EFFECT OF NITROGEN FERTILIZATION ON N_2O EMISSION IN DIFFERENT SOIL REACTIONS AND GROWN GRASS SPECIES

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Abstract. This research was carried out in order to determine the effect of fertilization, or lack of it, on N_2O emission in different conditions of soil reaction and grown grass species. The pot experiment was conducted in a randomized block design with three replications in 2014–2016 (Poland). Four grass species were grown in mineral soil under mineral or organic fertilizing ($\Sigma = 150$ N kg), or lack of fertilization and different soil reaction. The effect of N-fertilization dose was determined, too. The difference of groundcover density of tested grass species was assessed. The results indicated that N_2O emission was reduced with subsequent doses by 13, 15 and 18% compared with the initial values. The application of mineral fertilization caused a higher increase of the grass biomass than the use of organic fertilizer – without any marked effect of soil reaction. The N_2O emission was influenced by the type and dose of nitrogen fertilization, the reaction of mineral soil and the biomass of the cultivated grasses. With an increase in biomass of grasses, N_2O emission was found to decrease, generally at neutral soil reaction. The highest groundcover density was found for *Festuca pratensis* and *Lolium perenne*, which translated into increased nitrogen uptake and, consequently, reduced N_2O emissions. It was determined as the optimum technique of grassland management for the reduction of N_2O emission.
Keywords: *greenhouse gas, grassland, mineral or organic fertilization, Lolium perenne, Festuca pratensis*

Introduction

Emission of N_2O is considered the most harmful emission depleting the ozone layer and, according to Ravishankara et al. (2009), it will remain such in the 21st century. The global warming potential related to the emission of this compound is 298 times greater than CO_2 emission (Myhre et al., 2013). The dangers of N_2O emission stem from the fact that its amount is not regulated under the Montreal Protocol (MP, 1987). The largest source of emissions of this anthropogenic greenhouse gas is agriculture (Bellido et al., 2015; Mori, 2016). Additionally, at the end of the 19th and the 20th century, the atmospheric accumulation rates of N_2O exceeded the projections regarding agricultural production as limited to N fertilizers and biologically fixed nitrogen fertilizers (Davidson, 2009). Out of agricultural activities, permanent grassland, i.e. meadows and pastures, contribute to a significant amount of nitrous oxide emission due to microbial nitrification and denitrification of nitrogen mineralisation in soil (Mu et al., 2009). From the perspective of environmental protection, management of grassland constitutes

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a challenge as it occupies 40% of the earth's surface and efficient shaping of its effect on the atmosphere is difficult (Maillard et al., 2017).

According to research by Chang et al. (2017), Europe has experienced a greater warming than that presented for the globe since the pre-industrial era and the warming trend is expected to increase at an even faster rate in the 21st century. Climate models for various climate scenarios project an increase of 1–5.5 °C in the annual average temperature in Europe in the period 2071–2100 as compared to 1971–2000. Grassland ecosystems play an important role in the global nitrogen cycle and provide vital ecosystem services for many species. Consequently, numerous activities have been taken to assess the pan-European reaction of grassland to anticipated climate change. The emission of N_2O can be reduced by introducing to soil the appropriate amount of mineral fertilization applicable for a given crop (Shimizu et al., 2013) or by application of manure and its subsequent mineralization (Mori and Hojito, 2015). Bouwman (1996) and Sosulski et al. (2014) identified several factors affecting N_2O emission, including soil conditions, type of crop, nitrogen (N) fertilizer type and soil and crop management. The authors highlight the fact that the observed N_2O emission from mineral soil was higher than from mineral soil – a fact also observed by Aurangojeb (2017). Dobbie et al. (1999) also pointed to the effect of nitrogen fertilization dose, temperature and amount of precipitation on N_2O emission. Signor et al. (2013) stress the role of soil reaction in N_2O emission. In turn, Koncz et al. (2017) examined the influence of grassland management depending on grazing and mowing treatments.

The literature on the subject confirms the increase in N_2O emission with an increase in the applied mineral or organic fertilizer (Bouwman, 1996; Dobbie et al., 1999; Soussana et al., 2007, 2010; Chang et al., 2015; Ciais et al., 2010; Zhang et al., 2010; Koncz et al., 2017; Aurangojeb, 2017). There is no to big information in the recent publications on the effect of split application of N fertilizer on N_2O emission levels in cultivation of species: *Dactylis glomerata* L., *Festuca pratensis* Huds., *F. rubra* L. and *Lolium perenne* L. The search for optimal techniques of grassland management for the purpose of decreasing N_2O emission led the authors of the present paper to conduct the analysis of N_2O emission and biomass of grass in the conditions of split application of nitrogen fertilizer depending on the type of fertilization and soil reaction.

The aim of the study was to determine the effect of applied fertilization, or lack of it, on nitrous oxide emission in different conditions of soil reaction and grown grass.

Material and methods

Experimental design

In the period 2014–2016, a pot experiment was conducted in a greenhouse on Faculty of Environmental Management and Agriculture of West Pomeranian University of Technology in Szczecin, Poland.

The granulometric composition of the soil material in a pot (11 kg each) corresponded to sandy loam (sand: 2–0.05 mm – 60.9%, silt: 0.05–0.002 mm – 35.1%, colloidal clay: 0.002 mm – 4.0%). It was also characterized by slightly acid reaction (pH = 5.9), total organic carbon – 9.6 $g \cdot kg^{-1}$, humus – 16.6 $g \cdot kg^{-1}$, organic substance – 37.6 $g \cdot kg^{-1}$, total nitrogen – 0.75 $g \cdot kg^{-1}$, and low content of available phosphorus – 28 $mg \cdot kg^{-1}$, potassium – 83 $mg \cdot kg^{-1}$ and magnesium – 24 $mg \cdot kg^{-1}$. Each of the four species (*Dactylis glomerata* L., *Festuca pratensis* Huds., *F. rubra* L., *Lolium perenne*

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L.) was sown in an amount of 270 seeds on ¼ of the study area of the pot under the variable fertilization and soil pH conditions.

In order to obtain variability of soil pH, calcium oxide was introduced to half of the pots producing pH = 7.0. In a growing season, the fertiliser was applied to pots three times (every six weeks, starting from the half of May, 10 days after the 1st and 2nd mowing) in two fertilization variants (mineral fertilizer – ammonium nitrate, organic fertilizer – fresh slurry) in the amount of 0.355 g of N per pot, corresponding to 50 $kg N \cdot ha^{-1}$. The control sample were pots with the analysed grass species without fertilization. The experiment was conducted in three repetitions in each subsequent year of the study. At the beginning of the experiment, phosphorus fertilization in the form of superphosphate was applied to all pots in the amount of 40 $kg P_2O_5 \cdot ha^{-1}$. In case of mineral fertilization, potassium level in the soil material was supplemented with a dose of this element in the form of K_2SO_4 to the value determined for fresh slurry, i.e. $4 kg \cdot m^{-3}$. Pot experiment was arranged in a randomized block design.

Climatic conditions of plant growth

The climate in Szczecin is moderately warm. The average annual temperature is 8.6 °C. During the experiment period of 2014–2016 the temperature varied from 9 to 24 °C, from 9 to 27 °C and from 8 to 26 °C for the respective years. The annual time of sun exposition in this period amounted to 1015 h, 955 h and 1001 h respectively. The grass was grown outdoors under controlled and optimum humidity conditions, i.e. 60% of field water capacity (the TRIME FM P3 Eikelkamp meter). Water deficits were compensated by watering and the pots were protected from atmospheric precipitation.

Measurement of N_2O fluxes

Measurements of nitrogen dioxide emission were conducted with the use of a closed static chamber after a few hours from the application of each different nitrogen fertilizer and before each mowing. Measurements of N_2O were carried out using photoacoustic field gas monitor INNOVA 1412, LumaSense Technologies, Inc.® (Burczyk et al., 2008). N_2O concentration in the chamber was recorded every minute and emission levels were noted every 15 min.

Measurement and analysis of biomass tested grass species

Each year of the experiment, during each of the three mowing treatments, a percentage density of ¼ of the study area covered by each of the tested grass species was determined on the basis of plant density. The assessment of plant density was based on the percentage share of covered area as determined according to step values (26%, 38%, 50%, 63%, 75%, 88%, 100%), partly modelled on Braun-Blanquet method (Dzwonko, 2007). Cut fresh weight (at the height of 1–2 cm) of all the tested grass species grown in each of the 18 pots in the three years of the experiment was weighed on an analytical weight. Additionally, yield of sward was determined for each mowing.

Calculations of N_2O fluxes in optimisation grassland management

The values of N_2O emission in reference to 1 kg yield of the three mowing treatments were calculated according to particular stages of the experiment as presented in *Figure 1*. Total time period covered by the calculations amounted to 4.1 months,

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starting from the application of the 1st dose to 10 days after the last mowing. Determination of the optimum management conditions was based on the calculated smallest N_2O emission in reference to 1 kg of grass biomass for all three mowing treatments.

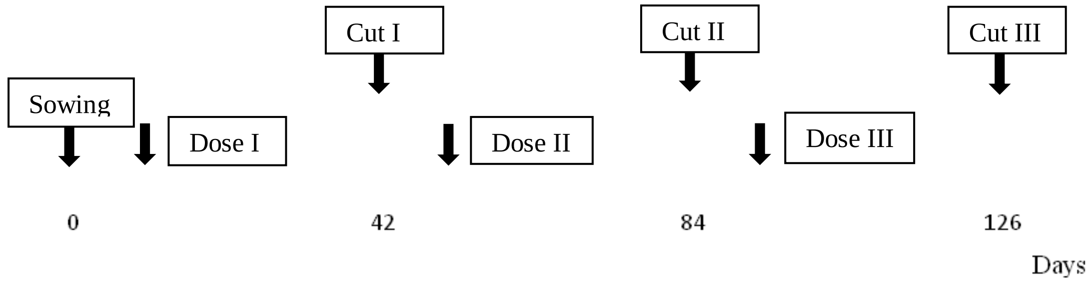

Figure 1. *Scheme of experimental design of the study: a time of sowing and a time application N-fertilizer and measurements of N_2O emission after cutting and application N-fertilizers*

Statistical analysis

Results obtained of N_2O and grass biomass were elaborated statistically with the method of two-factor analysis of variance (ANOVA: 1st factor – type of fertilization, 2nd factor – type of soil reaction) and one-way analysis of variance (dose N-fertilizer) *Statistica 12* software (StatSoft Poland). The linear correlation between analysed gas grass biomass was performer. The significance of differences between mean values and the value of Pearson correlation coefficient were evaluated with the HSD Tukey's test at $P < 0.05$. To assess the area covered by the tested grass species, a radar chart showing mean values of the nine measurement of the area covered by grass was used. Mean value of the area coverage was used for the one-way analysis of variance (grass species).

Results and discussion

Emission of N_2O fluxes from soil and after cutting of grasses

The main threats due to greenhouse gases relate to organic soil, however more and more studies address the issue in relation to mineral soil (Dobbie et al., 1999; Mu et al., 2009; Skiba et al., 2012; Aurangojeb, 2017). The experiment conducted as a part of the present study contributes to the discussion on the role of environmental factors of N_2O emission from mineral soil.

Numerous management techniques are introduced for the purpose of limiting N_2O emission, among others: split application of fertilizer (Weerasekara et al., 2018), introducing legumes to sward (Fuchs et al., 2018), injecting fresh slurry to a specific depth (Webb et al., 2010), control of soil density (Langevin et al., 2015), and the use of nitrification inhibitors (Dai et al., 2013; Duan et al., 2017).

Imer et al. (2013) report that N_2O emission is shaped by the type of climatic zone, type of soil and its pH as well as the source of nitrogen supply to soil. Moreover, emission of nitrogen greenhouse gas is additionally determined by the plant cover of soil. Consequently, lack of the plant cover results in increased emission levels. Linn and

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Doran (1984) reported, that emission of N_2O from N-fertilized, no-tillage soils was 9.4 times greater than with conventional tillage. Nitrous oxide production from nonfertilized soil was slightly greater for no tillage.

Organic nitrogen fertilizers foster the release of significant amount of ammonia and nitrous oxide (Ball et al., 2007). In our conducted experiment, following the first application of fertilizer (50 $kg \cdot N \cdot ha^{-1}$) to soil without plant cover, the highest N_2O emission levels were recorded in the conditions of acidic soil reaction and application of fresh slurry (*Fig. 2*). Under the same conditions in this experiment, mineral nitrogen fertilization applied in the same dose as organic nitrogen fertilization was found to produce a statistically lower emission of this gas. Emission levels following application of mineral and organic fertilizers to soil material of neutral pH were comparable. In the controlled conditions (without nitrogen fertilization), the value of nitrous oxide emission was approximately 50% lower than the values recorded for each type of fertilization. Merbold et al. (2014) report that following tillage and harrowing there was a pronounced increase in N_2O emission. Žurovec et al. (2017) demonstrated that with fertilization in the amount of 67.5 $kg \cdot N \cdot ha^{-1}$, reduced soil cultivation, similarly to lack of cultivation treatments, can bring favourable environmental effect due to a decrease in N_2O emission levels as compared with the conventional soil cultivation.

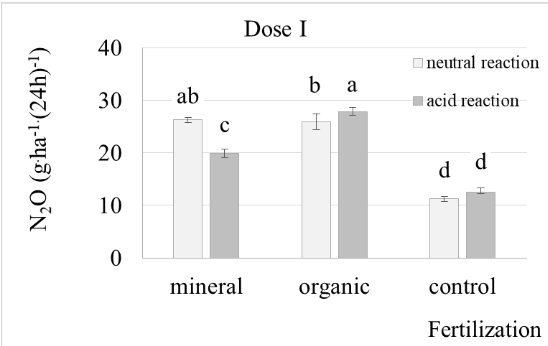

***Figure 2.** N_2O emissions from soil without a plant soil cover; different letters (a, b, c, d) indicate significant difference at $P < 0.05$ among different N-fertilizer in the same variety*

Data presented in the literature on the subject show that the highest levels of N_2O emission were recorded on different dates from fertilizer application, for example following a week (Flechard et al., 2007), two weeks (Merbold et al., 2014) or on the day of organic fertilizer application (Imer et al., 2013). In our experiment, the N_2O emission in consecutive doses associated with increasing fertilizer dose (I cut – 50 kg $N \cdot ha^{-1}$ – 24.98 g N_2O ha^{-1} 24 h^{-1}; II cut – 100 kg $N \cdot ha^{-1}$ – 15.22 g N_2O ha^{-1} 24 h^{-1}; III cut – 150 kg $N \cdot ha^{-1}$ – 12.61 g N_2O ha^{-1} 24 h^{-1}) differed significantly ($F = 162$; $P < 0.000$). The measurements of nitrous oxide emission was conducted a few hours after application of nitrogen fertilizer, therefore it is difficult to conclude whether the recorded emission levels were indeed the highest. Following the application of the second dose of nitrogen (*Fig. 3*), it was found that in the controlled conditions the N_2O emission with respect to both types of fertilization was 75% lower. It is important to note that N_2O emission for both fertilizer forms was 50% lower than that recorded in the first measurement. In turn, after the second mowing and application of the third and the last fertilizer dose, N_2O emission was found to decrease, on average, by 20% in both types of fertilization. In the

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control conditions, the values remained at the same level with respect to the effect observed following the application of the second dose. Differences in terms of N_2O emission depending on soil pH to which a given nitrogen fertilizer was applied were not remarkable, yet statistically significant following the 1st and the 2nd mowing and application of the second dose.

In our experiment, the results indicated that N_2O emission was reduced with subsequent doses by 13%, 15% and 18% as compared with the initial values.

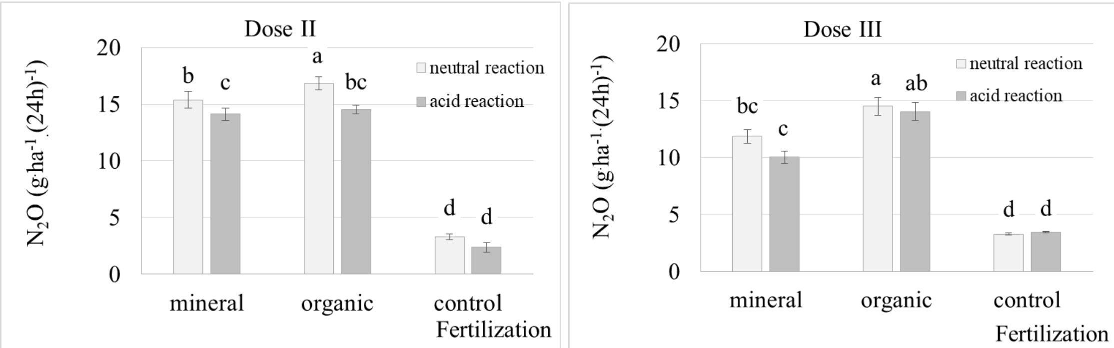

Figure 3. *N_2O emissions under conditions of 10 days after 1st and 2nd cut; different letters (a, b, c, d) indicate significant difference at $P < 0.05$ among different N-fertilizer in the same variety*

Emission of N_2O fluxes from soil after cutting grasses and grasses biomass

Grass seeds need mineral nutrients, including nitrogen, to germinate and grow (Springer, 2017). In our experiment, a 50% reduction in N_2O emission levels was observed on the day of the 1st mowing in comparison with the measurements taken following the application of the first dose of fertilizer (*Figures 2* and *4*). This resulted from nitrogen uptake by developing grass species as well as natural processes in the soil connected with nitrogen cycle and release of its gaseous form to the atmosphere (Kering et al., 2012). In our experiment, the results of N_2O emission on the day of the 1st mowing showed that this parameter was approximately 10 times greater with mineral nitrogen fertilization than organic fertilization or lack of fertilization – in this particular case, regardless of soil material pH (*Fig. 4*). Merbold et al. (2014) observe that following mineral fertilization the emission, as compared with background, increased 4–10 times. The smaller N_2O emission for slurry is due mainly to less inorganic N in slurry as compared with synthetic fertilizer as the same as reported Mori and Hojito (2011) for emission factor of manure. Similarly, the results obtained by Dobbie et al. (1999) show that nitrous oxide fluxes varies widely. In our experiment, fluxes generally increased sharply after N-fertilizer additions before tailing off to very low values, which continued until the next fertilizer addition (*Figs. 2, 3* and *4*).

In our experiment, the N_2O emission in consecutive cuts associated with increasing fertilizer dose (I cut – 50 kg N·ha^{-1} – 3.181 g N_2O ha^{-1} 24 h^{-1}; II cut – 100 kg N·ha^{-1} – 3.687 g N_2O ha^{-1} 24 h^{-1}; III cut – 150 kg N·ha^{-1} – 1.316 g N_2O ha^{-1} 24 h^{-1}) differed significantly ($F = 16.7$; $P < 0.000$). In the conditions of the second mowing in comparison with the first mowing, N_2O emission decreased by 30% with the application of mineral fertilizer, however, there was a ten-fold increase when organic fertilizer was used. The controlled conditions showed a two-fold increase in emission levels (*Fig. 4*). The results of the second moving (12–15 kg N_20-N per 100 kg N) showed higher values

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than that obtained by Dobbie et al. (1999) for cut grassland (0.3–5.8 kg N_20-N per 100 kg N) grown on mineral soils. In the last mowing, in our experiment, N_2O flux following mineral fertilization was almost 33% lower than in the previous cutting, and with organic fertilization the values were 25% lower. The emission from soil without fertilization showed a decrease, however only by 50% (*Fig. 4*).

Another feature of soil which could have an effect on N_2O emission is soil pH which can affect nitrification, denitrification and N_2/N_2O ratio. It is generally believed that N_2O reduction is inhibited at low pH (Bouwman et al., 1993). However, other authors stress that the same soil modified for different pH values showed no measurable differences in terms of N_2O emission. These results are contrary to data obtained by Sajeev et al. (2018) who observed a 21– 55% reduction in N_2O emission due to change in soil pH.

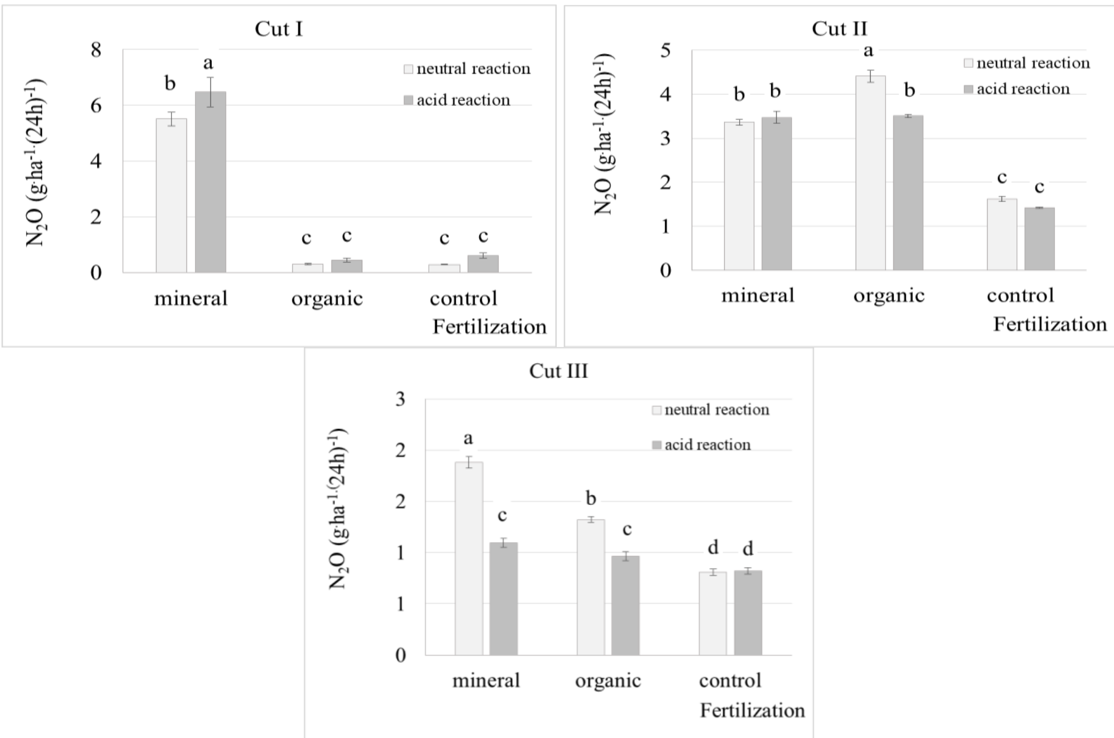

Figure 4. *N_2O emission from soil under I, II and III cut conditions; different letters (a, b, c, d) indicate significant difference at $P < 0.05$ among different N-fertilizer in the same variety*

Signor et al. (2013) demonstrated that N-fertilization implies a higher plant biomass production. In our experiment, on the basis of statistical analysis, it was found that grass biomass was dependent on the type of fertilization and soil material pH. From analysed grasses, *Lolium perenne* L. was provided with very favourable conditions for development with respect to mineral soil, as indicated by other authors (Bernal et al., 1998; Baggs and Blum, 2004; Harkot et al., 2015) and appropriate soil reaction, both slightly acidic as well as neutral, and nitrogen availability (*Fig. 5*). Hebeisen et al. (1997) pointed that high viability of the plant, even in the conditions of low nitrogen availability, is connected with the possibility of developing the root system to a depth of as much as 1.5 m and, therefore, obtaining the element from deeper layers of soil.

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Similarly to ryegrass, the conditions of *Festuca pratensis* Huds. development in our experiment also were favourable as the plant favours compact and medium compact mineral soils with sufficient moisture. Moreover, Borawska-Jarmułowicz et al. (2016) indicated that the plant's root system is well developed and reaches 1.2 m deep into the soil profile. Sosnowski (2012) demonstrated that in the conditions of nitrogen fertilization *Festuca pratensis* Huds., *Lolium perenne* L. and *Dactylis glomerata* L. showed an increase in yield – as is confirmed by the our results obtained in the 1st, 2nd and 3rd mowing treatment with the use of both types of nitrogen fertilization in comparison with the control (*Fig. 5*).

Dactylis glomerata L. shows tolerance to acidic as well as alkaline pH of mineral soil. Due to developing greater number of generative shoots (Borawska-Jarmułowicz et al., 2016), this plant is capable of a specific physiological adaptation to variable levels of mineral nutrients in soil (Crossley and Bradshaw, 1968; Zarzycki and Bedla, 2017) and shows a higher mean average increase in sward height as compared with *Festuca* variation. However, in the conditions of the experiment conducted as a part of this study, the biomass and groundcover density were found to be significantly lower as compared with *Lolium perenne* L. and *Festuca pratensis* Huds (*Fig. 5*).

In comparison to other tested grass species, *Festuca rubra L.* is characterised by a considerably lower growth rate (Zarzycki and Bedla, 2017), which translated into insignificant groundcover density observed in our experiment (*Fig. 5*).

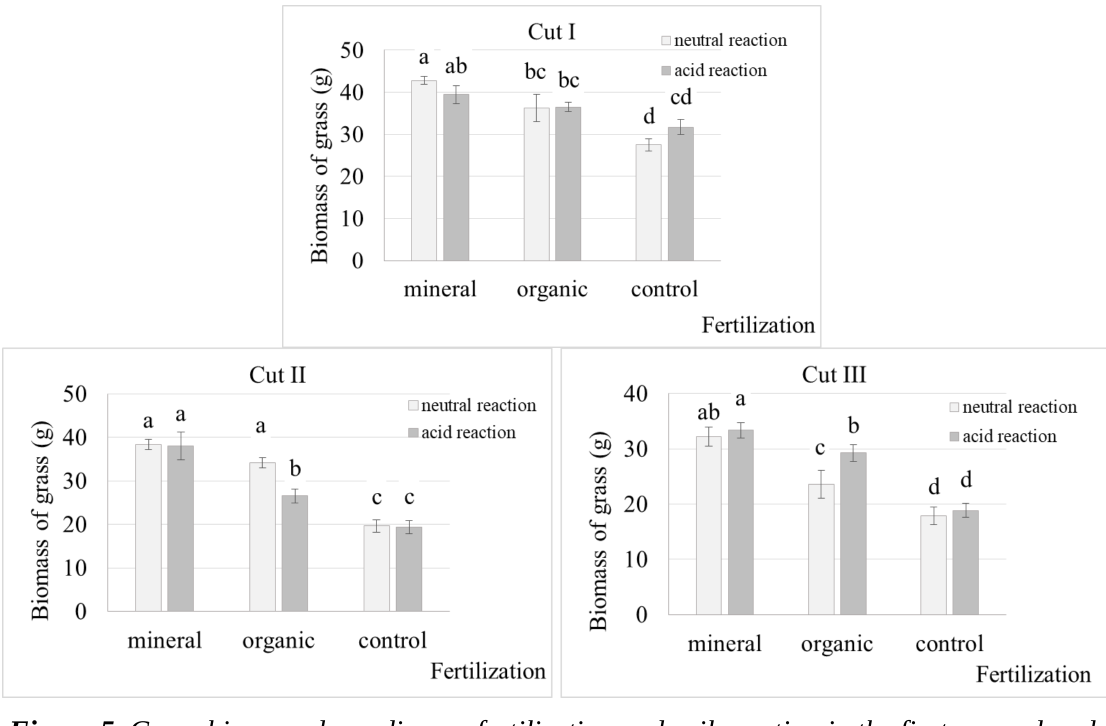

Figure 5. *Grass biomass depending on fertilization and soil reaction in the first, second and third cut; different letters (a, b, c, d) indicate significant difference at P < 0.05 among different N-fertilizer in the same variety*

Temperature and duration of sun exposition are important factors for plant yielding. Zhang et al. (2017) pointed that the amount of hours of daily sun exposition has an effect on the yield of grass only in periods of drought. In our experiment, constant soil

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moisture was maintained. The mean air temperature recorded in the subsequent years for the period between seeds sowing and the first cut was 15, 14 and 16 °C, for the period between the first and second cut, respectively 18, 18 and 19 °C, and for the period between second and third cut 18, 19 and 18 °C (*Fig. 6*). Biomass of the analysed grass for given moving treatments was connected with life process of the grown species and air temperature.

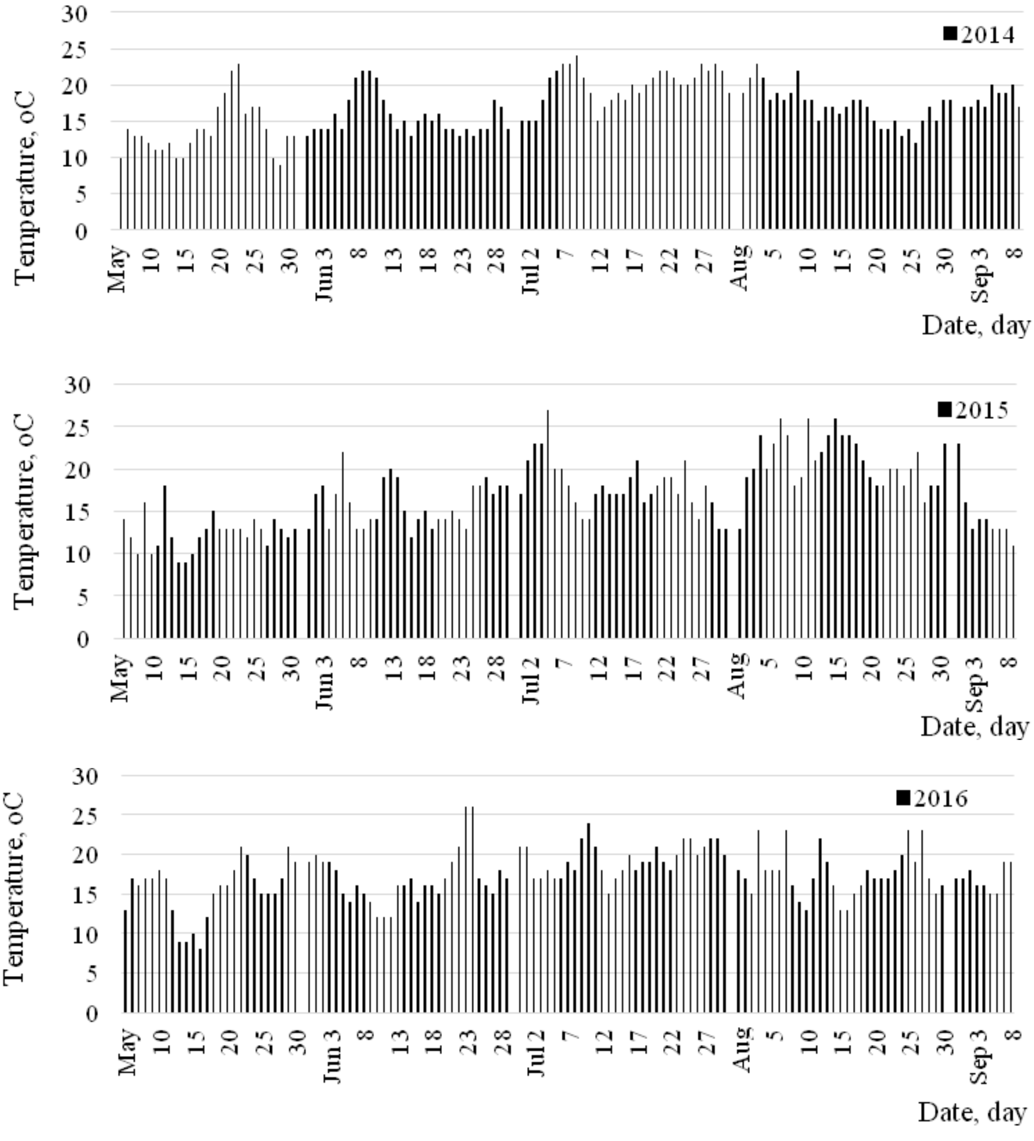

Figure 6. *Average daily air temperature from 16 May to 30 September in 2014–2016*

The lowest biomass yield for each of the mowing treatments was obtained in the conditions without nitrogen fertilization (*Fig. 5*). Markedly increased biomass in acidic soil pH was found only for the first mowing. On average, as compared with the first mowing, biomass showed a 33% and 38% decrease with subsequent mowing treatments. Biomass of the tested grass at application of mineral fertilization was higher than with the use of organic fertilizer – without any marked effect of soil material reaction. In subsequent mowing treatments, under the aforementioned fertilizing

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conditions, biomass showed a decline of 8% and 34%. In the case of organic fertilization, the effect of soil pH on biomass amount was found to be more pronounced. This was observed for the 2nd and 3rd moving with a reverse trend in terms of biomass amount. For subsequent mowing treatments, biomass showed a decrease of 18% and 26%.

Share of grass species in the cover of the study area

From sowing to the last cut the temperature and the number of sunshine hours had a positive effect on the development of grasses. Despite the diversity of the grass species and their sensitivity to temperature variability, optimal habitat conditions, in particular air temperature, were maintained during the experiment. Nevertheless, the rate of growth and sensitivity differed strongly between red fescue and ryegrass as indicated by Jankowska-Huflejt (2013) and Jankowski et al. (2018).

Out of the tested grass species, in the conducted experiment the best yield was obtained for *Festuca pratensis* Huds. and *Lolium perenne* L. *Dactylis glomerata* L. showed substantially worse results and *Festuca rubra* L. the worst. The share of the latter two species amounted to, on average, 1/3 of the biomass obtained from individual mowing treatments.

Generally, for all cuts it was found that *Lolium perenne* L. (average in the range of 75% to 88%) and *Festuca pratensis* Huds. (average in the range of 75% to 88%) significantly ($F = 19904$; $P < 0,000$) occupied a larger area than *Dactylis glomerata* L. (average in the range of 50% to 63%) and *Festuca rubra* L. (average in the range of 38% to 50%). The density of the tested grass species showed an increasing tendency in the second mowing, and decreasing in the third as compared to the first mowing treatment (*Fig. 7*). The lowest density in subsequent mowing treatments was recorded for *Festuca rubra* L. (50%/50%/38%), followed by *Dactylis glomerata* L. (63%/63%/50%), and *Lolium perenne* L. with *Festuca pratensis* Huds. (75%/88%/63%). There was no effect of the type of fertilization and soil pH on density of the tested grass species ($P > 0.05$). However, biomass analysis shows that for particular mowing treatments there was a statistically significant effect of fertilization and soil reaction on the development of the tested grass species (*Fig. 5*).

In individual mowing treatments, mean area covered by the tested grass species amounted to 27, 21 and 39% which could affect N_2O emission levels (*Fig. 4*), as is also shown in studies by Linn and Doran (1984). They suggested that this gas emission from non-fertilized soil was slightly greater for no tillage.

Assessment of the relationship between N_2O emission and biomass

It was found that out of 18 variants tested in the course of the experiment, 14 showed a statistically significant linear correlation – 13 out of which were negative (*Table 1*). With an increase in biomass of the tested grass species, N_2O emission was found to decrease, always at neutral soil reaction. In the control conditions and with organic fertilization only in the first mowing treatment there were no significant correlations at acidic pH of soil material. In the case of mineral fertilization, such correlation was recorded in all mowing treatments. Negative correlations confirm the relationship between high nitrogen dependence of species such as *Dactylis glomerata* L. and the resultant decreased N_2O emission alongside an increase in plant biomass – an observation which has also been made by other authors (Abalos et al., 2018).

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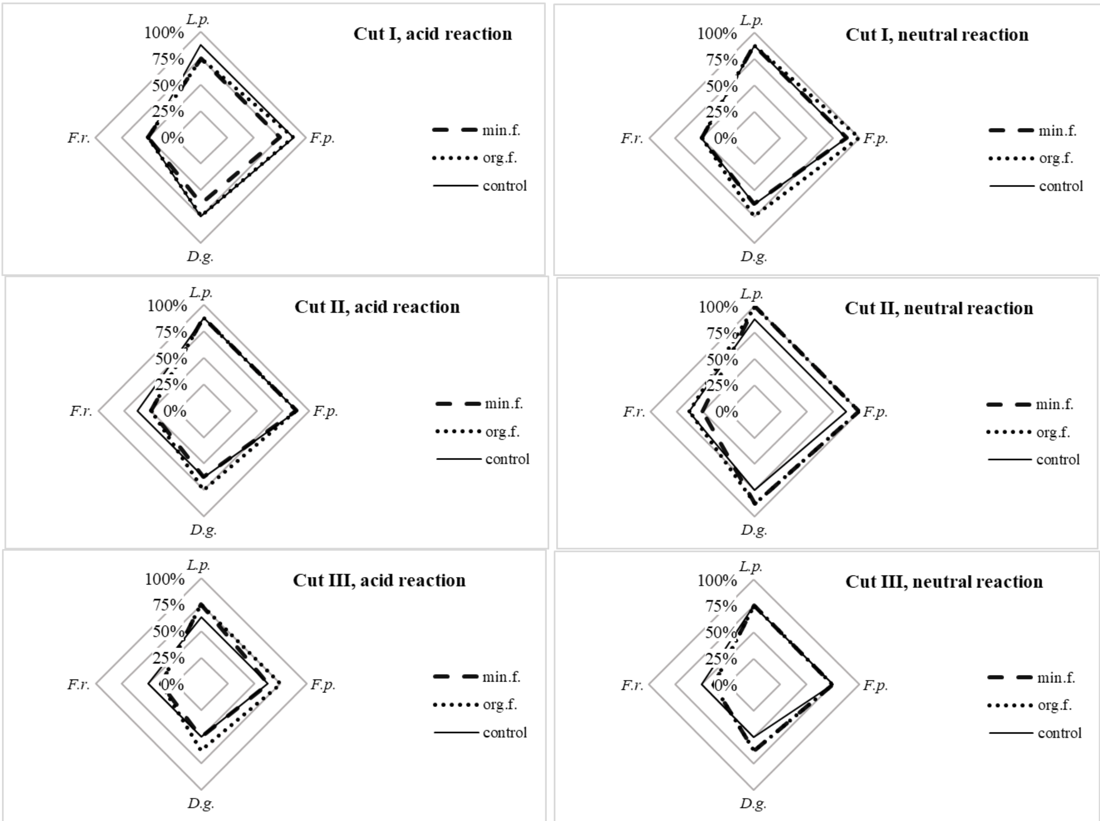

Figure 7. *Percentage of grass species in the coverage of the study surface depending on fertilization and soil reaction in individual cuts; Lolium perenne (L.p.), Festuca pratensis (F.p.), Dactylis glomerata (D.g.), Festuca rubra (F.r.); mineral fertilizer (min.f.), organic fertilizer (org.f.)*

Table 1. *Linear correlation between N_2O emission ($mg \cdot ha^{-1} \cdot (24\ h)^{-1}$) and grass biomass (g)*

Cut	Reaction	Type of fertilization		
		Control	**Mineral fertilization**	**Organic fertilization**
I	Neutral	<u>y = 658.9 – 11.73*x</u> r = -0.8053; p = 0.0088	<u>y = 18017 – 163.6*x</u> r = -0.6819; p = 0.0431	<u>y = 644.1 – 9.337*x</u> r = -0.9730; p = 0.00001
	Acid	<u>y = -157.9 + 27.73*x</u> r = 0.8543; p = 0.0034	y = 7030 + 149.9*x; r = 0.1450; p = 0.7097	y = 1080.7 – 17.64*x r = -0.9887; p = 0.00000
II	Neutral	<u>y = 2307 – 35.13*x</u> r = -0.9735; p = 0.00001	<u>y = 4892 – 39.95*x</u> r = -0.6797; p = 0.0440	<u>y = 7453 – 88.94*x</u> r = -0.7593; p = 0.0176
	Acid	<u>y = 1556 – 6.805*x</u> r = -0.6962; p = 0.0372	y = 3952 – 12.56*x r = -0.3062; p = 0.4229	<u>y = 3885 – 14.37*x</u> r = -0.7262; p = 0.0267
III	Neutral	<u>y = 1049 – 13.39*x</u> r = -0.6723; p = 0.0473	<u>y = 2897 – 30.46*x</u> r = -0.9166; p = 0.0005	<u>y = 1562 – 8.186*x</u> r = -0.7100; p = 0.0321
	Acid	<u>y = 1163 – 18.14*x</u> r = -0.7693; p = 0.0154	y = 807.8 + 8.8876*x r = 0.2840; p = 0.4590	<u>y = 1530 – 24.01*x</u> r = -0.8258; p = 0.0061

Statistically significant correlations were underlined

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Annual of N_2O fluxes

Due to specific management strategies, organic farming may have an effect on reduction of greenhouse gases emission – including nitrous oxide (Lorenz and Lal, 2016). Grassland cultivation can mobilize large pools of N in the soil, with the potential for N leaching and N_2O emissions. Numerous studies report various N_2O emission levels from grassland in particular regions of the European Union depending on, among others, manner of use (Ciais et al., 2010; Koncz et al., 2017). For example Soussana et al. (2007) and Flechard et al. (2007) reported that 9 sites reached 1.0 ± 0.5 kg N-N_2O ha^{-1} yr^{-1}. Similar results were obtained by Chang et al. (2015) who demonstrated that indirect N_2O emissions from fertilized grassland soils were 1.2 ± 0.4 kg N-N_2O ha^{-1} yr^{-1}. In turn, Zhang et al. (2010) observed a several times lower N_2O flux from meadows located in mountain regions in the western part of China: 0.26, 0.14 and 0.38 kg N-N_2O ha^{-1} yr^{-1}. Calculated annual emission of our analysed experiment showed very different values (mineral fertilization: acid reaction 0.4–7.3 and neutral reaction 0.7–9.6; organic fertilization: 0.2–10.2 and 0.1–10.5, without fertilization: 0.2–4.6 and 0.1–4.1 N-N_2O ha^{-1} yr^{-1}, respectively).

On the basis of the equation by Bouwman (1996): $E = 1 + 1.25 * F$, with fertilization dose in the amount of 50 kg·N·ha^{-1}, annual N_2O emission level was identified at 63.5 kg·N·ha^{-1}. The results of our studies showed that for the first mowing, there was a variability in N_2O emission which was dependent on the type of nitrogen fertilization (Nmin-69.6 kg·N·ha^{-1} and Norg-4.3 kg·N·ha^{-1}). The greater was the dose of nitrogen fertilization, the more the results differ from the values calculated with the equation by Bouwman (1996) for mineral soil. A decrease in N_2O flux in subsequent mowing treatments was most likely connected with phenological development of given grass species and with temperature decrease, particularly in the third mowing. The effect of temperature on N_2O emission levels was indicated by, among others, Dobbie et al. (1999), Soussana et al. (2010), Aurangojeb (2017).

Soil pH is another important factor influencing N_2O emissions, because nitrous oxide reductase is inhibited by low pH and in the presence of O_2. Generally, if denitrification is the main source of N_2O, higher pH values decrease the soil N_2O emissions, but if nitrification is the main process of N_2O production, then an increase in the soil pH stimulates the N_2O production (Signor et al., 2013). In our experiment, this effect was observed from the moment of the application of the second fertilizer dose, through the second mowing, third fertilizer dose and third mowing treatment. As emissions increased with temperature and rainfall, Ball et al. (2007) recommended to restrict tillage operations to cool, dry conditions, and being aware of possible soil compaction which may also promote denitrification. Soussana et al. (2010) pointed to the fact that emission factor of N_2O from mowed synthetically fertilised permanent grassland increased in the range from 0.1% to 5.7% alongside an increase in mean average air temperature from 5.2 to 15.8 °C, and atmospheric precipitation in the range 1160–2595 mm yr^{-1}.

Assessment of the possibilities of management optimization on crop of grasses

The search for optimum techniques of grassland management in order to reduce nitrous oxide emission led the authors to conduct the analysis of N_2O flux and biomass of grass due to split application of nitrogen fertilizer. On the basis of estimates and the adopted method of N_2O flux assessment with respect to 1 kg of grass biomass for each

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of the three mowing treatments in a 4.1 month long period, optimum management conditions were determined (*Table 2*). It was found that with the application of 50 kg·N·ha^{-1} dose at the beginning of the experiment, the expected effect can be achieved with the use of mineral fertilizer in a slightly acidic soil material (0.083 g N_2O·kg^{-1} yield grass; 10.9 Mg·ha^{-1} grass yield). An increase in gas emission was recorded using 100 kg·N·ha^{-1} in aggregate, in a system: 1st and 2nd dose – mineral fertilization in a slightly acidic environment (0.096 g N_2O·kg^{-1} grass yield; 13.6 Mg·ha^{-1} grass yield). An even greater increase of the analysed emission was recorded using 150 kg·N·ha^{-1} in a system: 1st, 2nd and 3rd dose – mineral fertilization, always in a slightly acidic environment (0.097 g N_2O·kg^{-1} grass yield; 15.6 Mg·ha^{-1} grass yield).

Table 2. *Emission of N_2O on grass yield and grass yield*

Cut	Reaction	Type of fertilization					
		Control		Mineral fertilization		Organic fertilization	
		N_2O emission (kg N_2O·kg^{-1}gy)	Grass yield (kg·ha^{-1})	N_2O emission (kg N_2O·kg^{-1}gy)	Grass yield (kg·ha^{-1})	N_2O emission (kg N_2O·kg^{-1}gy)	Grass yield (kg·ha^{-1})
I	neutral	0.000094	3875	0.000149	6030	0.000163	5107
	acid	0.000091	4468	0.000126	5561	0.000174	5140
II	neutral	0.000044	2762	0.000098	5395	0.000121	4818
	acid	0.000033	2719	0.000091	5358	0.000134	3732
III	neutral	0.000045	2512	0.000088	4538	0.000144	3316
	acid	0.000045	2653	0.000071	4694	0.000112	4115

Conclusions

In conclusion, the N_2O emission was influenced by the type and dose of nitrogen fertilization, the reaction of mineral soil and the biomass of cultivated grasses. With an increase in biomass of the tested grass species, a decrease in N_2O emission was observed, especially in neutral soil reaction. Among the tested grass species, in the experimental conditions, the highest density of ground coverage was found for *Festuca pratensis* and *Lolium perenne*, which contributed to an increased nitrogen uptake and, consequently, reduced N_2O emissions. It was established as the optimum technique of grassland management in order to reduce N_2O emission. The use of 50 kg·N·ha^{-1} mineral fertilizers resulted in the smallest emission of nitrous oxide per 1 kg of yield in a slightly acidic soil. Second application of the same fertilizer dose induced a 16% increase in emission per 1 kg of yield when the second dose was the mineral fertilizer. When the dose was applied three times (1st, 2nd and 3rd – mineral fertilizer in a slightly acidic environment), the emission increased only by 1% per 1 kg of yield. In the next study we would like to test and describe the influence of soil moisture on N_2O emission.

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