P content (r = 0.851) was observed in the third year of the study. It is noteworthy that the content of NO_3^--N and NH_4^+-N in soil are strongly correlated with the N content in grain of winter and spring wheat and in winter rapeseed. A positive correlation was also observed between the yield of grain and the NO_3^--N and NH_4^+-N content in soil. The only exception was no correlation observed between the NH_4^+-N content and the yield of spring wheat grain in 2009.

CONCLUSIONS

After meat and bone meal (MBM) was used every year at 2.0 and 2.5 t ha^{-1}, the content of mineral N was found to increase 2.33- and 2.56-fold compared to unfertilized soil. Each additional 0.5 t MBM above 1.0 t ha^{-1} resulted in an increase in the mineral N content in soil by 4 mg. The highest rate of release of NO_3^--N was observed after MBM was applied at 2.0 and 2.5 t ha^{-1} in all the years of the study, whereas for NH_4^+-N the highest rate was in the first 2 yr of the study. NO_3^--N and NH_4^+-N released in the mineralization process caused the crop yield to increase after the largest dose of MBM (2.5 t ha^{-1}) was used in all the years of the study. The study found that the content of NO_3^--N and NH_4^+-N was not correlated with the total N content or, in most cases, with other parameters responsible for soil fertility. It is noteworthy that there is a strong correlation between NO_3^--N and NH_4^+-N content in soil and nitrogen content in winter and spring wheat grain and in winter rapeseed. Moreover, the grain yield was also positively correlated with the content of NO_3^--N and NH_4^+-N in soil.

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