

# Preliminary assessment of the impact of soil microorganisms on greenhouse gas emissions expressed in CO<sub>2</sub> equivalent and grass biomass

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**Abstract:** The pot experiment was conducted to access the soil microorganisms biomass (physiological method – Substrate Induced Respiration) and emissions of N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> (photoacoustic infrared detection method), and grasses biomass (weight method). The obtained results of analysed gases were converted to CO<sub>2</sub> equivalent. There was no effect of the microorganisms biomass on the N<sub>2</sub>O emissions. The increase in CO<sub>2</sub> emissions was accompanied by an increase in the microorganisms biomass ( $r = 0.48$ ) under the conditions of the I swath and acid soil reaction, as well as the II swath and neutral reaction ( $r = 0.94$ ). On the other hand, in the case of CH<sub>4</sub> emission, such a relationship was noted both swaths under the conditions of neutral reaction ( $r = 0.51$ ), but a negative correlation ( $r = -0.71$ ) was noted for the acid reaction only at the II swath. The increase in the grasses biomass with the increase in the microorganisms biomass was recorded only at the II swath in neutral reaction ( $r = 0.91$ ). In a short period of time, with the neutral soil reaction with the increase in the soil microorganisms biomass, an increase in CO<sub>2</sub> sequestration and biomass of cultivated grasses was noted. Information on the determination of the microorganisms groups responsible mainly for the transformation of carbon compounds and CO<sub>2</sub> and CH<sub>4</sub> emissions from the soils of grasslands would be valuable scientifically.

**Keywords:** greenhouse gases, methan, nitrous oxide, soil microorganisms, soil reaction

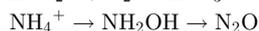
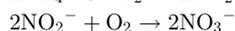
## INTRODUCTION

Microorganisms are dominant in soil habitats in terms of diversity, biomass, and their effects on basic soil processes [BAHRAM *et al.* 2018; MAĆIK *et al.* 2020]. Soil microbial diversity helps to keep its healthy character and fertility, as well as to mitigate climate change. This is not only conducive to good-quality soil environment, but also allows to maintain a high yield potential [CONSTANCIAS *et al.* 2015; DELGADO-BAQUERIZO *et al.* 2017], as microorganisms support plant growth and development through the production of agroactive substances [MAJEED *et al.* 2015]. The plant root system provides a surface for the colonisation of microorganisms, contributing to the increased metabolic activity of microorganisms, supporting their growth, respiration and metabolism by providing carbon [KUZYAKOV *et al.* 2019; SRIVASTAVA 2021].

As much as 99% of soil microbial species and their functions remain unknown, so they are known as the Earth's "microbial dark matter" [LLOYD *et al.* 2018]. Their diversity (bacteria, fungi, actinomycetes) and numerical diversity within the studied groups cause a different reaction of the physical and chemical environmental factors of the soil (i.e. availability of nutrients, reaction of soil, humidity, organic compounds and vegetation) [BURCZYK *et al.* 2016; GEISSELER, SCOW 2014; MAĆIK *et al.* 2020; ROUSK *et al.* 2010]. In 1 g of soil there may be from 4 000 to 7 000 different species, and the biomass density may be from 30 to 30 000 kg·ha<sup>-1</sup> [WU *et al.* 2020].

Soil acidity influences soil carbon and nitrogen cycles by controlling activities of microorganisms involved in the transformations of these two elements [KUNHIKRISHNAN *et al.* 2016; ZHANG *et al.* 2020a], and therefore CO<sub>2</sub> and N<sub>2</sub>O emissions [CHENG 2020]. The use of nitrogen fertilisers, increasing the activity of soil

microorganisms, determined the efficiency of microbial nitrification and denitrification processes [WU *et al.* 2020]. Nitrification is an aerobic process, and denitrification is anaerobic [FENG *et al.* 2019]. The final nitrification reaction is nitrates(V) ( $\text{NO}_3^-$ ), and the denitrification – molecular nitrogen ( $\text{N}_2$ ). The nitrification reaction takes place in several stages: in the first stage, the bacteria of the *Nitrosomonas* group oxidise ammonia to nitrites, then to nitrates. When the access to air (oxygen) is limited and the oxidation of ammonium is incomplete, nitrogen oxide is formed in indirect or side reactions instead of nitrates(V).



Apart from natural factors influencing chemical reactions taking place in soil, anthropogenic factors are also important. Greenhouse effect by elevation of temperature shifts soil N cycling from microbial immobilisation to enhanced nitrification and denitrification leading to an increase in  $\text{N}_2\text{O}$  emissions of up to 227%. The use of N fertilisers increased the activity of microorganisms and the production of  $\text{N}_2\text{O}$  [DAI *et al.* 2020; WU *et al.* 2020].

Emissions of greenhouse gases (GHG), such as carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) have great impact on global warming and pollution of atmosphere, hydrosphere and pedosphere [HUNTINGFORD *et al.* 2020], contributing to approx. 60, 20, and 6% of the greenhouse effect, respectively. Pollution from agriculture has environmental consequences at local and global scales [CHITRA, PRIYA 2020; JANTKE *et al.* 2020]. Global reductions of agricultural methane emissions until 2030 of up to 48%, relative to 2010, and of nitrous oxide emissions by up to 26% are required to limit global warming to 1.5°C [IPCC 2018].

That is why proper management is crucial to maintain the so-called Climate-Smart Agriculture (CSA) [GAŁCZYŃSKA *et al.* 2016], which remains a difficult task due to the scattered sources and complex relationships between water and atmospheric emissions in agroecosystems [BHATTACHARYYA *et al.* 2020; HUNDERTMARK *et al.* 2021]. Grassland management should favour the microbiological diversity of soils, and keeping agricultural soils pH above 5.5 maximises the energy efficiency of microorganisms [JONES *et al.* 2019]. This is conducive to maintaining the balance of the entire ecosystem and allows to shape greenhouse gas emissions [DAI *et al.* 2021]. It is also important to act to increase the storage of carbon dioxide in the soil. Less intensive management practices in near-neutral soils have a greater carbon storage potential due to the increased growth efficiency of microorganisms, while in acidic soils the growth of microorganisms undergoes a greater reduction in the rate of degradation [MALIK *et al.* 2018].

Taking into account the small scale of the assessment of greenhouse gas emissions, e.g. from the surface of a meadow, several factors should be analysed: the amount of nitrogen fertilisation, the type of fertilisation, the type and moisture of the soil and its pH. The increasing concentrations of  $\text{CO}_2$  in the atmosphere account for the majority of greenhouse gases,  $\text{N}_2\text{O}$  and  $\text{CH}_4$  are equally important for their radiative properties and long residence time in the atmosphere, making their Global Warming Potential (GWP) 298 and 25 times greater than  $\text{CO}_2$  over 100 years, respectively [MYHRE *et al.* 2013].  $\text{CH}_4$  and  $\text{N}_2\text{O}$  are greenhouse gases with a much greater greenhouse potential than

$\text{CO}_2$  [WANG *et al.* 2021]. The temporal GWP20 has shown the necessity to include anthropogenic and non-anthropogenic emissions in the analyses in order to be able to define priorities in future action strategies [SKYTT *et al.* 2020], but also to learn about the reaction of the microbial composition of the soil to environmental factors.

The development of soil microorganisms depends on the physico-chemical properties of the soil environment [BAHRAM *et al.* 2018; GROSS, ROBBINS 2000; MALIK *et al.* 2018; MAĆIK *et al.* 2020]. Microorganisms determine the transformation of nitrogen and carbon compounds, and thus influence the emission of such gases as:  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  [DAI *et al.* 2020; GEISSELER, SCOW 2014; SRIVASTAVA 2021; WANG *et al.* 2019]. Therefore, it was assumed that in a short period of time on undegraded soils (pH > 5) [SMREZAK *et al.* 2020] it will be possible to observe the effect of soil microorganism biomass on the emission of these gases under variable environmental conditions (pH, humidity and fertilisation). The aim of the study was an attempt to determine the impact of microorganisms on the individual and total emission of the analysed gases ( $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$ ) and grass biomass under variable soil environment conditions.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

The pot experiment was conducted in a greenhouse on Faculty of Environmental Management and Agriculture of West Pomeranian University of Technology in Szczecin, Poland, according to the methodology presented by GAŁCZYŃSKA *et al.* [2018].

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 characterised by slightly acid reaction (pH = 5.9), total organic carbon – 9.6 g·kg<sup>-1</sup>, humus – 16.6 g·kg<sup>-1</sup>, organic substance – 37.6 g·kg<sup>-1</sup>, total nitrogen – 0.75 g·kg<sup>-1</sup>, and low content of available phosphorus – 28 mg·kg<sup>-1</sup>, potassium – 83 mg·kg<sup>-1</sup> and magnesium – 24 mg·kg<sup>-1</sup>. Soil material dedicated to the research characterised, by the criteria of IUNG, slightly acidity and low content of available phosphorus, potassium and magnesium (SIEBIELEC 2017). Each of the four species (*Dactylis glomerata* L., *Festuca pratensis* Huds., *F. rubra* L., *Lolium perenne* L. – Photo 1) was sown in an amount of 270 seeds on 1/4 of the study area of the pot (0.071 m<sup>2</sup>) under the variable fertilisation, moisture (wet, optimum, dry), 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 fertilisers was applied to pots two times (every six weeks, starting from the half of May, 10 days after the 1st haymaking) in two fertilisation variants (mineral fertiliser – ammonium nitrate, organic fertiliser – fresh slurry) in the amount of 0.355 g of N per pot, corresponding to 50 kg N·ha<sup>-1</sup>. The control sample were pots with the analysed grass species without fertilisation.

Measurements of soil microbial biomass and emissions of  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{CH}_4$  were carried out at the beginning of June and mid-July in the next day after I and II swaths. The experiment was conducted in three repetitions. At the beginning of the experiment, phosphorus fertilisation in the form of superphosphate was applied to all pots in the amount of 40 kg P<sub>2</sub>O<sub>5</sub>·ha<sup>-1</sup>.



**Photo 1.** Pot experiment; 1 – *Dactylis glomerata*, 2 – *Festuca pratensis*, 3 – *Festuca rubra*, 4 – *Lolium perenne* (phot. R. Gamrat)

In case of mineral fertilisation, potassium level in the soil material was supplemented with a dose of this element in the form of K<sub>2</sub>SO<sub>4</sub> to the value determined for fresh slurry, i.e. 4 kg·m<sup>-3</sup>. Pot experiment was arranged in a randomised block design.

### MEASUREMENT AND ANALYSIS OF BIOMASS OF SOIL MICROORGANISMS

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 Substrate Induced Respiration (SIR) method, developed by ANDERSON and DOMSCH [1978].

The SIR method characterises the current presence of the microorganisms in the soil. This method is often used in combination with measurements of CO<sub>2</sub> 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 CO<sub>2</sub> evolved after three hours. Microbial biomass was calculated using the equation authors methods:

$$x = 40.4y + 0.37$$

where:  $x$  = the amount of carbon contained in the biomass of microorganisms per 100 g d.m. soil (mg);  $y$  = maximum initial production CO<sub>2</sub> (cm<sup>3</sup>·(100 g soil)<sup>-1</sup>·h<sup>-1</sup>).

### MEASUREMENT OF GREENHOUSE GASES (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) FLUXES

Measurements of greenhouse gases (GHG): dinitrogen oxide (N<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) emission were conducted with the use of a closed static chamber after a few hours from the application of each different nitrogen fertiliser and before each mowing. Gas measurements were carried out using photoacoustic field gas monitor INNOVA 1412, [BURCZYK *et al.* 2008]. Gases concentration in the chamber was recorded every minute and emission levels were noted every 15 min. Each gas concentration was recalculated.

### CLIMATIC CONDITIONS OF GRASS GROWTH

The climate in Szczecin is moderately warm. The average annual temperature is 8.6°C. During the experiment in 2014 the

temperature varied from 9 to 24°C. The grass was grown outdoors. Water deficits were compensated by watering and the pots were protected from atmospheric precipitation.

### CALCULATION OF CO<sub>2</sub> EQUIVALENT FOR MEASURED GREENHOUSE GAS EMISSIONS

Calculation of the CO<sub>2</sub> equivalent for the measured greenhouse gas emissions is based on three different values from years 1995, 2007 and 2014 according to MYHRE *et al.* [2013] – Table 1.

**Table 1.** Global warming potential (GWP) values relative to CO<sub>2</sub>

Gas	GWP values for 100-year time horizon based on values from		
	1995	2007	2014
CO <sub>2</sub>	1	1	1
CH <sub>4</sub>	21	25	28
N <sub>2</sub> O	310	298	265

Source: own elaboration acc. to MYHRE *et al.* [2013].

### STATISTICAL ANALYSIS

Results obtained of GHG emission expressed in CO<sub>2</sub> equivalent, and results of biomass of microorganisms and grasses were elaborated statistically by the linear correlation between analysed factors using Statistica 13 software (StatSoft Poland). Pearson correlation coefficient were evaluated at  $p < 0.05$ . The strength of Pearson's  $r$ -correlation was characterised after GUILFORD and FRUCHTER [1977]: none  $r = 0$ , slight  $r \leq 0.1$ , weak  $r \in (0.1; 0.3>$ , average  $r \in (0.3; 0.5>$ , high  $r \in (0.5; 0.7>$ , very high  $r \in (0.7; 0.9>$ , almost full  $r \in (0.9; 1.0)$ , full  $r = 1$ .

The total emission of greenhouse gases was calculated depending on the pH of the soil and the term of the grass swath. The percentage share of emissions of all analysed gases (CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>) expressed in CO<sub>2</sub> equivalent was also assessed. In all calculations, three different values of conversion factors for CH<sub>4</sub> and N<sub>2</sub>O were taken into account.

### RESULTS AND DISCUSSION

Generally, in a neutral soil environment, greater numbers of bacteria and actinomycetes were recorded than in the acidic environment (Fig. 1). On the contrary, the number of fungi was greater in acidic soil than in neutral soil. Taking into account the biomass of soil microorganisms, their higher value was also found in a neutral environment. In the II swath, all of the discussed groups of organisms were more numerous than in the I swath. The phenomenon of the influence of pH on the number of soil microorganisms is described in various ways in the literature [WANG *et al.* 2019]. SMRE CZAK *et al.* [2020] emphasise that most of the bacteria involved in the transformation of organic substances in the soil environment are active in the range from slightly acidic to neutral pH. The increase in acidification of soils is conducive to the appearance of numerous species of fungi [GROSS, ROBBINS

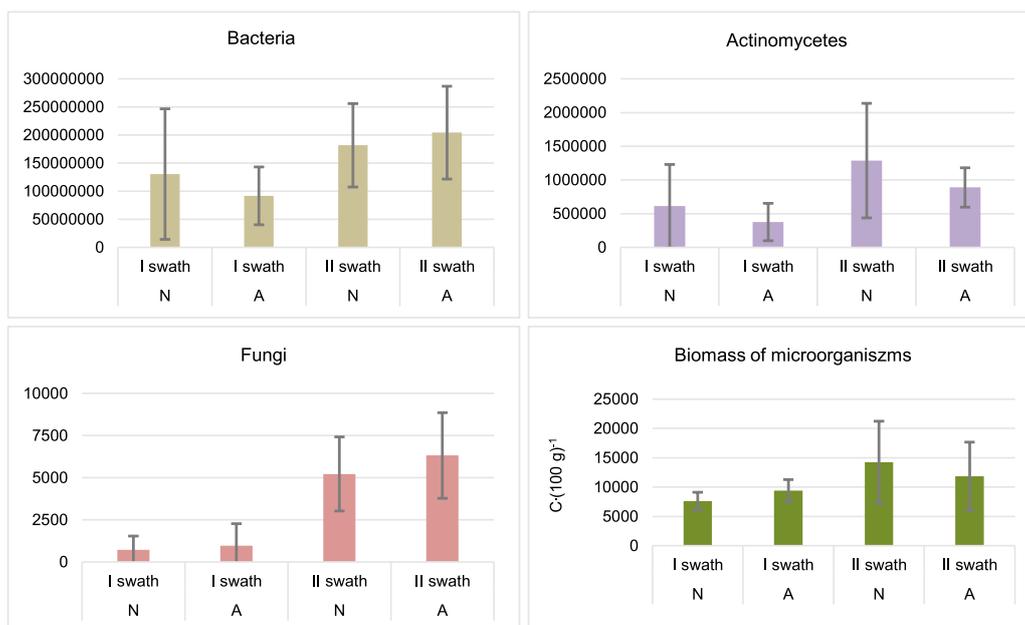


Fig. 1. The number of different organisms in soil in I and II swaths, biomass of microorganisms for acidic (A) and neutral (N) reaction; source: own study

2000; LIU *et al.* 2020], which also thrive in a close to neutral environment (e.g. *Actinomucor elegans* (Eidam) C.R. Benj. & Hesselt pH range from 3.3 to 8.0, *Monilinia fructicola* L.R. Batra from 3.0 to 7.0, *Mortierella minutissima* Tiegh. 1878 from 3.8 to 8.6, *Mucor racemosus* Fresen from 2.0 to 9.2, *Rhizopus stolonifer* (Ehrenb.) Vuill. from 3.0 to 7.0, *Zygorrhynchus moelleri* Vuill. from 3.1 to 8.8).

Environmental Protection Agency (EPA) updates the GWP values according to updated scientific estimates of the energy absorption or lifetime of the gases or to changing atmospheric concentrations of GHGs that result in a change in the energy absorption of 1 additional ton of a gas relative to another. The EPA considers the GWP estimates presented in the most recent IPCC scientific assessment to reflect the state of the science. CO<sub>2</sub>, by definition, has a GWP of 1 regardless of the time period used, because it is the gas being used as the reference. Over the years, the value for CH<sub>4</sub> has been increased and the value for N<sub>2</sub>O decreased [BIERNAT *et al.* 2020; MYHRE *et al.* 2013; ZHANG *et al.* 2020b]. CH<sub>4</sub> emitted today lasts about a decade on average, which is much less time than CO<sub>2</sub>. But CH<sub>4</sub> also absorbs much more energy than CO<sub>2</sub>. N<sub>2</sub>O has a GWP 265–298 times that of CO<sub>2</sub> for a 100-year timescale and emitted today remains in the atmosphere for more than 100 years, on average [TIMMA *et al.* 2020; TROTTIER 2015].

The calculated total CO<sub>2</sub> emission for the recommended CO<sub>2</sub> equivalent coefficients (from 2014) of the analysed gases was the highest in relation to the calculated total CO<sub>2</sub> emissions using the previously accepted coefficients used in the calculations (from 1995 and 2007 years). The EPA considers the GWP estimates presented in the most recent IPCC scientific assessment to reflect the state of the science [MYHRE *et al.* 2013].

The selection of coefficients for calculating the CO<sub>2</sub> equivalent emission for individual greenhouse gases has a different impact on the assessment of CO<sub>2</sub> emissions under variable soil pH conditions [AGUILERA *et al.* 2015; WIŚNIEWSKI, KISTOWSKI 2020]. For example, for the I swath, the difference between the total CO<sub>2</sub> emission for the recommended CO<sub>2</sub> equivalent coefficients and

the emission for the earliest established coefficients (1995) amounted to approx. 3,500 g·m<sup>-2</sup>·d<sup>-1</sup> at acidic soil reaction. On the other hand, under the conditions of neutral soil reaction, the discussed difference in the emissions of the analysed greenhouse gases expressed as CO<sub>2</sub> equivalent amounted to less than 2,000 g·m<sup>-2</sup>·d<sup>-1</sup>. For the II swath, the difference between the total CO<sub>2</sub> emission for the recommended coefficients of CO<sub>2</sub> equivalent and the emission for the earliest established coefficients (1995) was less than 3,000 g·m<sup>-2</sup>·d<sup>-1</sup> at acidic soil reaction, and approx. 2,500 g·m<sup>-2</sup>·d<sup>-1</sup> for neutral soil.

The total GHG emissions expressed in CO<sub>2</sub> equivalent were almost twice as high in the II swath as compared to the I swath. For the I swath, with acidic soil reaction, higher greenhouse gas emissions expressed as CO<sub>2</sub> equivalent (33,892–37,320 g·m<sup>-2</sup>·d<sup>-1</sup>) compared to neutral pH (21,722–23,659 g·m<sup>-2</sup>·d<sup>-1</sup>) were found. This difference was approx. 60% (Fig. 2). Regardless of the soil reaction, CO<sub>2</sub> emissions had the largest share in the total emission of these gases, followed by CH<sub>4</sub>, and N<sub>2</sub>O to the lowest extent. Comparing the results between soil reactions, changes in the proportion of the percentage of CO<sub>2</sub> equivalent calculated for N<sub>2</sub>O and CO<sub>2</sub> alone are also observed. For the neutral reaction, the percentage of CO<sub>2</sub> equivalent calculated for N<sub>2</sub>O as compared to the acidic reaction was almost twice as high. On the other hand, no changes were observed with regard to the percentage of CO<sub>2</sub> equivalent calculated for CH<sub>4</sub> for both neutral and acidic pH. According to OCHAL *et al.* [2017] as a consequence of the multiple effects of liming on soil properties, both its production potential increases and the negative impact on the environment is reduced. An increase in the soil pH to the values optimal for agricultural soils, i.e. pH from 5.6 to 7.2, will increase CO<sub>2</sub> sequestration and reduce nitrogen losses to adjacent ecosystems (water, air).

For the II swath with acidic soil reaction, lower greenhouse gas emissions expressed as CO<sub>2</sub> equivalent (55,604–58,473 g·m<sup>-2</sup>·day<sup>-1</sup>) compared to the neutral reaction (61,133–63,871 g·m<sup>-2</sup>·day<sup>-1</sup>) were found. This difference was approximately 10% (Fig. 3). Regardless of the pH of the soil, just like in the case of the

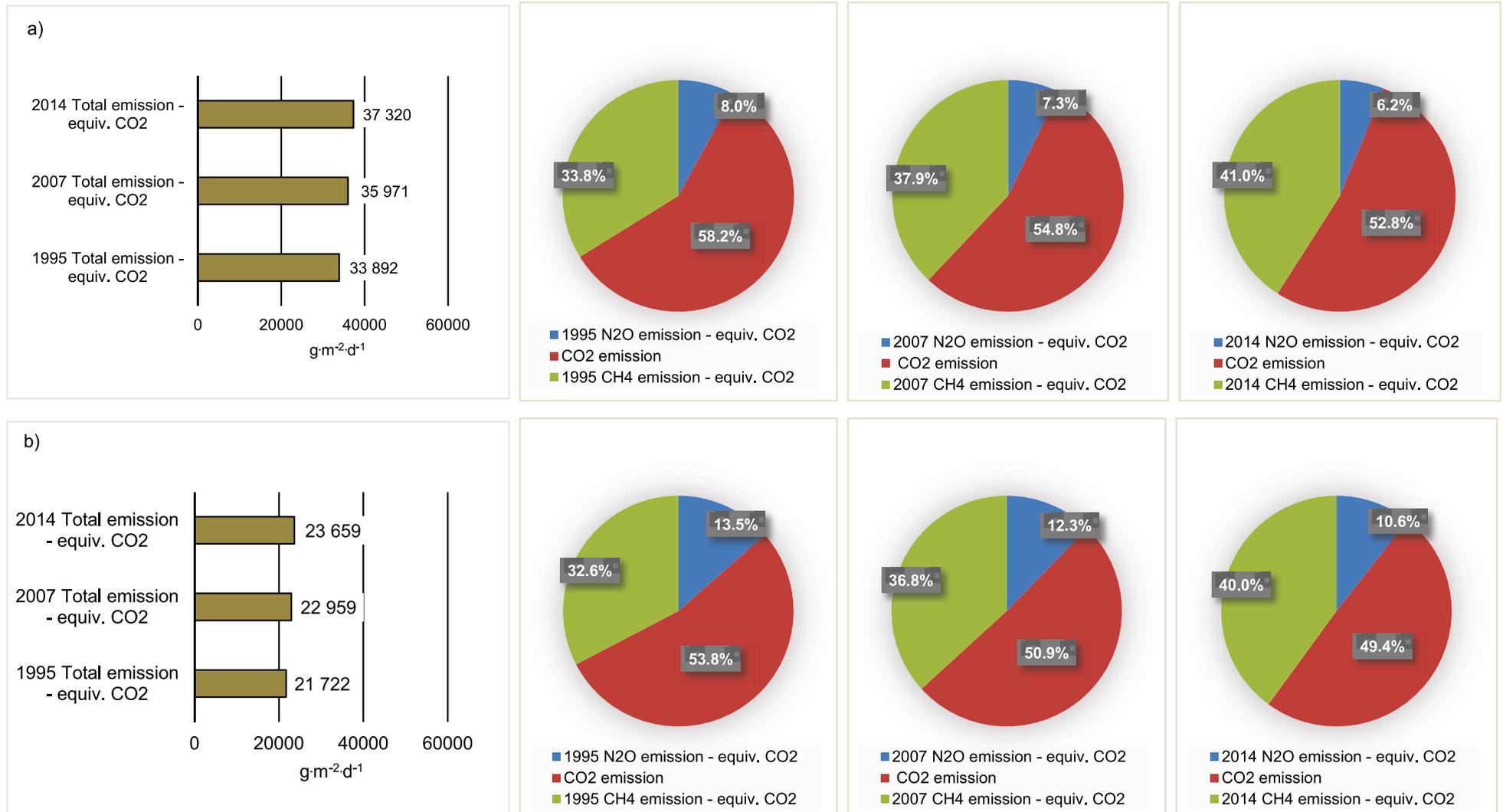


Fig. 2. Percentage of greenhouse gas emissions as CO<sub>2</sub> equivalent and total emissions from soil in I swaths grass mixture for different reaction: a) acidic, b) and neutral; source: own study

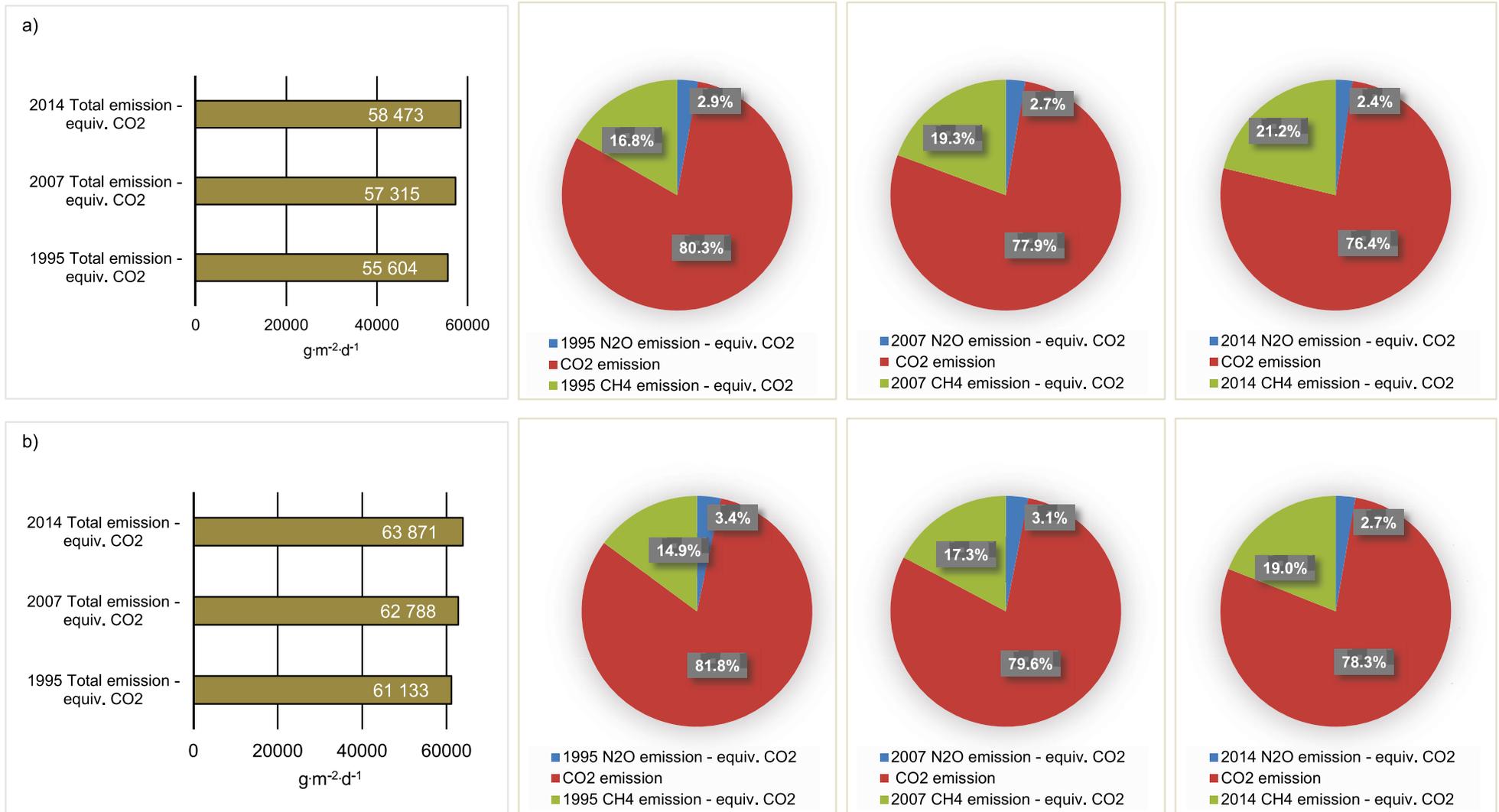


Fig. 3. Percentage of greenhouse gas emissions as CO<sub>2</sub> equivalent and total emissions from soil in II swath grass mixture for different reaction: a) acidic, b) and neutral; source: own study

**Table 2.** Correlation between biomass of microorganisms (x) and grass biomass and greenhouse gas emissions as CO<sub>2</sub> equivalent from soil in the cultivation of a mixture of grasses (y)

Factor (y)	Year as a base	Biomass of microorganisms (x)				
		swath I		swath II		generally
		neutral reaction	acidic reaction	neutral reaction	acidic reaction	
Grass biomass	-	$y = 39.1431 - 0.0010x$	$y = 35.4621 - 0.0002x$	$y = \mathbf{8.9099 + 0.0014x}$	$y = 20.6322 + 0.0002x$	$y = 25.3632 + 0.0003x$
		$r = -0.2940; p = 0.237$	$r = -0.0570; p = 0.822$	$r = \mathbf{0.9068}; p = \mathbf{0.000}$	$r = 0.1580; p = 0.531$	$r = 0.1680; p = 0.159$
Total emission – equivalent CO <sub>2</sub>	1995	$y = 2.1E7 - 314.5x$	$y = 5.7E7 - 2799.4x$	$y = \mathbf{-2.3E6 + 4213.7x}$	$y = 3.5E7 + 1096.2x$	$y = \mathbf{1.1E7 + 2789.9x}$
	2007	$y = 2.3E7 - 401.2x$	$y = 6.1E7 - 2802.3x$	$y = \mathbf{-2.2E6 + 4320.2x}$	$y = 3.9E7 + 917.8x$	$y = \mathbf{1.2E7 + 2799.0x}$
	2014	$y = 2.4E7 - 481.2x$	$y = 6.2E7 - 2824.0x$	$y = \mathbf{-2.3E6 + 4404.4x}$	$y = 4.1E7 + 783.0x$	$y = \mathbf{1.3E7 + 2806.2x}$
		$r = -0.0750; p = 0.767$	$r = -0.3410; p = 0.166$	$r = \mathbf{0.8949}; p = \mathbf{0.000}$	$r = 0.2540; p = 0.309$	$r = \mathbf{0.5270}; p = \mathbf{0.000}$
N <sub>2</sub> O emission– equivalent CO <sub>2</sub>	1995	$y = 6.1E5 + 193.1x$	$y = -9.2E4 + 251.7x$	$y = 2.4E6 - 54.6x$	$y = 1.1E6 + 12.8x$	$y = 1.8E6 - 4.5x$
	2007	$y = 5.9E5 + 185.6x$	$y = -8.9E4 + 242.0x$	$y = 2.5E6 - 52.5x$	$y = 1.0E6 + 12.3x$	$y = 1.7E6 - 4.3x$
	2014	$y = 5.2E5 + 165.0x$	$y = -7.9E4 + 215.2x$	$y = 2.1E6 - 46.7x$	$y = 9.1E5 + 11.0x$	$y = 1.5E6 - 3.8x$
		$r = 0.2430; p = 0.330$	$r = 0.3840; p = 0.116$	$r = -0.2940; p = 0.237$	$r = 0.0878; p = 0.729$	$r = -0.0140; p = 0.907$
Emission CO <sub>2</sub> – equivalent CO <sub>2</sub>	-	$y = 1.1E7 - 91.3x$	$y = \mathbf{4.8E7 - 3086.7x}$	$y = \mathbf{-5.7E6 + 3720.0x}$	$y = 1.3E7 + 2017.3x$	$y = \mathbf{3.9E5 + 2747.3x}$
		$r = -0.0290; p = 0.909$	$r = \mathbf{-0.4780}; p = \mathbf{0.045}$	$r = \mathbf{0.9400}; p = \mathbf{0.000}$	$r = 0.3959; p = 0.104$	$r = \mathbf{0.5730}; p = \mathbf{0.000}$
Emission CH <sub>4</sub> – equivalent CO <sub>2</sub>	1995	$y = \mathbf{9.5E6 - 416.2x}$	$y = 1.1E7 + 35.6x$	$y = \mathbf{9.3E5 + 548.3x}$	$y = \mathbf{2.1E7 - 934.0x}$	$y = 8.4E6 + 47.1x$
	2007	$y = \mathbf{1.1E7 - 495.5x}$	$y = 1.3E7 + 42.4x$	$y = \mathbf{1.1E6 + 652.7x}$	$y = \mathbf{2.5E7 - 1089.8x}$	$y = 1.0E7 + 56.1x$
	2014	$y = \mathbf{1.2E7 - 554.9x}$	$y = 1.4E7 + 47.5x$	$y = \mathbf{1.2E6 + 731.0x}$	$y = \mathbf{2.5E7 - 1111.8x}$	$y = 1.1E7 + 12.1x$
		$r = \mathbf{-0.5292}; p = \mathbf{0.024}$	$r = 0.0200; p = 0.936$	$r = \mathbf{0.5030}; p = \mathbf{0.034}$	$r = \mathbf{-0.7145}; p = \mathbf{0.001}$	$r = 0.0390; p = 0.744$

Explanations: bold = statistically significant correlations.

Source: own study.

I swath, CO<sub>2</sub> emissions had the highest share in the total emission of these gases, followed by CH<sub>4</sub>, and N<sub>2</sub>O to the lowest extent. However, when comparing the results between soil reactions, there is no real change in the proportion of the percentage of CO<sub>2</sub> equivalent calculated for N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> alone.

The biomass of the cultivated grasses in the I swath (mean ± standard deviation 393.3 ± 8.9 g neutral reaction; 410.1 ± 10.5 g acidic reaction) was higher than in the case of the II swath (360.7 ± 8.9 g neutral reaction; 284.4 ± 5.6 g acidic reaction). Plant productivity did not reveal significant relationships with microbial diversity [ZHALNINA *et al.* 2015]. In our studies, a statistically significant increase in the biomass of cultivated grasses with an increase in the biomass of microorganisms at the level of almost full linear correlation ( $r = 0.91$ ) was recorded only at the II swath under the conditions of neutral soil reaction (Tab. 2). Under the same conditions, a very high correlation ( $r = 0.89$ ) was found for the relationship between the total gas emissions expressed in CO<sub>2</sub> equivalent and the biomass of microorganisms. Taking all research results into account, the correlation strength decreased to high ( $r = 0.53$ ).

No linear relationship was found between the N<sub>2</sub>O emission expressed in CO<sub>2</sub> equivalent and the biomass of microorganisms in all of the tested variants. The increase in CO<sub>2</sub> emissions dependent on the biomass of microorganisms was encountered in the conditions of the I swath and acidic soil reaction at the level of the average correlation ( $r = 0.48$ ), then in the conditions of the II swath and neutral soil pH at the level of almost full correlation ( $r = 0.94$ ) and for all results at the high correlation level ( $r = 0.57$ ).

The increase in CH<sub>4</sub> emissions expressed in CO<sub>2</sub> equivalent was accompanied by an increase in the biomass of soil microorganisms both at the I and II swath under the conditions of neutral soil pH, at the level of high ( $r = 0.53$ ) and average ( $r = 0.50$ ) correlation, respectively. A negative and, at the same time, very high correlation ( $r = -0.71$ ) was recorded at the II swath for the acidic reaction of the soil.

## CONCLUSIONS

In a short period of time, on undegraded soils, the pH of the soil influenced the development possibilities of various groups of soil microorganisms, and thus determined the emission of the analysed greenhouse gases. The overlapping nitrification and denitrification processes as well as the changing soil pH did not make it possible to observe the relationship between the biomass of soil microorganisms and the emission of N<sub>2</sub>O under the changing environmental conditions of the experiment. Raising the soil pH to neutral increased the CO<sub>2</sub> sequestration. Also, under the conditions of neutral soil pH, an increase in the biomass of cultivated grass species was noted with an increase in the biomass of soil microorganisms.

Analysing of the soil productivity is key to managing greenhouse gas emissions from grassland. Reducing nitrogen fertilisation in subsequent doses for growing grasses may limit the emissions of the tested gases, and thus reduce their negative impact on the greenhouse effect. In further studies on the discussed issue, it would be advisable to analyse the groups of microorganisms responsible mainly for the transformation of carbon compounds in terms of the reaction of undegraded and degraded soils characterising permanent grasslands.

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