THE EFFECT OF MINERAL AND LIQUID ORGANIC FERTILISERS ON SOIL MINERAL NITROGEN AND CHLOROPHYLL CONTENT IN WINTER WHEAT LEAVES

PhD Stud. Danute Kvasoviene - Petraityte [1], Dr. Jurgita Ceseviciene [1], Dr. Ausra Arlauskiene [2], Dr. (HP) Alvyra Slepetiene [1]

[1] Chemical Research Laboratory, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Lithuania
[2] Joniskelis Experimental Station, Lithuanian Research Centre for Agriculture and Forestry, Lithuania

E-mail: danute.kvasoviene@lammc.lt, jurgita.ceseviciene@lammc.lt

Abstract: *The aim of the present study was to compare the effects of mineral and liquid organic fertilisers on the accumulation of nitrate and ammonium nitrogen in a clay loam Endocalcari Endohypogleyic Cambisol and on chlorophyll content in common winter wheat (Triticum aestivum L. emend. Fiori et Paol.) leaves. Ammonium nitrate was used as a mineral fertiliser. Liquid bio-fertilisers – pig slurry and anaerobic digestate – were studied with and without nitrification inhibitor (DMPP). In spring, after resumption of winter wheat vegetation, the investigated fertilizers were applied at N_{120} rate. Soil samples were taken from the 0-30 and 30-60 cm soil depths one and a half months after fertilisation. Dry weather prevailed during the study period. Results showed that the mineral nitrogen content in the soil varied depending on the fertiliser used and a slight effect of DMPP was observed. Compared to the unfertilised treatment, the highest and significant increase in mineral (nitrate and ammonium) nitrogen content was determined in the deeper soil layer of the mineral fertilisation treatment.* Neither nitrogen rate nor fertilisers of different composition had influence on leaf chlorophyll content.

Keywords: AMMONIUM NITRATE, PIG SLURRY, LIQUID ANAEROBIC DIGESTATE, DMPP, SOIL NITRATE AND AMMONIUM NITROGEN, CHLOROPHYLL CONTENT.

1. Introduction

The interaction among balanced economic development, sustainable farming and natural environment has been receiving increasing attention recently. The efficiency of synthetic N fertilizers is declining as only 47 % of mineral fertilizer nitrogen is converted into products compared with 68 % in 1960 (Ladha et al., 2016). In addition, significant amounts of nitrogen are lost to the environment, wasting resources, endangering air, water, soil and biodiversity, and generating greenhouse gas emissions (Godinot et al., 2014; Lassaletta et al., 2014). Farming systems should be less reliant on resource imports and negative environmental effects should be minimized (Thorup-Kristensen, 2012), with the ultimate goal being production quality not quantity and soil productivity (Sutton et al., 2013).

When using nitrogen from various organic sources (plant residues, green manure, farmyard manure, slurry, waste products of bioenergy production (digestates), composts, etc.), the uptake of nitrogen compounds and its intensity have a marked effect on plant nutrition and productivity (Opheusden et al., 2012) and on soil quality (Bhowmik et al., 2016). According Johansen et al. (2013), plant and animal-derived digestates stimulate mineralization processes in the soil and increase mineral N content by 30-40 % compared with cattle slurry.

The use of N fertilizers, especially organic liquid, can lead to a loss of nitrogen (N) during and after fertilization, which can have negative environmental effects. Depending on the environmental and soil conditions, ammonium ($N\text{-}NH_4$) nitrogen is converted into nitrates ($N\text{-}NO_3$). Microbial nitrification and denitrification processes can result in losses of nitrogen oxide (N_2O) causing greenhouse gas emissions. Moreover, $N\text{-}NO_3$ is more mobile in the soil than $N\text{-}NH_4$, and can therefore leach into deeper soil layers. N-stabilizing nitrification inhibitors capable of slowing $N\text{-}NH_4$ conversion to $N\text{-}NO_3$ and reducing NO_2 emission are used to reduce potential N losses and increase the value of organic fertilizers (Huérfano – Salinas et al., 2016; Kong et al, 2016; Torralbo et al, 2017; Corrochano-Monsalve et al., 2018). Livestock slurry and biogas digestate have recently been used in large quantities throughout Europe as valuable fertilizers (Insam et al., 2015). It is maintained that digestate can be an effective way to reduce nitrogen losses compared with mineral fertilizers when using the same N rates (Verdi et al., 2019); however, the yield may not always differ significantly (Šimon et al., 2015; Verdi et al., 2019), and the positive effect of total N incorporated with organic fertilizers may be felt after several years (Cavali et al., 2016).

The aim of present study is to determine the accumulation of mineral (nitrate and ammonium) nitrogen in a clay loam soil and chlorophyll content in common winter wheat *(Triticum aestivum L. emend. Fiori et Paol.)* leaves by comparing the effect of mineral fertiliser and liquid organic fertilisers, pig slurry and anaerobic digestate, using them with and without a nitrification inhibitor.

2. Materials and methods

2.1. Site and soil description

The field experiment was conducted in 2018/2019 in the northern part of Central Lithuania's lowland (56°12′ N, 24°20′ E) at the Joniskelis Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry (LAMMC). Research was carried out on a drained clay loam with deeper lying sandy light loam whose parental rock is limnoglacial clay on morenic loam. According to the classification of Lithuania's soils, it is assigned as *Endocalcari Endohypogleyic Cambisol* (Cmg-n-w-can). The topsoil (0–30 cm) pH is close to neutral (6.8), moderate in humus (28.1 g kg^{-1}) and total nitrogen (Ntot 1.83 g kg^{-1}); medium in available phosphorus (P_2O_5 141 mg kg^{-1}) and high in available potassium (K_2O 387 mg kg^{-1}).

2.2. Experimental design and details

In the autumn, pre-sowing, complex mineral fertilizers $N_{32}P_{32}K_{32}$ were applied in the experimental field. Fertilizer rates were chosen according to the status of soil available phosphorus and potassium. A winter wheat cultivar 'Patras' was sown at a rate of 4.5 million seeds ha^{-1}. A preceding crop was cereal.

The treatments were laid out in a randomized design with three replications, where every replication is in a separate band. The size of each replicate plot area was 75 m^2 (15×5 m).

Winter wheat was fertilised after resumption of spring vegetation on March 25, 2019; at BBCH 23-25 growth stage. Fertiliser rate per ha was calculated according to total nitrogen. The experiment included six fertilisation treatments:

1. Control;
2. N_{120} mineral fertiliser – ammonium nitrate (AN);
3. N_{120} pig slurry (PS);
4. N_{120} pig slurry (PS) + DMPP;
5. N_{120} liquid anaerobic digestate (LD);
6. N_{120} liquid anaerobic digestate (LD) + DMPP.

Ammonium nitrate was used for mineral fertilisation, nitrogen (N 344 g kg^{-1}) composition in the fertilizer is 50 % $N\text{-}NH_4$ and 50 % $N\text{-}NO_3$. Liquid bio-fertilisers were pig slurry and anaerobic digestate, obtained under the controlled biological decomposition of pig slurry and residues of agriculture crops. Both liquid fertilizers are based on ammonium. A detailed nutrient composition of the applied bio-fertilisers is provided in Table 1.

Table 1. Characteristics of liquid bio-fertilisers

Ferti-liser	pH	DM	Corg	Ntot		N-NH_4	N-NO_3	P	K
		g kg^{-1}	g kg^{-1}	g kg^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	g kg^{-1}	g kg^{-1}
PS	7.5	14.3	4.53	2.32	2.37	1.90	0.01	0.23	1.44
LD	8.2	8.0	1.82	1.81	2.13	1.58	0.01	0.07	1.14

3,4-dimethylpyrazole phosphate (DMPP) base product Vizura ® (BASF, Germany) was used as a nitrification inhibitor. It was mixed with liquid bio-fertilisers at a rate of 2 L ha^{-1}.

2.3. Methods of analysis and sampling

Primary soil analyses. The pH was determined by the potentiometric method (C5020, Consort, Belgium) in 1M KCl (1:2.5, w/v). The content of humus was calculated using organic carbon conversion factor 1.72, while after wet combustion organic carbon was determination by a spectrophotometric measurement at 590 nm (UV/Vis Cary 50, Varian Inc., USA) with glucose as a standard (Nikitin, 1999). The content of Ntot was determined after the wet digestion process with sulfuric acid (H_2SO_4) by the Kjeldahl method using a spectrophotometric measuring procedure at 655 nm wavelength. Available potassium (K_2O) and phosphorus (P_2O_5) extractions were done according to the Egner-Riehm-Domingo (A-L) method; measurements were carried out using the flame atomic absorption spectrometry (AAnalyst 200, Perkin Elmer, USA) in accordance with the manufacturer's instructions and spectrophotometery by colour development with molybdate-vanadate at 430 nm, respectively.

Composition of organic fertilisers. The pH of organic fertilisers was measured by the potentiometric method immediately after the homogenization of the fresh sample. Organic carbon (Corg) content was determined in the same way as in soil samples (see above). Ammonium and nitrate nitrogen was analysed spectro-photometrically using LCK 302 and LCK 339 cuvette tests (DR3900, HACH Lange, Germany) by the standard procedure. Before determination of total nitrogen, phosphorus and potassium, the samples were wet digested: for nitrogen and phosphorus with sulphuric acid (H_2SO_4), for potassium with nitric acid (HNO_3) plus hydrogen peroxide (H_2O_2). The content of Ntot was determined by the Kjeldahl method. Total phosphorus concentrations were quantified spectrophotometrically by a colour reaction with ammonium molybdate vanadate reagent at wavelength 430 nm. The total potassium content was determined by the flame atomic absorption.

Fertilisation trial. Soil sampling and analysing. Soil samples were taken from the 0-30 and 30-60 cm soil layers ~ one and a half months after fertilisation (May 13, 2019; BBCH 34-35). Five cores were randomly collected from each plot, crushed and stored in a deep freezer (−18◦C) until analysis. The concentrations of nitrate (N-NO_3) nitrogen were determined by the potentiometric method in a 1 % extract of $KAl(SO_4)_2 \times 12H_2O$ (1:2.5, w:v), and ammonium (N-NH_4) nitrogen using a spectrophotometric measurement procedure at a wavelength of 655 nm in a 1M KCl extract (1:2.5, w:v). Soil mineral N content was calculated as the sum of N-NH_4 and N-NO_3.

All chemical analyses of soil and liquid bio-fertilisers were conducted at the Chemical Research Laboratory of the Institute of Agriculture, LAMMC. All concentrations of elements and compounds are expressed on a DM basis.

The relative chlorophyll content in wheat leaves was determined using the SPAD 502 (Konica Minolta, Japan) device, Fig. 1. Wheat plants (15 plant leaves per plot) in the field were monitored twice – at the beginning of stem elongation (BBCH 28-30; April 18, 2019) and in the middle of heading stage (BBCH 54-55; Jun 2, 2019).

2.4. Meteorological conditions

The data show that as little as 10 mm of rainfall fell during the period from winter wheat spring fertilisation to soil sampling (7 weeks). The long-term average monthly precipitation of April is 37.4 mm, but in 2019 there was no rain in April so at the end of the month the soil was extremely dry. There was more abundant rainfall only at the end of May. April was not only dry, but also warm with the mean air temperature exceeding the long-term average by 2.0°C, the temperature of May was close to the long-term average. Nevertheless this month stood out by several days of heat wave with the temperatures typical of summer.

Fig. 1. Determination of chlorophyll content in leaves with SPAD 502.

2.5. Data analysis

The research data are reported as average values of replications and standard errors. The data were statistically processed using the analysis of variance method (ANOVA) with the SELEKCIJA software package (Raudonius, 2017). Statistical significance of treatments was determined according to F-test probability (*P*) level and least significant difference (LSD_{05}) values.

3. Results and discussion

3.1. Distribution of mineral nitrogen and its components in the soil

The amount of mineral nitrogen (N-NH_4 and N-NO_3) present in the soil is very important for plant nutrition. Changes in nitrogen content in the soil occur not only through its use by plants, but also due to soil microorganism activity, ambient temperature, soil moisture, organic matter content, fertilisation technologies and other factors (Haynes, 2012). It is therefore very important to know how the amount of mineral N components in the soil changes due to application of different nitrogen fertilisers under respective conditions.

In our research, the effect of fertilisation treatments (Factor B) on soil mineral N components was significant (Table 2). In addition, the amount of nitrogen accumulated at different soil depths (Factor A) differed substantially: significantly higher N-NH_4 and mineral N contents were found in the deeper layer. So, the variation of mineral N in the soil was mainly due to ammonium dynamics.

Such increases in mineral and ammonium nitrogen in the deeper soil layers are essentially characteristic of fertilised soils (Fig. 2). Evident differences were observed in all fertiliser treatments. And this shows that nitrogen applied with fertilisers can migrate from upper soil layers to lower ones not only in the presence of excess moisture (as N-NO_3), but also during periods of moisture shortage (as N-NH_4).

Table 2. Probability (*P*) level of factors and distribution of soil mineral N content and its components in the soil layers

	$N\text{-}NH_4$	$N\text{-}NO_3$	Mineral N
Soil layer (Factor A)	<0.000**	0.316ns	0.001**
Fertilisation (Factor B)	0.003**	<0.000**	<0.000**
Interaction	0.077ns	0.004**	0.027*
Factor A	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
0-30 cm	2.61	8.29	10.9
30-60 cm	6.34	9.03	15.4

*Note: $N\text{-}NO_3$ – soil nitrate nitrogen, $N\text{-}NH_4$ – soil ammonium nitrogen; ** – $P \leq 0.01$, * – $P \leq 0.05$, ns – $P > 0.05$.*

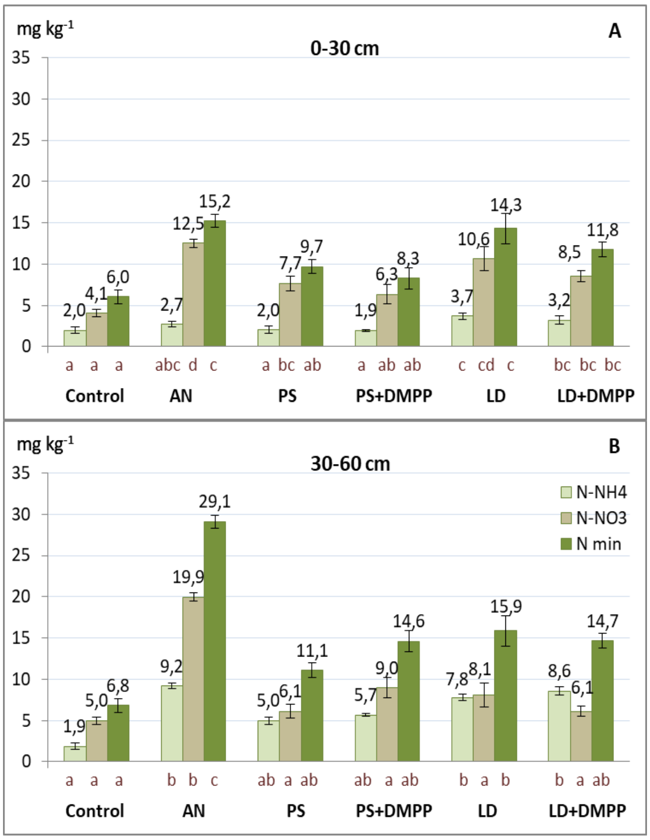

Fig. 2. The influence of fertilisers on mineral nitrogen (Nmin) and distribution of its components ($N\text{-}NH_4$ and $N\text{-}NO_3$) in diferent soil layers: A. 0-30 cm, B. 30-60 cm. *Note: Different letters indicate significant differences among the fertiliser treatments (one way ANOVA, LSD_{05}). Values are means of 3 field replications with error bars as standard errors of the mean*.

Comparison of different fertilisers for their nitrogen distribution in the soil showed the highest and significant increase of $N\text{-}NO_3$ and $N\text{-}NH_4$ contents (4.0 and 4.8 times, respectively) in a deeper soil layer when using mineral fertilisation, compared to unfertilised treatment. Fertilisation with $N\text{-}NH_4$-based liquid bio-organic fertilisers also increased the content of mineral N components, but the increase was not as pronounced as in the case of ammonium nitrate fertilisation and not always significant compared with the control. The difference of soil mineral N content in deeper (30-60 cm) soil layer, was insignificant between the liquid fertilizers used but when comparing the influence of these fertilisers in the top soil (0-30 cm), significantly higher mineral N ($P < 0.05$) contents were detected in the anaerobic digestate treatments.

In both organic fertilisers with the nitrification inhibitor DMPP, it tended to reduce the content of $N\text{-}NO_3$ in the top soil compared to equally fertilised treatments without the inhibitor. This finding agrees with that obtained by French researchers in a study dealing with soil tillage and wheat fertilization in which nitrification inhibitors substantially reduced nitrogen losses to the levels in the control in both once and twice fertilized wheat (Corrochano-Monsalve et al., 2018). Kong et al. (2016) have reported that the nitrification inhibitor DMPP was effective in reducing ammonia oxidation activity.

3.2. Chlorophyll content in winter wheat leaves

The results of winter wheat leaf chlorophyll content measured with SPAD are presented in Fig 3. The chlorophyll content ranged from 51.2 to 55.8 units, and significantly higher values (by on average 4.0), likely due to the drought, were found in leaves at the begining of stem elongation stage (BBCH 30) than in the middle of heading stage (BBCH 55). Fertilisation did not have significant influence on chlorophyll content (*P* level – 0.28 and 0.61 at BBCH 30 and BBCH 55 respectively). This partly confirms the results of other researchers who found that nitrogen fertilisers of different composition did not always have a positive effect on the chlorophyll index (Smalstienė et al, 2017).

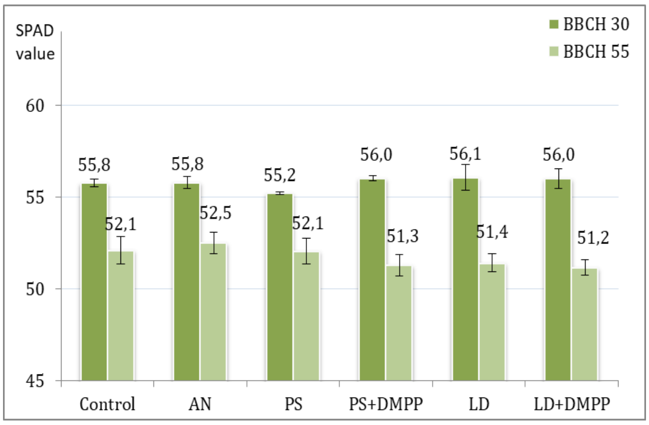

Fig 3. The influence of fertilisers on winter wheat leaf chlorophyll content at diferent plant growth stages. *Note: Values are means of 15 measurement readings with error bars as standard errors of the mean.*

4. *Conclusions*

Results showed that soil mineral nitrogen content varied depending on the fertiliser. The higher content of mineral and ammonium nitrogen in the deeper soil layer is characteristic of N_{120} fertilised soils.

The highest and significant increases in mineral (ammonium and nitrate) nitrogen contents were determined in the deeper (30-60 cm) soil layer when using mineral fertilisation. The differences between organic liquid fertilisers, pig slurry and anaerobic digestate, were not significant, the treatments fertilised with anaerobic digestate tended to have a slightly higher top soil mineral N accumulation.

The denitrification inhibitor DMPP tended to reduce the content of nitrate nitrogen in the top soils, compare to equally fertilised treatments without the inhibitor.

The wheat leaf chlorophyll contents measured with SPAD during the dry weather period were higher at the beginning of stem elongation stage than in the middle of heading stage. Neither nitrogen rate nor fertilisers of different composition had influence on leaf chlorophyll content.

Acknowledgments. This work was part of the long-term research programme "Biopotential and quality of plants for multifunctional use" implemented by the Lithuanian Research Centre for Agriculture and Forestry. The last author acknowledges the project "Quality diagnostics of biogas production by-product (digestate) for innovative use as a biofertilizer" No. DOTSUT-217 (01.2.2-LMT-K-718-01-0053) funded by the Research Council of Lithuania.

5. *References*

Bhowmik A., A.M. Fortuna, L. Cihacek, A. Bary, C.G. Cogger Use of biological indicators of soil health to estimate reactive nitrogen dynamics in long-term organic vegetable and pasture systems – Soil Biology and Biochemistry, 103, 2016, 308-319.

Cavalli D., J. Cabassi, L. Borrelli et al. Nitrogen fertiliser value of digested dairy cow slurry, its liquid and solid fractions, and of dairy cow slurry – Italian Journal of Agronomy, 2014, 9 (2), 71-78.

Corrochano-Monsalve M., X.F. Huérfano, T. Uertes-Mendizabal et al. in: 20th N Workshop and Side event, June 25-27 2018, Rennes, France, 253-254.

Godinot O., M. Carof, F. Vertčs, P. Leterme SyNE: An improved indicator to assess nitrogen efficiency of farming systems – Agricultural Systems 127, 2014, 41-52.

Haynes R. 2012. Mineral Nitrogen In The Plant-Soil System. London, Academic Press, 2012.

Huérfano-Salinas X., T. Fuertes-Mendizábal, K. Fernández-Díez et al. The new nitrification inhibitor 3.4-dimethylpyrazole succinic (DMPSA) as an alternative to DMPP for reducing N_2O emissions from wheat crops under humid Mediterranean conditions – European Journal of Agronomy, 80, 2016, 78-87.

Insam I., M. Gómez-Brandón, J. Aschera Manure-based biogas fermentation residues – Friend or foe of soil fertility? – Soil Biology and Biochemistry, 84, 2015, 1-14.

Johansen A., M.S. Carter, E.S. Jensen et al. Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO_2 and NO_2 – Applied Soil Ecology, 63, 2013, 36-44.

Kong X., Y. Duan, A. Schramm, J. Eriksen, S.O. Petersen 3.4-Dimethylpyrazole phosphate (DMPP) reduces activity of ammonia oxidizers without adverse effects on non-target soil microorganisms and functions – Applied Soil Ecology, 105, 2016, 67-75.

Ladha J.K., A. Tirol-Padre, C.K. Reddy et al. Global nitrogen budgets in cereals: A 50-year assessment for maize, rice and wheat production systems – Scientific reports, 2016, 6-19355.

Lassaletta L., G. Billen, B. Grizzetti, J. Anglade, J. Garnier 50 year trends in nitrogen use efficacy of world cropping systems: the relationship between yield and nitrogen input to cropland – Environmental Research Letters, 9 (10), 2014, 1-9.

Nikitin B.A. Metod opredeleniya gumusa pochvy [Method for determination of soil humus] – Agrokhimiya, 5, 1999, 91-93.

van Opheusden, A.H.M., G.J.H.M. van der Burgt, P.I. Rietberg. Decomposition rate of organic fertilizers: effect on yield, nitrogen availability and nitrogen stock in the soil. No. 2012-033 LbP. Louis Bolk Instituut, 2012.

Raudonius S. Application of statistics in plant and crop research: important issues – Zemdirbyste-Agriculture, 104 (4), 2017, 377-382.

Smalstienė V., I. Pranckietienė, R. Dromantienė, G. Šidlauskas The influence of different nitrogen forms and application time on winter wheat – Žemės Ūkio Mokslai [Agricultural Sciences], 24 (3), 2017, 81-90.

Šimon T., E. Kunzová, M. Friedlová The effect of digestate, cattle slurry and mineral fertilization on the winter wheat yield and soil quality parameters – Plant Soil Environment, 61 (11), 2015, 522-527.

Sutton M.A., A. Bleeker, C.M. Howard et al. Our Nutrient World: The challenge to produce more food and energy with less pollution, Edinburgh, Centre for Ecology and Hydrology, 2013.

Thorup-Kristensen K., D.B. Dresbøll, H.L. Kristensen Crop yield, root growth and nutrient dynamics in a conventional and three organic cropping systems with different levels of external inputs and N re-cycling through fertility building crops – European Journal of Agronomy, 37 (1), 2012, 66-82.

Torralbo F., S. Menéndez, I. Barrena, J.M. Estavillo, D. Marino, C. González-Murua Dimethyl pyrazol-based nitrification inhibitors effect on nitrifying and denitrifying bacteria to mitigate N_2O emission – Scientific report, 7, 2017, 13810

Verdi L., P.J. Kuikman, S. Orlandini et al. Does the use of digestate to replace mineral fertilizers have less emissions of N_2O and NH_3? – Agricultural and Forest Meteorology, 269-270, 2019, 112-118.