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Various textured soil as nitrous oxide emitter and consumer

T. Włodarczyk[1], *W. Stępniewski*[2], *M, Brzezińska*[1], *and U. Majewska*[1]

[1]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland
[2]University of Technology, Nadbystrzycka 40b, 20-618 Lublin, Poland

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A b s t r a c t. Changes of NO_3, and N_2O concentrations during 34 days hypoxic incubation of eleven nitrate amended samples of the Eutric Cambisols developed from sand, silt and loam were studied. It was found that initial generation of nitrous oxide was followed, after a maximum by its subsequent absorption. The absorption rate was correlated with the efflux. Both were negatively correlated with sand content and positively with silt, clay and C_{org}. The percentage of nitrate reduced equals 35, 97, and 100% for Eutric Cambisols developed from sand, loam and silt, respectively. The highest N_2O efflux was observed from silty soils, the lowest one from sandy soils. Total N_2O consumption ranged between 3.3 and 66.5 mg N kg^{-1}, and consisted 32.9, 99.2, and 100% of the produced N_2O for the sandy, loamy and silty soil samples, respectively. The tested soils were characterized by various ratio of the N_2O emitted to the consumed. Most of sandy samples are characterized by a weak capacity to N_2O production and consumption, while loamy and silty soils are characterized by a good or very good capacity to N_2O production and consumption.

K e y w o r d s: soil, nitrous oxide, emission, consumption, nitrate reducing

INTRODUCTION

Greenhouse gas emissions to the atmosphere and their contribution to climate change have attracted world-wide attention. Concentrations of atmospheric greenhouse gases (GHGs), such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which can alter the Earth climate, have risen dramatically during the past century. This has resulted in an urgent need for process-based understanding of the main factors influencing the exchange of these gases between the land and atmosphere at a range of scales, as a route to developing effective mitigation technologies. Accordingly, the measurement and reduction of these gaseous emissions have become a global imperative, attracting the interest of researchers and policy makers alike (Kutilek and Nielsen, 2010; Saggar, 2010). Aside from carbon dioxide, the most significant are methane, and nitrous oxide that have a potential for global warming because of increasing man-made emission (Khalil, 2000). The global warming potential of nitrous oxide is about 320 times higher than that of CO_2. Current atmospheric abundance is alarmingly increasing in various sectors (Verma *et al*., 2006). The residence time of N_2O in the atmosphere is estimated to be about 150 years (Khalil and Rasmussen, 1992). This indicates that there are neither reactions with other chemicals present in the atmosphere nor removal with precipitation water (Verma *et al*., 2006).

Denitrification, or dissimilative nitrate reduction, is an anaerobic process used by some bacteria for energy generation. This process is important in many aspects, but its environmental implications have been given particular relevance. Nitrate accumulation and release of nitrous oxide in the atmosphere due to excess use of fertilizers in agriculture are examples of two environmental problems where denitrification plays a main role (Tavares *et al.,* 2006).

Under certain aeration conditions in soil, nitrous oxide is formed (Huang *et al.,* 2007; Li *et al*., 2006). It can be generated or absorbed in soil depending on the redox potential (Gliński and Stępniewski, 1985). Nitrous oxide can be produced at a certain soil layer and absorbed in the deeper, more reduced horizons or oxidized during its migration to the atmosphere. Closer to the surface it can be produced again due to nitrification. Thus the net emission or absorption of nitrous oxide at the soil surface will depend on soil aeration status (Seo and DeLaune, 2010; Stępniewski and Stępniewska, 2009).

*Corresponding author's e-mail: teresa@ipan.lublin.pl

Despite the importance of nitrous oxide as a greenhouse gas and its role in destroying stratospheric ozone, its global budget remains poorly understood (Khalil, 2000). Two aspects of N_2O research in agricultural ecosystems that limit our understanding of and the ability to accurately predict and manage N_2O are:
– the information on N_2O emission from plants and,
– atmospheric N_2O consumption by soils.

The aim of the present paper was: to characterize:
– the nitrate reduction to N_2O in Ap horizon samples from the Eutric Cambisols under limited oxygen availability,
– subsequent their N_2O consumption in the headspace.

MATERIALS AND METHODS

Soil samples were taken from 11 Ap horizons (0-30 cm) and used in the study after drying pretreatment. The Eutric Cambisols were developed from different parent materials: sands (Nos 1-4), silts (Nos 5-7) and loams (Nos 8-11). The soil texture was determined by standard sedimentation method. The Eutric Cambisols showed a large variation of the soil texture, $C_{org.}$, endogenous NO_3^- content and pH (Table 1).

The 5 g portions of air-dried sieved (1 mm sieve) the Eutric Cambisols were placed in 38 cm^3 glass vessels and enriched with KNO_3 at the rate of 100 mg NO_3^--N kg^{-1} dry soil. This N rate corresponded approximately to 300 NO_3^--N kg ha^{-1} (calculated on 20 cm layer basis). The soil/water ratio was 1:1 (w/w). There was about 0.5 cm of stagnant water on soil surface. The vessels with suspensions were tightly sealed with rubber stoppers and incubated in the atmosphere diluted with gaseous nitrogen in order to simulate the lower oxygen content in soil air as compared to the atmospheric one, and giving the opportunity to initiate dissimilative nitrate reduction. The initial concentration of O_2 in the gas headspace at the beginning of the incubation was 10 % v/v ± 0.5 (50% of the volume of soil headspace was replaced by N_2). Paraffin films were used on the stoppers to ensure hermetic seals. The headspace gas was sampled through the stopper with a gas – tight syringe. The Eutric Cambisols were incubated at 20°C for 21-34 days without acetylene block (Włodarczyk *et al*., 2005). The incubation was finished before the 34th day if nitrous oxide was exhausted earlier.

A set of 33 incubation vessels was prepared for each the Eutric Cambisol. Three vessels of these were used for the determination of gas concentration. The other vessels were opened in three replications successively during each measurement day (after 1, 2, 3, 7, 10, 14, 21, 28, 32 and 34 days of incubation) for determination of pH and nitrate concentration in the suspension.

After opening of the vessels and the measurements of pH (with pH- meter, Radiometer, Copenhagen) the soil suspensions were quantitatively transferred into plastics flasks, shaken for 1h with 250 ml of 0.01 M $CaCl_2$, and filtered through filter paper for nitrate determination (FIA Star 5000 autoanalyzer FOSS, Tecator) as described previously (Włodarczyk *et al*., 2005). The determination of $C_{org.}$ in the soil samples was based on the reduction of the $Cr_2O_7^{2-}$ ion by organic matter, wherein the unreduced excess $Cr_2O_7^{2-}$ was measured by titration (Balashov *et al*., 2010).

During the measurement days, the concentration of N_2O in the headspace gas was determined with a gas chromatograph (Shimadzu GC-14, Japan) equipped with an electron capture detector at 300°C. The gas components were

T a b l e 1. Basic properties of the Eutric Cambisols under investigation (modified table of Włodarczyk *et al*., 2005)

Parent material	Soil No.	Particle size fraction (%)			$C_{org.}$ (%)	NO_3-N_0* (mg kg^{-1})	pH_0* in KCl
		>0.05 mm	0.05-0.002 mm	<0.002 mm			
Sand	1	95	5	0	0.67	4.53	4.69
	2	78	22	0	0.92	58.2	4.95
	3	85	12	3	0.32	6.47	7.35
	4	74	21	5	0.44	29.94	7.38
Silt	5	52	43	5	2.31	32.93	5.37
	6	32	60	8	2.85	65.76	3.94
	7	27	67	6	1.24	7.87	4.71
Loam	8	63	23	14	0.77	9.46	6.85
	9	71	21	8	0.57	17.88	7.61
	10	69	28	3	0.88	9.78	5.56
	11	55	41	4	1.89	5.37	3.83

*Values at the start of incubation.

separated on a column (2 m long) packed with a Porapak Q and maintained at 80°C. The carrier gas was He flowing at a rate of 40 ml min^{-1}. The temperature of injector was 120°C. The contents of N_2O-N were corrected for gas dissolved in the water using literature values of Bunsen absorption coefficient (α) of 0.629 at 20°C (Gliński and Stępniewski, 1985).

As the nitrous oxide content in the headspace showed an initial phase of increase and, after reaching a maximum it started to decrease, the results related to the denitrification of nitrate to N_2O were expressed as:

– maximum cumulative N_2O efflux (maxN_2Oe),
– an average daily N_2O efflux (aN_2Oe),
– the highest daily N_2O efflux (hN_2Oe), whereas the results related to N_2O consumption phase were expressed as:
– total N_2O consumption (N_2Oc),
– an average daily N_2O consumption (aN_2Oc), and
– the highest daily N_2O consumption (hN_2Oc).

The interrelations between efflux and consumption were analyzed in terms of the ratio of N_2O emission to N_2O consumption (e/c ratio) for an average and highest daily effluxes to an average and highest daily consumption (ae/ac ratio and he/hc ratio). Moreover the total nitrate (native + added) reduced (NO_3-R) and denitrified do N_2O (NO_3-D) were calculated.

The average daily efflux (aN_2Oe) was calculated by considering the maximal cumulative N_2O content in the headspace divided by the length of the emission period. The average daily consumption (aN_2Oc) was calculated by considering N_2O drop in the headspace (after its maximum content) to the end of incubation divided by the length of this period. The ratio of N_2O emission to N_2O consumption (e/c ratio) was calculated by division the average (or highest) daily effluxes by average (or highest) daily consumption (ae/ac and he/hc).

The linear, multiplicative, exponential or logarithmic models were used in the regression analysis, and in each case the model with the highest R^2 was selected as the best fit for the experimental data (Statgraphics 5.0).

RESULTS AND DISSCUSION

The changes of NO_3, and N_2O concentrations during incubation of the Eutric Cambisols developed from sand, silt and loam under soil hypoxia and nitrate treatment are shown in Figs 1-3. The Eutric Cambisols under consideration showed a high variability of all the investigated features. Calculated values varied among the Eutric Cambisols developed from the same and different parent materials. The investigated soils were characterized by a very large variation of original nitrate content. The reasons for this may be numerous. For example, the lower nitrate content in the heavy textured soils (loams) may be associated with a prevalence of denitrification over nitrification. In the light textured soils we can observed the opposite situation.

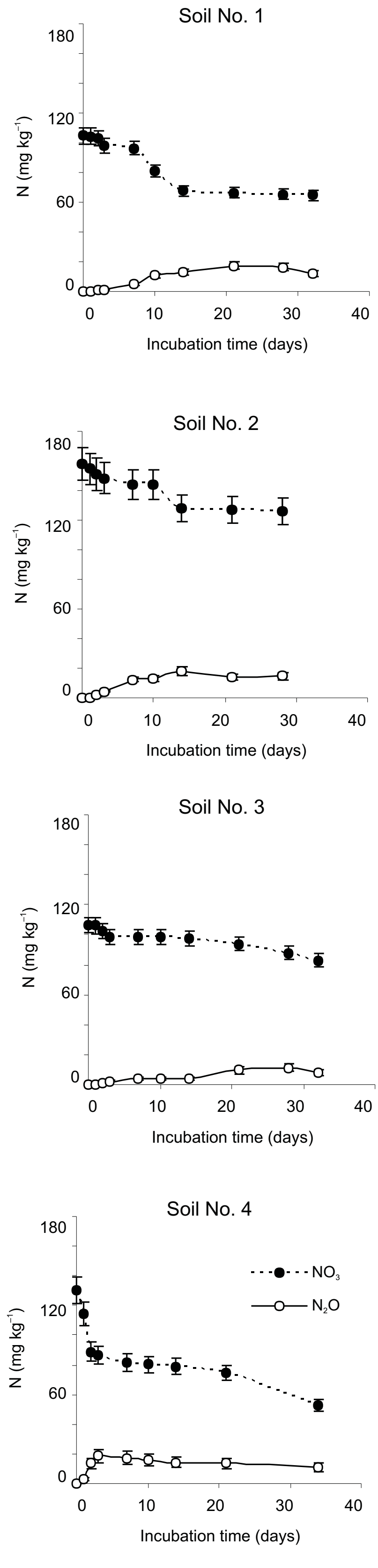

Fig. 1. Cumulative curves N_2O efflux and nitrate concentration in the Eutric Cambisols developed from sand.

Some authors concluded that denitrifying bacteria show a lack of certain reductases in the reductive pathways. In this case, the lack of nitrate reductase may lead to accumulation of nitrates. Frequently missing enzymes in this pathway are nitrate and nitrous oxide reductases (Robertson and Kuenen, 1991). However Klemedtson and Svensson (1988) paying attention to the phenomenon say that not all bacteria have a set of enzymes capable to convert nitrates to molecular nitrogen, N_2O is sometimes the main product, while others start from the reduction of nitrite rather than nitrate. All these factors may affect the nitrate content of the Eutric Cambisols in greater or lesser extent.

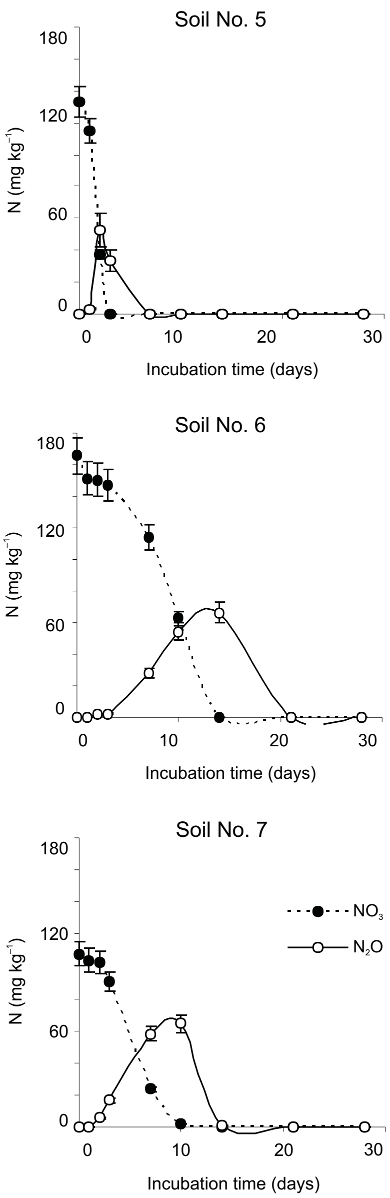

Fig. 2. Cumulative curves N_2O efflux and nitrate concentration in the Eutric Cambisols developed from silt.

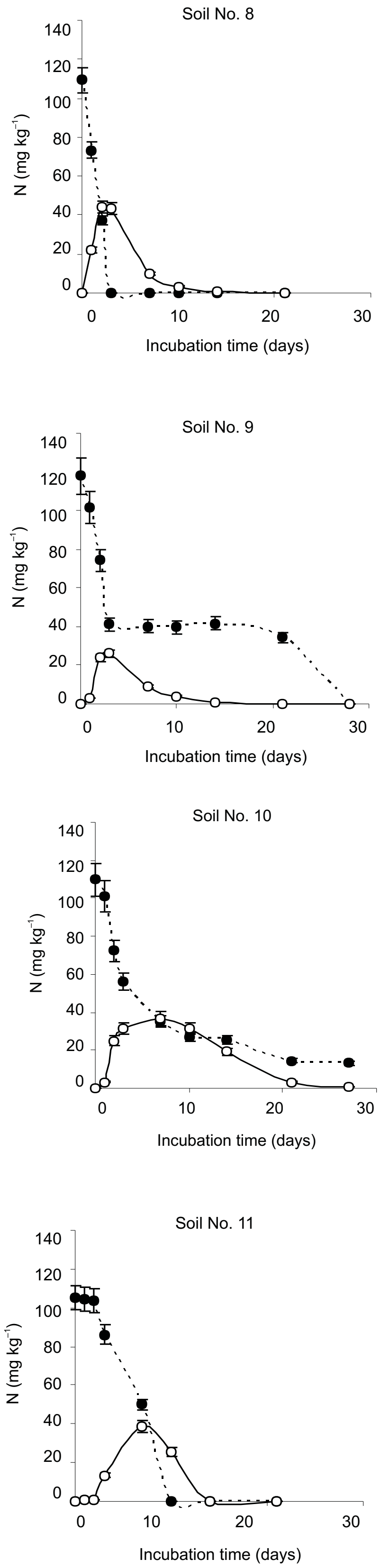

Fig. 3. Cumulative curves N_2O efflux and nitrate concentration in the Eutric Cambisols developed from loam.

The highest NO_3-R was found in the soils developed from silt, lower activity in the soils developed from loam, and the lowest one in the soils developed from sand. The percentage of NO_3-R (Table 2) differed especially among the soils developed from sand (35%) and those developed from both from loam (97%) and silt (100%). Such a large difference in the reduction of nitrate between the Eutric Cambisols developed from sand and developed from silt may be caused by the oxygenation status under natural conditions before sampling. In other words, the history of the soil before it is sampled is very important (Włodarczyk, 2000).

Another inhibitory factor the process of nitrate reduction can also be deficiency of ammonia form in the soils studied. Denitrifying bacteria cannot use nitrate as a nitrogen source and require ammonia form. Moreover the investigated soils were characterized by large variations in $C_{org.}$ content, which had a definite influence on the amount of nitrate reduction.

Nitrate denitrified to N_2O (NO_3-D) was calculated in two ways (Table 2). In the first one the nitrate denitrified to N_2O form (NO_3-D) was expressed as percentage of total initial nitrate content. It varied for particular Eutric Cambisols between 11 and 60%. The highest percentage of NO_3-D was observed in the soils developed from silt (46% in average), 1.4 times lower value was observed in the case of soils developed from loam (33% in average) and 3.5 times lower in soils developed from sand (13% in average).

In the second way the amount of nitrate denitrified to N_2O form (NO_3-D) was calculated as percentage of the total nitrate reduced and resulted in 22 to 60% as the percentage of nitrous oxide in the reduced nitrate (Table 2). The highest percentage of NO_3-D was observed in the soils developed from silt (46% in average), nearly the same value was observed in the case of soils developed form sand (43% in average), and 1.3 times lower value occurred in soils developed from loam (34% in average).

The first way of calculating the loss of nitrate informs us of the total losses of NO_3^- in the Eutric Cambisols. It is a very important information from the agricultural point of view. Under flooding conditions the microorganisms compete with the plants for nitrates.

The second way of calculating the losses of nitrate informs us of the conversion of NO_3^- to the form of nitrous oxide. In turn, this information is extremely important from the standpoint of natural environment protection. As it is clear from this data the ability to release N_2O in the Eutric Cambisols is similar in soils with coarser particles (sand and silt) than in the loams. Soils of coarser grain-size distribution

T a b l e 2. NO_3-N reduced (NO_3-R) and denitrified (NO_3-D) for the investigated Eutric Cambisols

Parent material	Soil No.	NO_3-N: Reduced: Total (NO_3-R) (mg kg^{-1})	Total (NO_3-R) (%)*	Reduced to N_2O form (NO_3-D), calculated with respect to total NO_3^- (%)**	reduced NO_3^- (%)***
Sand	1	39.9	36.5	16.5	43.3
	2	31.9	20.2	11.3	55.9
	3	23.6	22.1	10.7	48.4
	4	77.2	59.4	14.2	23.8
Average		43.2	34.6	13.2	42.8
Silt	5	132.9	100	39.3	39.3
	6	165.8	100	40.1	40.1
	7	107.9	100	59.5	59.5
Average		135.5	100	46.3	46.3
Loam	8	109.5	100	40.5	40.5
	9	117.9	100	22.1	22.1
	10	96.5	86.4	33.5	38.1
	11	105.4	100	36.7	36.7
Average		107.3	96.6	33.2	34.4

*Percent of nitrate reduced (NO_3-R) was calculated by considering the decrease of the total nitrate concentration (native + added). **Percent of nitrate denitrified to N_2O form (NO_3-D) was calculated to total nitrate concentration (native + added). ***Percent of nitrate denitrified to N_2O form (NO_3-D) was calculated to reduced nitrate under natural conditions are better aerated. Easier access of oxygen causes that the denitrification process is interrupted at the level of N_2O because nitrous oxide reductase is particularly sensitive to the presence of oxygen. These soils may be dominated by bacteria not adapted to full denitrification. This ability is the result of influence of various factors under natural conditions, including soil type, which are revealed after flooding of the air-dry soil.

Under the experimental conditions, the highest N_2O efflux was observed from the soils developed from silt and the lowest one from the soil developed from sand (Figs 1-3). The value of maximum cumulative N_2O efflux (maxN_2Oe) ranged between 11.4 and 66.5 mg N_2O-N kg^{-1} and was observed between 2nd and 28th day of the experiment (Table 3). Silva *et al.* (2008) investigated nitrous oxide production in sieved air-dried sandy loam soil under 100% WHC (water holding capacity) and found maximum production about 1 mg N_2O-N kg^{-1} in unamended and about 1.5 mg N_2O-N kg^{-1} in NH_4^+ amended soil after first day of incubation. Deyan and Changchum (2010) investigated N_2O emission from sieved fresh and amended with ammonium nitrate (5 mg N g^{-1} soil) meadow soil. Cumulative N_2O emission was equal 41.86 mg N kg^{-1} from soil after the 65-day of incubation. Yanai *et al.* (2008) studied nitrous oxide production in sieved fresh and amended with KNO_3 (8 g l^{-1}) grassland fields under flooded conditions. The examples of production and N_2O emissions show enormous adaptability of soil microorganisms to the extremely different soil moisture conditions.

The average daily N_2O efflux (aN_2Oe), ranged between 0.4 to 26.1 mg N kg^{-1} d^{-1}. The aN_2Oe was lowest in the sandy soils and the highest in the soils developed from silt .

The highest daily N_2O efflux (hN_2Oe) ranged between 1.8 and 24.5 mg N kg^{-1} d^{-1} and reached maximum values in the soils developed from loam while the lowest ones were observed in the sandy soils (Table 3). The hN_2Oe showed a large variation among the Eutric Cambisols.

T a b l e 3. N_2O efflux and consumption for the investigated Eutric Cambisols

Parent material	Soil No.	N_2O-N								
		Efflux			Consumption				ae/ac *****	he/hc ******
		Total (NO_3-D) (mg kg^{-1})	Average daily* (aN_2Oe) (mg kg^{-1} d^{-1})	Highest daily** (hN_2Oe) (mg kg^{-1} d^{-1})	Total N_2O form (mg kg^{-1})	% of effluxed	Average daily*** (aN_2Oc) (mg kg^{-1} d^{-1})	Highest daily**** (hN_2Oc) (mg kg^{-1} d^{-1})		
Sand	1	17.3	0.8	1.9	5.2	30.0	0.5	1.1	1.6	1.7
	2	17.8	1.3	2.1	3.3	18.4	0.2	0.5	6.5	4.2
	3	11.4	0.4	1.8	3.4	30.2	0.9	0.9	0.4	2.0
	4	18.4	6.1	10.4	7.3	52.9	0.3	0.5	20.3	20.8
Average		16.2	2.2	4.1	4.8	32.9	0.5	0.8	7.2	7.2
Silt	5	52.3	26.1	24.5	52.3	100	2.8	18.7	9.3	1.3
	6	66.5	4.8	8.7	66.5	100	9.5	9.5	0.5	0.9
	7	64.1	6.1	11.1	64.1	100	3.1	15.7	2.0	0.7
Average		61.0	12.3	14.8	61.0	100	5.1	14.6	3.9	1.0
Loam	8	44.3	22.2	22.2	43.9	99.2	2.3	8.4	9.7	2.6
	9	26.0	8.7	20.9	25.9	99.3	1.0	4.2	8.7	5.0
	10	36.7	5.3	21.8	36.2	98.4	1.8	3.1	2.9	7.0
	11	38.7	5.5	12.2	38.7	100	2.8	6.4	2.0	1.9
Average		36.4	10.4	19.3	36.2	99.2	2.0	5.5	5.8	4.1

*The average daily efflux was calculated by considering the total cumulative N_2O increase in the headspace divided by the length of this period. **The highest rate of daily efflux was calculated by considering the highest rate of cumulative N_2O increase in the headspace divided by the length of this period. ***The average daily consumption was calculated by considering N_2O drop in the head space after its maximum content to the end of incubation divided by the length of this period. ****The highest rate of daily consumption was calculated by considering N_2O drop in the head space after its maximum content to the day of highest rate of N_2O consumption divided by the length of this period. *****aN_2Oe/ aN_2Oc – daily average efflux/daily average consumption, ******hN_2Oe/ hN_2Oc – highest daily average efflux/highest daily average consumption.

Four Eutric Cambisols: one silty (No. 5) and three loamy (Nos 8, 9 and 10) were very active in N_2O release, their hN_2Oe was more than 20 mg N_2O-N kg^{-1} d^{-1}. These four Eutric Cambisols reached maximum daily nitrous oxide efflux during first two days. These four soils showed the best adaptation to the conditions of limited access of oxygen, when some kind of bacteria change aerobic respiration to nitrate. In these four soils we observed the phenomenon of rapid adaptation of microorganisms to changes in electron acceptor and nitrate reductase synthesis. This is undoubtedly a result of adaptation to natural conditions, forced by the state of oxygenation, especially in the case of loamy soils. Błaszczyk (1992) by examining the activity of denitrifying, 2 strains of *Pseudomonas* and one strain of the genus *Paracoccus* in cultures with the addition of NO_3 and NO_2 found that the bacteria need time for the synthesis and activation of nitrite reductase by changing the electron acceptor. Simultaneous incubation of these strains in the substrate with the addition of nitrate and nitrite showed three different induction systems reductases: nitrate and nitrite.

Cumulative N_2O curves show an increase in the initial period and then, after maximum, a decrease as a result of nitrous oxide consumption (Figs 1-3). The longest period till the start of nitrous oxide consumption was 28 days for the soil developed from sand (No. 3), while the shortest (2 days) for one soil developed from silt (No. 5) and one soil developed from loam (No. 8). Time needed for beginning of N_2O consumption was longest for soils developed from sand (3-28 days), then for soils developed from silt (2-14 days), and the shortest one for the loamy soils (2-7 days).

The Eutric Cambisols under study were characterized by different ability to nitrous oxide consumption (N_2Oc). The soils developed from silt consumed all the produced N_2O (100%), loamy soils consumed almost all produced nitrous oxide (99.2%), and sandy soils consumed only 32.9% of the N_2O produced. The average values of the total nitrous oxide consumption ranged between 4.8-61.0 mg N kg^{-1} for the investigated groups (Table 3).

The average N_2O consumption (aN_2Oc) ranged between 0.2 to 9.5 mg N kg^{-1} d^{-1} and was much lower than the highest N_2O consumption (hN_2Oc), especially in the silty and loamy soils (Table 3). The aN_2Oc was lowest in sandy soils , but the highest one in silty ones.

The average hN_2Oc ranged between 0.8 - 14.6 mg N kg^{-1} d^{-1} and was highest in the soils developed from silt. Two silty soils (Nos 5 and 7) were very active in N_2O consumption. Their daily N_2O consumption was more than 15 mg N kg^{-1} (Table 3).

The relationships presented in Fig. 4 show that max N_2Oe was strongly negatively correlated with the sand fraction content (R^2= 0.8928) and positively correlated with finer grain size fractions *ie* with silt (R^2= 0.8366) and weekly with clay (R^2= 0.2933, not shown). The values of average nitrous oxide consumption (aN_2Oc) are also negatively correlated with sand fraction and positively with the silt and clay fraction (clay fraction not shown in Fig. 5). Both maximum cumulative efflux of nitrous oxide and average consumption are positively correlated with the C_{org}. Of special interest is Fig. 6 presenting highly positive correlation (R^2= 0.9759) between efflux of nitrous oxide and its consumption.

Incubation of nitrate amended Eutric Cambisols under hypoxia gave under laboratory conditions 16.2 mg N kg^{-1}, 61.0 and 36.4 mg N kg^{-1} losses of nitrogen through denitrification to N_2O for the soils developed from sands, silts and loams, respectively (Table 3). This indicates that about 13, 46, and 33% of initial nitrate content was reduced to N_2O in the sandy, silty and loamy Eutric Cambisols, respectively. However, total nitrate-N decrease (NO_3-R) comprising reduction to N_2O, N_2, and NH_4^+ and other products including N incorporated into biomass was 43.2, 135.5, and 107.3 mg N kg^{-1} for the Eutric Cambisols developed from sand, silt and loam, respectively (Table 2). Matthews *et al*. (2010) showed that the main soil property influencing N_2O fluxes was nitrate concentration. Vilain *et al*. (2010) found that nitrous oxide was significantly correlated with the highest range of NO_3^- content in soils, but no relationship with the lowest range of NO_3^- content.

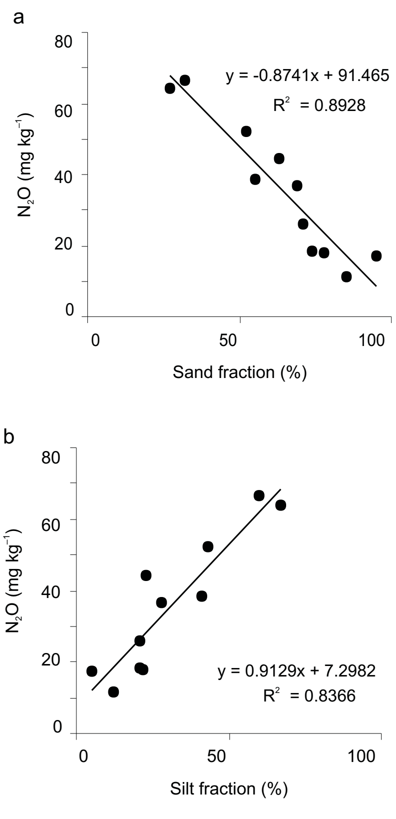

Fig. 4. Maximum cumulative N_2O efflux as a function of the particle size fractions: a – sand fraction, b – silt fraction (modified figure of Włodarczyk *et al*., 2005).

Taking into consideration the maximum of cumulative nitrous oxide efflux and its average daily consumption, the investigated Eutric Cambisols were divided as follows:
- 'weak emitter' – efflux below 20 mg N_2O-N kg^{-1};
- 'good emitter' – 20 to 40 mg N_2O-N kg^{-1};
- 'very good emitter' – more than 40 mg N_2O-N kg^{-1};
- 'weak consumer' – absorbed below 1 mg N_2O-N kg^{-1} d^{-1};
- 'good consumer' – 1 to 5 mg N_2O-N kg^{-1} d^{-1};
- 'very good consumer' – more than 5 mg N_2O-N kg^{-1} d^{-1}.

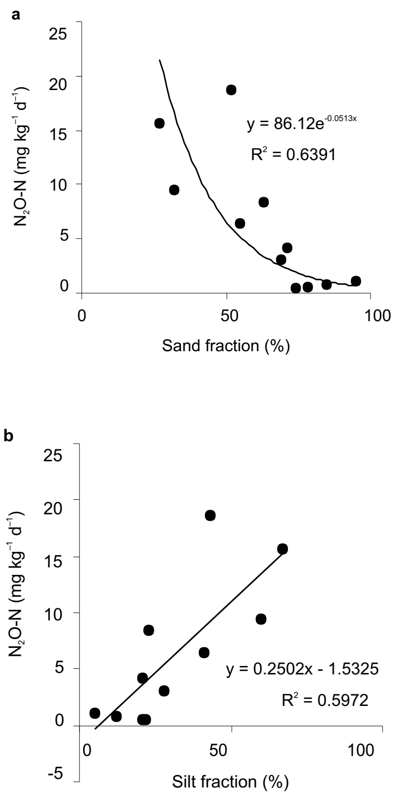

Fig. 5. Average daily N_2O consumption as a function of the particle size fractions: a – sand fraction, b – silt fraction and (modified figure of Włodarczyk *et al*., 2005).

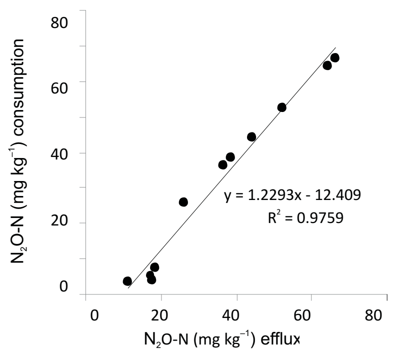

Fig. 6. Maximum N_2O consumption versus maximum cumulative N_2O efflux.

According to this, the Eutric Cambisols were divided into four groups (Table 4):
- 'weak emitter' and 'weak consumer', inside this group were all soils developed from sand (Nos 1-4),
- 'good emitter' and 'good consumer', which represent loamy soils (Nos 9, 10, 11),
- 'very good emitter' and 'good consumer' – soils No. 8 (loam), 5, 7 (silt), and
- 'very good emitter' and 'very good consumer' – soil No. 6 (silt).

This is the result of the high correlation (R^2 = 0.9759) between the emission of nitrous oxide and its consumption presented in Fig. 6. This relationship is a very important from the standpoint of environmental protection because of N_2O has a big impact on the greenhouse effect and ozone layer destruction.

Regression analysis of maxN_2Oe and aN_2Oc, as a function of granulometric composition showed that the analyzed indexes were closely related to coarser (sand and silt) granulometric soil fractions; negatively only to sand fraction (>0.05 mm, p<0.001) and positively to silt (0.05-0.002 mm, p<0.001) and colloidal fraction (<0.002 mm, p<0.05) (Figs 4 and 5). Włodarczyk *et al*. (2005) investigated 16 soils developed from sand, silt and loam, found very high correlation between granulometric composition and both nitrous oxide efflux and daily nitrous oxide consumption. The authors observed that the total N_2O evolution and daily nitrous oxide consumption showed a high negative linear correlation with the content of the fraction >0.05 mm, a positive linear correlation with 0.05-0.002 mm fraction and the lower one with <0.002 mm fraction.

In our opinion, the particle size distribution explains very low N_2O efflux and consumption from soils developed from sand and much higher from the soil developed from finer fraction (0.005-0.002). Vilain *et al*. (2010) concluded that nitrous oxide emission from riparian sites depended on particle size, soil type, N input *etc*. Water-filled pore space and NO_3^- soil concentration explained most of the N_2O flux variability during the entire sampling period. Lamers *et al.* (2007) showed a large variability between the different soil

Table 4. Eutric Cambisols characteristics in terms of their ability to N_2O efflux and consumption

Soil characteristics	Sandy soils				Silty soils			Loamy soils			
	1	2	3	4	5	6	7	8	9	10	11
WE WC	+	+	+	+							
GE GC									+	+	+
VGE GC					+		+	+			
VGE VGC						+					

WE – weak emitter, GE – good emitter, VGE– very good emitter, WC – weak consumer, GC – good consumer, VGC – very good consumer.

types in N_2O fluxes. Data demonstrate that the variability between soil types is mainly driven by the high dynamic water regime at the study site, which causes a highly variable spatial distribution of N_2O production conditions ranging from optimal to unfavorable. The environment in which denitrifying bacteria are developing, has a significant impact on their selection and activity of denitrifying species. Błaszczyk (1997), Błaszczyk *et al.* (1985) and Włodarczyk (2000) investigated the seasonal differentiation of dehydrogenase activity of Orthic Luvisol developed from silt and for comparison the same soil samples stored under air-dry conditions for about 7 years. The activity of air-dried soils was higher but the seasonal differentiation remained. It seems that dehydrogenase activity is affected by the environmental properties in the field so strongly that it 'keeps' its initial activity for long time. Measurements of its activity represent immediate metabolic activities of the soil microorganisms at the time of the test. These studies seem to confirm the strong influence of history of the soil on the currently measured soil denitrifying activity. Therefore, in terms of flooding, soil structure is not as significant for denitrification as in conditions of lower humidity, when it decides on the aeration status of the soil.

Closed system incubation gave us the opportunity to compare the capability of soil to nitrous oxide production and then release into the headspace, and its followed consumption. The ratio of 'the average daily effluxes' to 'the average daily consumption' (ae/ac ratio) showed that two Eutric Cambisols (one developed from sand – No. 3 and one developed from silt – No. 6) consumed more N_2O than released per day (ae/ac ratio = 0.4/0.9 and 4.8/9.5 mg kg^{-1} d^{-1} for the soils Nos 3 and 6, respectively). The highest ae/ac ratio (7.2) was observed in the Eutric Cambisols developed from sand. It means that sandy soils released 7 times more nitrous oxide than consumed per day. Lower ae/ac ratio (5.8) was observed in the loamy soils. The lowest one (3.9) was found in the soils developed from silt. Clough *et al.* (2006) studied consumption of the $^{15}N_2O$ in soil columns filled with sivied silt loam soil (sampled from 0-5 cm) *via* passive diffusion. Investigation showed that nitrous oxide consumption equal to 0.48 ng N_2O g^{-1} soil h^{-1}.

Considering the relation of the hN_2Oe and the hN_2Oc (he/hc ratio) it was shown that two Eutric Cambisols developed from silt (Nos 6 and 7) daily consumed more N_2O, than released (he/hc ratio = 8.7/9.5 and 11.1/15.7 mg kg^{-1} d^{-1}) The highest he/hc ratio (7.2) was observed in the Eutric Cambisols developed from sand. It means that sandy soils released daily 7.2 times more nitrous oxide than consumed per day. About twice lower he/hc ratio (4.1) was observed in the loamy soils. The lowest ratio (1.0) was found in the soils developed from silt. This means that all the produced nitrous oxide by silty soils theoretically may be consumed under field conditions provided that it remains within the gas soil phase or dissolved in soil suspension before it escapes to the atmosphere.

The different N_2O efflux and its consumption might be due some reasons:

– due to the differences in granulometric composition. In our experiment this effect is visible in all the investigated indexes but especially in he/hc ratio, where the two Eutric Cambisols with the index below one (0.9 and 0.7) are characterized by the highest content of silt fractions among the Eutric Cambisols (Tables 1 and 3). Generally, higher nitrate reduction and N_2O and consumption was observed in the Eutric Cambisols with higher silt fraction content;
– the results in Figs 7 and 8 points to the significant relationship between total N_2O efflux, daily N_2O consumption and $C_{org.}$ content;
– under the conditions of incubation experiment, both $maxN_2Oe$ and hN_2Oc occurred in the wide range of pH and equaled 4.63-7.23 for $maxN_2Oe$ and 4.51-7.60 for hN_2Oc. There were found large differences of pH values among the Eutric Cambisols developed from sand, silt and loam. The highest $maxN_2Oe$ was observed at pH 6.55, 5.01 and 7.23 but highest hN_2Oc was found at pH 4.9, 5.23 and 6.5 for the sandy, silty and loamy Eutric Cambisols.

Generally it can be concluded that in the Eutric Cambisols developed from sand and loam the N_2O efflux occurred at higher pH values than nitrous oxide consumption. Soil

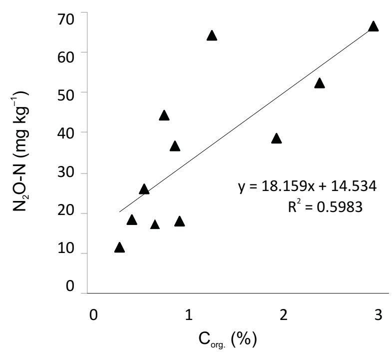

Fig. 7. Maximum cumulative N_2O efflux as a function of organic carbon content.

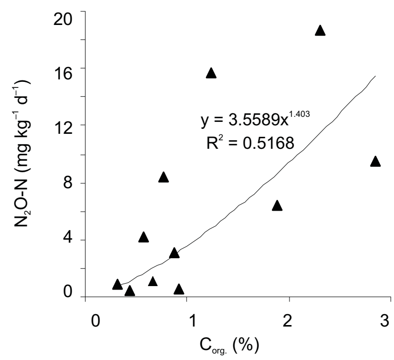

Fig. 8. An average daily N_2O consumption as a function of organic carbon content.

reduction processes are accompanied by changes in its pH towards the neutral. In other words, acidic soil pH increases while that of alkaline soils decreases (Gliński and Stępniewski, 1985). The increase in pH of acid soils on submergence is explained by H^+ uptake in most of the reduction processes. The decrease in pH of alkaline soils is caused by accumulation of CO_2 under anoxia (Ponnameruma, 1972). This phenomenon was also observed during incubation of the studied soils. Soil pH value for the soil developed from sand and loam was significantly higher (the average pH value was close to 6) than that of the soils developed from silt (the average pH value was close to 4.5 at the beginning of incubation). This means that in the case of sandy and loamy soils the N_2O efflux occurred at the pH value in the phase of decline but in silty soils in the phase of increasing pH. It seems that nitrous oxide reductase is more sensitive to alkaline environment, then followed by the accumulation of N_2O. Therefore better consumption of N_2O was observed under slightly acid conditions. The opposite situation was observed in the Eutric Cambisols developed from silt, although the difference in pH value was small and insignificant.

Taking into consideration all the Eutric Cambisols the highest maxN_2Oe was observed at pH 5.01 (66.5 mg N kg^{-1}) but hN_2Oc at pH 5.23 (18.7 mg N kg^{-1} d^{-1}) (data not shown and concern soils developed from silt). It means that there was not found the linear relationship between both max N_2O-e and hN_2O-c and pH value. Scholefield *et al*. (1997) showed that denitrification decreased with increasing soil pH within the range 5.1-9.4. Šimek *et al*. (2002) found that populations of soil denitrifies might be adopted to prevailing natural soil pH in the sense that they exhibited the highest denitrifying enzyme activity (DEA) at or near natural pH. If the pH was artificially changed (either decreased or increased) the DEA was lowered. Some researchers have suggested that soil microbial population dynamics may be more important factor than soil physical and soil chemical factors in explaining the characteristics of nitrous oxide production from soil (Abou-Seada and Ottow, 1985; Granli and Bøckmen, 1994; Pawlson *et al*., 1988).

CONCLUSIONS

1. Incubation of nitrate amended the Eutric Cambisols under hypoxic conditions was accompanied by an initial increase of headspace nitrous oxide and after reaching a maximum its subsequent decrease, even at the presence of nitrates.

2. The appearance of nitrous oxide was observed after 1-3 days and its maximum occurred after 2-28 days from the start of the incubation.

3. Investigated soils were characterized by different native nitrate content and the ability to its reduction. The percentage of nitrate reduced during incubation was: 35, 97, and 100% for the Eutric Cambisols developed from sand, loam and silt, respectively.

4. The highest N_2O efflux was observed from the Eutric Cambisols developed from silt and the lowest from the Eutric Cambisols developed from sand. The value of maximum cumulative N_2O efflux for particular soils ranged between 11.4-66.5 mg N kg^{-1}.

5. The Eutric Cambisols under study were characterized by different ability to nitrous oxide consumption which was 32.9, 99.2, and 100% of the N_2O evolved for the sandy, loamy and silty soils, respectively. The average value of nitrous oxide consumption for investigated groups ranged between 4.8-61.0 mg N kg^{-1}.

6. The Eutric Cambisols ability to produce nitrous oxide was correlated with its absorption.

7.The Eutric Cambisols capability to generate and to absorb nitrous oxide was correlated negatively with sand content and positively with silt, clay and organic carbon content.

8. Taking into account the soil ability to emission and consumption of nitrous oxide the investigated Eutric Cambisols may be classified as follows: 'weak emitters' and 'weak consumers'; 'good emitters' and 'good consumers'; and 'very good emitters' and 'good consumers'.

9. Three of the tested Eutric Cambisols showed greater activity in consumption of nitrous oxide than in its effluxing.

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