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EFFECT OF NITROGEN FERTILIZATION TYPES ON THE SOIL MICROBIAL BIOMASS AND GREENHOUSE GASES EMISSION

SUMMARY

Global climate models shows patterns of temperature and precipitation changes worldwide. Soil moisture and type of fertilization are key determinants of the microbial processes that determine the fluxes of gases from soil. There are not many research activities including the assessment how land conversion to the grassland can influence the greenhouse effect. The aim of this study was to determine the biomass content of microorganisms in soil and CO₂ and CH₄ emissions in conditions of diversified nitrogen fertilization and soil moisture in the cultivation of pot grass mixtures. The results of the study were treated by two-factor analysis of variance. The linear correlation between analysed gases and between microbial biomass and CO₂ or CH₄ emissions was performed. The volume of soil microbial biomass in the cultivation of grass mixtures was affected by the type of nitrogen fertilization and the level of soil moisture. Approximately 1.5 times bigger microbial biomass was found after fertilization than under control conditions. The same relationship occurred in the comparison between the microbial biomass during wet and dry conditions. Only the volume of CO₂ emission in this pot experiment was affected by the type of nitrogen fertilization. Higher emission of CO₂ was accompanied by increased emission of CH₄. In humid conditions, both mineral and organic fertilization affected positively on soil microbial biomass and the volume of CO₂ emission. From the viewpoint of reducing greenhouse gases emission, inorganic fertilizers used in dry conditions during the land conversion to the grassland, would be the best grassland cultivation method.

Keywords: Grassland, microbial biomass, carbon dioxide, methane

INTRODUCTION

Effect of agriculture on the environment involves a number of factors such as the release of chemicals from soil to water and air (Galczyńska and Kot, 2010;

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Liu *et al.*, 2014). Gaseous carbon compounds such as: dioxide carbon (CO₂) and methane (CH₄) are two the most important greenhouse gases (GHG), present in the atmosphere, which are produced in part by natural sources.

Because GHG prevent heat emitted by the Earth from escaping to space, changes in their atmospheric concentrations can alter the energy balance of the climate system.

According to FAO, in 2010, agriculture was the third largest contributor to global emissions by sector, with CH₄ accounting for just under half of total agricultural emissions, nitrous oxide (N₂O) for 36%, and CO₂ for some 14% (Reynolds, 2013). In 2012 agricultural activities in the EU-28 generated 470.6 million tonnes of CO₂ equivalent (evaluated for CH₄ and N₂O), corresponding to about 9.6% of total greenhouse gas emissions (information on land use, land use change and forestry – LULUCF is excluded).

GHG emissions from agriculture have increased by approximately 23.8% since 1990 (Eurostat, 2016). LULUCF covers GHG emissions into the atmosphere and removal of carbon from the atmosphere resulting from our use of soils, trees, plants, biomass and timber.

Forests and agricultural lands naturally hold large stocks of carbon, preventing its escape into the atmosphere. For example ploughing up grassland generates emissions but conversion of arable land into grassland can result in protection of carbon stocks or even carbon sequestration (Doblas-Miranda *et al.*, 2013).

Many papers suggested, that soil moisture and type of fertilization are key determinants of the microbial processes (Natywa *et al.*, 2014), that determine the fluxes of GHG from soil (Nannipieri *et al.*, 2003; Skiba *et al.*, 2013).

The aim of this study was to determine the biomass content of microorganisms in soil and two gases emissions (carbon dioxide and methane) in conditions of nitrogen fertilization and soil moisture in the cultivation of pot grass mixtures.

MATERIAL AND METHODS

The pot experiment was conducted in 2014 in the greenhouse on West Pomeranian University of Technology in Szczecin. A mix of grasses was grown in the conditions of mineral and organic fertilization and from two humidity levels (dry and wet) at the same time (30 and 60 % of field water capacity).

In the experiment, the soil material collected from a 30 cm deep layer of soil not used for agricultural production for 15 years (sand: 2-0.05 mm - 60.9%, silt: 0.05-0.002 mm - 35.1%, colloidal clay: 0.002 mm - 4.0%) granular metric composition was sandy clay.

Soil material dedicated to the research characterized, by the criteria of IUNG (Obojski and Strączyński, 1995), slightly acidity and low content of available phosphorus, potassium and magnesium (Table 1).

Table 1. Characteristic of soil material

Reaction pH _{KCl}	Salinity g NaCl·dm ⁻³	TOC g·kg ⁻¹	Humus g·kg ⁻¹	Organic substance g·kg ⁻¹	N _{tot} g·kg ⁻¹	Content of bioavailable forms mg·kg ⁻¹		
						P	K	Mg
5.9	0.37	9.6	16.6	37.6	0.75	28	83	24

*Source: Own study

Vases of the soil material (11 kg) were fed in the middle of May a mineral fertilizer (ammonium nitrate) and organic (slurry) at a dose of 0.355 g N per vase, which corresponds to 50 kg N·ha⁻¹. After a few days grass mixtures were sown to vases. Measurements of soil microbial biomass and emissions of carbon dioxide and methane were carried out at the beginning of June and mid-July in the next day after grass cutting. The second dose of nitrogen fertilization was applied (at the same amount) after the first grass cutting.

In the soil samples collected from the vases (in triplicate) the biomass of living micro-organisms in the soil was determined. The measurements were performed with the use of a physiological method defined in the literature as the SIR method (Substrate Induced Respiration), developed by Anderson and Domsch (1978).

The SIR method characterizes the current presence of the microorganisms in the soil. This method is often used in combination with measurements of CO₂ emission (Liu *et al.*, 2014). For this purpose, the soil samples were analysed with a mass of 10 g, which is enriched with extra carbon source in the form of a mixture of glucose and talc (weight ratio 1:5). The amount of glucose was determined by taking into account the initial deviation values for the matrix used. The prepared samples were then transferred to the columns of the analyser Ultragas U4S and measured the amount of CO₂ evolved after three hours. Microbial biomass was calculated using the equation authors methods:

$$x = 40,4y + 0,37$$

were:

x – the amount of C contained in the biomass of microorganisms per 100 g d.m. soil, mg;

y – maximum initial production CO₂, cm³·(100 g soil·h)⁻¹.

Measurements of carbon dioxide and methane were carried out using photoacoustic field gas monitor INNOVA 1412 (Burczyk *et al.*, 2008).

The results of the study were obtained with by a two-factor analysis of variance (1st factor – type of fertilization, 2nd factor – level of soil moisture). The linear correlation between analysed gases and between microbial biomass and carbon dioxide or methane emissions was performer.

The significance of the differences between means (Tukey test) and the value of Pearson correlation coefficient at the confidence level of p = 0.05 was calculated using *Statistica 12*.

RESULTS AND DISCUSSION

Soil microorganisms constitute less than 0.5% (w/w) of the soil mass, but they play a key role in soil properties and processes. Microorganisms participate in oxidation, nitrification, ammonification, nitrogen fixation, and other processes which lead to decomposition of soil organic matter and transformation of nutrients. Natywa *et al.* (2014) reported, that soil moisture and type of fertilization are very important determinants of the microbial processes. In pot experiment, the volume of soil microbial biomass in the cultivation of grass mixtures was affected by the type of nitrogen fertilization and the level of soil moisture, too (Table 2).

Table 2. Statistical parameters of two-way of the variance analysis for microorganism biomass (A) and carbon dioxide (B) or methane (C) emissions.

Parameters	A or B or C	F	p	LSD _{0,05}
Level of soil moisture (I)	A	14.2	0.000	2950
	B	0.26	0.611	-
	C	0.29	0.594	-
Type fertilization (II)	A	5.10	0.009	3106
	B	3.81	0.027	1.6·10 ⁸
	C	0.44	0.647	-

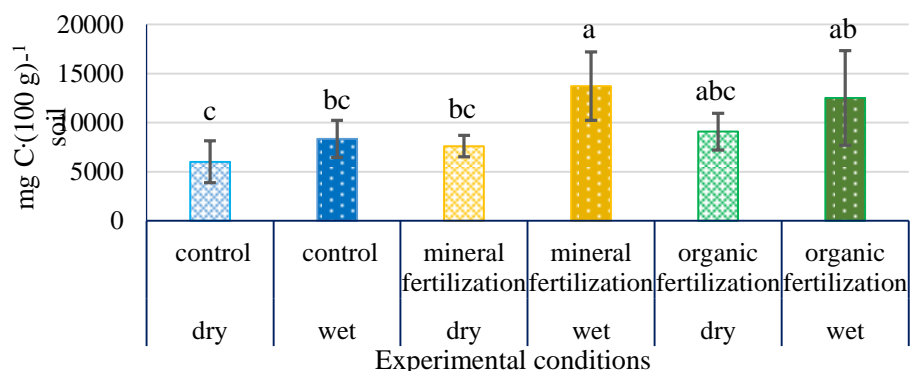
*Source: Own study

Approximately 1.5 times bigger microbial biomass (Fig. 1) was found after fertilization (10731 mg C(100 g)⁻¹) than under control conditions (7178 mg C(100 g)⁻¹). It was found that the applied dose of nitrogen fertilisation in the form of ammonium nitrate or slurry (50 kg N·ha⁻¹), generally stimulated bacterial growth (Fig. 1).

The influence of mineral fertilisation on the formation of microbial biomass is confirmed by other researchers (Kozanecka *et al.*, 1996; Barabasz and Voříšek, 2002). The inhibitory effect of NH₄NO₃ on the total number of bacteria in soil was observed by Kozanecka *et al.* (1996) at a high dose of such fertilization – 240 kgN·ha⁻¹.

Generally, it is assumed that slurry is a fertilizer which is comparable to mineral fertilizers in terms of effectiveness and period of activity. It decomposes intensely immediately after application to soil, therefore the long-term effects of slurry on properties of organic matter in soil are rather non-existent.

Yet, contrary to existing opinions, Dębska (2004) found that organic matter in slurry is relatively resistant to decomposition in soil, and research by Mazur and Mazur (2015) indicate that average increase of organic carbon as a result of fertilization in relation to the control group was 1.94 g·kg⁻¹ in lessive soil.



Source: Own study

Figure 1. Biomass soil microorganisms depending on the type of experimental conditions (Explanations: error bars are mean \pm standard deviation; a, b, ab are homogeneous groups)

Soil water content controls microbial activity and is a major factor that determines the rates of mineralization (Paul *et al.*, 2003, Yan *et al.*, 2015). In analysed experiment average 1.5 times bigger microbial biomass (Fig. 1) was found during wet ($11526 \text{ C} \cdot (100\text{g})^{-1}$) than during dry conditions ($7567 \text{ C} \cdot (100 \text{ g})^{-1}$). Due to the role of microorganisms in soil processes, their presence and biomass significantly affect the level of CO_2 emission from soils (Nannipieri *et al.*, 2003), which is the result of root respiration and physiological processes of the microorganisms involved in the decomposition of organic material. Emissions of CO_2 from soils appear to be highly variable in heterogeneous soil micro-sites, and they are influenced by the activity of roots, microbial processes, crop residue and litter content, microclimate and catalytic properties of clay colloids (Matteucci *et al.*, 2000).

In this pot experiment, besides two gaseous carbon compounds, only the volume of carbon dioxide emission was affected by the type of nitrogen fertilization (Table 2., Fig. 2 and Fig. 3).

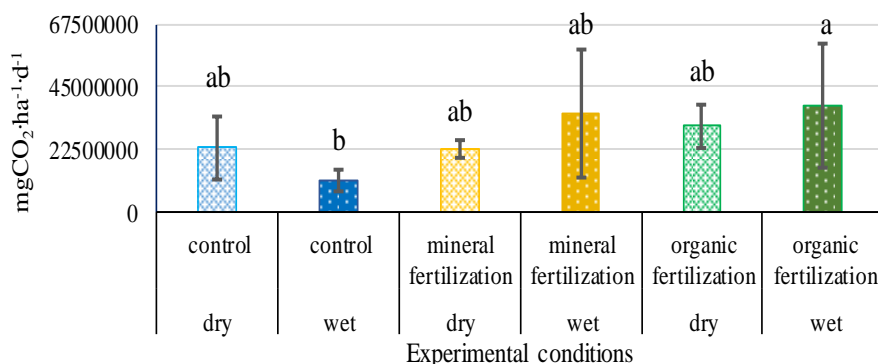


Figure 2. Carbon dioxide depending on the experimental conditions

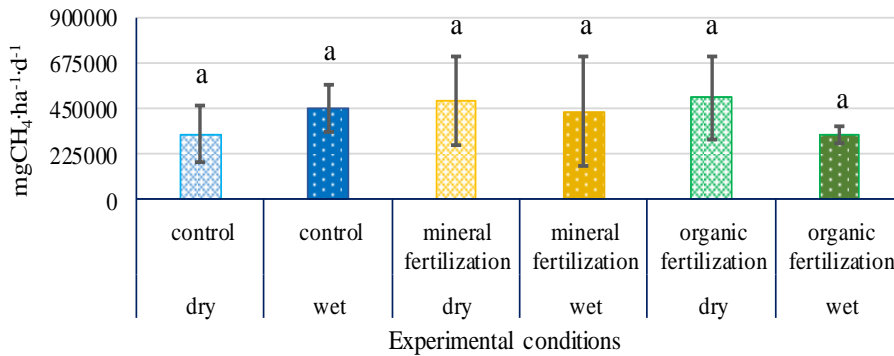


Figure 3. Methane emissions depending on the experimental conditions.

However, generally in dry conditions, higher emission of carbon dioxide was accompanied by increased emission of methane (Table 3).

Table 3. Parameters of statistically significant linear correlation between emissions analysed gases and between biomass soil microorganisms and CO₂ or CH₄ emissions were performed.

Relation, parameters		Experimental conditions					
		c/d	c/w	mf/d	mf/w	of/d	of/w
eCH ₄ = f(eCO ₂)	r	0.8251	-	-	-	0.8727	-
	p	0.0010				0.0002	
eCO ₂ = f(b)	r	-0.7346	-	-	0.9571	-	0.6342
	p	0.0065			0.0000		0.0268
eCH ₄ = f(b)	r	-	0.6317	-0.7013	-	-	-
	p		0.0276	0.0110			

Source: Own study

Explanations: e-emission; b-biomass soil microorganisms; c-control; mf-mineral fertilization; of-organic fertilization; d-dry; w-wet

The complex gas cycle of carbon compounds in mineral soil depending on the abiotic and biotic factors should be further researched.

Land cover change in agriculture shows highly contrasting trends in different areas of Europe. The main trend has been towards a conversion of arable land and permanent crops to pasture, set-aside and fallow land (EEA, 2005). The use of knowledge of the environmental results of changes in agricultural land use will affect the assessment of greenhouse gas emission.

CONCLUSIONS

In humid conditions, both mineral and organic fertilization affected positively on soil microbial biomass and the volume of carbon dioxide emission. From the viewpoint of reducing greenhouse gases emission, inorganic fertilizers used in dry conditions during the land conversion to the grassland, would be the best grassland cultivation method.

REFERENCES

- Anderson J.P.E., Domsch K.H. (1978). A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol. Biochem.*, 10: 215–221.
- Barabasz W., Voříšek K. (2002). Bioróżnorodność mikroorganizmów w środowiskach glebowych (Biodiversity of microorganisms in soil environments). In Barabasz, W. (ed.), *Aktywność drobnoustrojów w różnych środowiskach* (Microbial activity in different environments). Wydaw. AR, Kraków: 22–30.
- Burczyk P., Gamrat R., Gałczyńska M. (2008). The use of the photoacoustic field gas monitor for measurement of the concentration of gases in measurements of dinitrogen oxide emission from grassland's soils. *Pol. J. Environ. Stud.*, 17(3A): 105–108.
- Dębska B. (2004). Właściwości substancji humusowych gleby nawożonej gnojowicą (Properties of humic substances of soil fertilized with slurry). *Rozprawy nr 110* Wyd. Uczel. AT-R. Bydgoszcz, pp. 112.
- Doblas-Miranda E., Rovira P., Brotons L., Martínez-Vilalta J., Retana J., Pla M., Vayreda J. (2013). Soil carbon stocks and their variability across the forests, shrublands and grasslands of peninsular Spain. *Biogeosciences*, 10: 8353–8361.
- Efenberger M., Brzezińska-Błaszczyk E., Wódz K. (2014). Archeony – drobnoustroje ciągle nieznane (Archaeons – still unknown microorganisms). *Postępy Hig. Med. Dosw.*(Online), 68: 1452-1463.
- European Environment Agency (EEA) 2005. The European environment - State and outlook 2005. Copenhagen.
- Eurostat (2016). Agriculture, forestry and fishery statistics - 2015 edition. Luxembourg: Publications Office of the European Union.
- Gałczyńska M., Kot M. (2010). Influence of anthropoppression on concentration of biogenic compounds in water of small ponds in farmland. *J. Elementol.* 15(1): 53-63.
- Kozanecka T., Rokosz-Burlaga H., Russel S. (1996). Aktywność mikrobiologiczna gleby w sadzie jabłoniowym w zależności od sposobu jej utrzymania, nawożenia azotem i wapnowania (Microbial activity of the soil in an apple orchard depending on its maintenance, nitrogen fertilization and liming). *Rocz. Glebozn.* no 47, supl.: 75–84.
- Liu Q., Liu X., Bian C., Ma C, Lang K, Han H., Li Q. (2014). Response of soil CO2 emission and summer maize yield to plant density and straw mulching in the North China Plain. *The Scientific World Journal* 2014, Art. ID 180219: pp. 8 <http://dx.doi.org/10.1155/2014/180219> Accessed on 18/06/2016.
- Matteucci, G., Dore S., Rebmann C., Stivanello S., Buchmann N. (2000). Soil respiration in beech and spruce forest in Europe: trends, controlling factors, annual budgets and implications for the ecosystem carbon balance. In Schulze, E.D. (ed.), *Carbon and nitrogen cycling in European forest ecosystems*. Springer Verlag, Berlin, Germany: 217-236.
- Mazur Z., Mazur T. (2015). Organic carbon content and its fractions in soils of multi-year fertilization experiments. *Pol. J. Environ. Stud.*, 24(4): 1697-1703.
- Nannipieri P., Ascher J., Ceccherini M.T., Landi L., Pierratellara G., Renella G. (2003). Microbial diversity and soil function. *Eur. J. Soil Sci.*, 54: 655-670.
- Natywa M., Selwet M., Maciejewski T. (2014). Wpływ wybranych czynników agrotechnicznych na liczebność i aktywność drobnoustrojów glebowych (Effect

- of some agrotechnical factors on the number and activity soil). *Fragmenta Agronomica* 31(2): 56-63.
- Obojski J., Strączyński S. (1995). Odczyn i zasobność gleb Polski w makro- i mikroelementy (The pH of the soil and the abundance of Polish macro- and microelements). Wydaw. IUNG, Puławy: pp. 48.
- Paul K.I., Polglase P.J., O'Connell A.M., Carlyle J.C., Smethurst P.J., Khanna P.K. (2003). Defining the relation between soil water content and net nitrogen mineralization. *Eur. J. Soil Sci.*, 54: 39-47.
- Reynolds L. (2013). Agriculture and livestock remain major sources of greenhouse gas emissions. <http://vitalsigns.worldwatch.org/vs-trend/agriculture-and-livestock-remain-major-sources-greenhouse-gas-emissions>. Accessed on 18/06/2016.
- Skiba U., Jones S.K., Drewer J., Helfter C., Anderson M., Dinsmore K., McKenzie R., Nemitz E., Sutton M.A. (2013). Comparison of soil greenhouse gas fluxes from extensive and intensive grazing in a temperate maritime climate. *Biogeosciences*, 10: 1231-1241.
- Yan N., Marschner P., Cao W., Zuo C., Qin W. (2015). Influence of salinity and water content on soil microorganisms. *ISWCR*, 3, 316-323.