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PRODUCTION AND UPTAKE OF NITROUS OXIDE (N_2O) AS AFFECTED BY SOIL CONDITIONS

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187

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CONTENT

1. INTRODUCTION

Nitrous oxide (N_2O) is one of the greenhouse gases. Its concentration in the atmosphere is small (about 1,000 times lower than that of carbon dioxide, CO_2), but the efficiency of sorption of infrared radiation is up to 296 times higher. Furthermore, the dissociation of N_2O in the stratosphere is a source of nitric oxide (NO), which contributes the destruction of the ozone layer. It is assumed that the contribution of N_2O in enhancing the greenhouse effect is 6% (IPCC 2001). The increase of N_2O concentration in the troposphere form about 270 ppbv in the period before industrialization, to 314 ppbv in 1998 (Flückiger *et al.* 1999), and 350 ppbv in 2003 (Takaya *et al.* 2003) is a consequence of its elevated emissions from natural and agricultural ecosystems. The use of nitrogen fertilizers and cultivation of legumes have been regarded to strongly influence these changes. It is estimated that nitrogen losses from organic fertilizers (manure/compost) in result of N_2O emission may amount up to 1% of nitrogen introduced in the fertilizer. In addition, about 1% N fixed in legume plants undergoes denitrification to N_2O (Stalenga and Kawalec 2007).

Soils are the dominant source of N_2O (Davidson 1991, Khalil and Rasmussen 1992, Prinn 1994). Nitrous oxide emissions account for about 10% of global greenhouse gas emissions, with ~90% of these emissions derived from agricultural practices (Smith *et al.* 2007). It is estimated that annual N_2O emission from soils into the atmosphere is about 9.5 Tg N_2O-N, that is 65-70% of global emissions of N_2O, of which 3.5 Tg N $year^{-1}$ originate from agricultural soils, and 1 Tg N $year^{-1}$ – from grasslands (IPCC 2001). Nowadays, N_2O emission in temperate climates reaches about 2.2 kg N_2O-N ha^{-1} $year^{-1}$ (Sapek 2008). Calculated emission of N_2O from agriculture for Poland in 2005 has been shown in Table 1 (Zaliwski and Purchała 2007).

Table 1. N_2O emission (in Gg) from agriculture in Poland in 2005 as calculated according to the 2006 IPCC methodology (after Zaliwski and Purchała 2007)

Emission source	N_2O emission in 2005
Soils	28.8
Animal manure	34.0
Crop residue burning	0.0
Total from agriculture	62.8

Although not all of the scientific community share the opinion related to human participation in enhancing the greenhouse effect, studies to clarify the mechanisms of greenhouse gases are now popular and intensive. Addi-

tionally, in result of the initiatives contained in the UN Framework Convention on Climate Change on Earth, many countries are obligated to reduce emission of greenhouse gases in years 2008-2012 by about 5% compared to the level of 1999 (New York 1992, the Kyoto Protocol 1997).

Nitrous oxide is produced primarily by the activity of both denitrifying and nitrifying microorganisms that inhabit the soils, sediments, water reservoirs and sewage treatment plants. Other mechanisms related to emission of N_2O are: heterotrophic nitrification, aerobic denitrification and chemodenitrification.

The heterotrophic denitrification is considered the main source of N_2O. It occurs after oxygen depletion, with nitrate(V), (NO_3^-), used by facultative anaerobes as an alternative electron acceptors in the course of cell metabolism. N_2O is an intermediate product here. Studies on isolated enzymes involved in the process of denitrification suggest their strong sensitivity to oxygen. Inhibition in the presence of O_2, especially in the case of N_2O reductase that catalyzes the last step of denitrification (a reduction of N_2O to N_2). However, in a heterogeneous soil environment, where air-filled pores are located close to the anaerobic aggregates containing micropores saturated with water, microhabitats favourable to the development of various microbial populations occur. The confirmation of the possibility of denitrification process in aerated soil (in the presence of O_2) are reported with ^{15}N-labeled nitrogen. The distinction between processes of aerobic nitrification and anaerobic denitrification as N_2O source *in situ* is very difficult or even impossible. However, attempts to clarify the mechanism of nitrous oxide formation in the soil seem to be desirable not only because of the involvement of N_2O in creating the greenhouse effect, but also because this processes are involved in the loss of nitrogen fertilizer that were applied.

It is generally accepted that the main source of N_2O to the atmosphere are primarily wetland soils, and over fertilized soils. However, increasing attention is paid to estimating the contribution of nitrification to N_2O emissions. Moreover, it is not fully elucidated, which soil conditions determine the full or incomplete course of denitrification (to N_2 or ending at the stage N_2O, respectively). Due to the adaptation of denitrifying microorganisms for both aerobic and anaerobic conditions, their activity is characteristic for agricultural soils that have a wide spatial and temporal variability of air-water conditions

In the course of autotrophic nitrification, N_2O is a by-product. This aerobic process is based on the gradual oxidation of ammonium (NH_4^+) to the form of nitrates(V).

2. PROCESSES RELATED TO N_2O EMISSION AND SORPTION

Nitrous oxide is one of the elements that bind biogeochemical cycles in soil environment. N_2O appears in the course of several metabolic pathways of a large group microorganisms inhabiting the soil. Nitrous oxide in soils is produced largely by the microbial process of denitrification and to a lesser extent by nitrification. Nitrification is an aerobic process that oxidizes ammonium (NH_4^+) to nitrate (NO_3^-), with N_2O as a by-product, whereas dissimilatory nitrate reduction (denitrification) is an anaerobic process that reduces NO_3^- − to N_2, with N_2O as an obligatory intermediate (de Klein and Eckard 2008). Denitrifying bacteria are aerobes that substitute NO_3^- or NO_2^- for O_2 as the terminal electron acceptor when there is little or no O_2 available. Denitrifiers are diverse in terms of respiratory and nutrient requirements. Thus, the distinction between the processes responsible for the production of N_2O is often difficult or even impossible (Conrad, 1996). The problem is further complicated by the fact that physiologically defined groups of microorganisms are widespread in different taxonomic units. It includes proteobacteria, gram-positive and gram-negative bacteria, and fungi. Enzymatic basis for the emission and sorption of N_2O are not fully recognized. The best documented processes are denitrification and nitrification (Zumft 1993, Ferguson 1994, Ye *et al.* 1994).

2.1. Denitrification

Denitrification is one of the most important sources of nitrous oxide (Webster and Hopkins 1996, Paul and Clark 1998). In the process (Fig. 1), N_2O is an intermediate product in the sequential reduction of nitrate(V) (Zumft 1993, Ferguson 1994, Ye *et al.* 1994). Under appropriate conditions, N_2O is further reduced to molecular nitrogen, N_2 (Firestone 1982). This last step of denitrification is responsible for the sorption (uptake) of N_2O. Thus, nitrous oxide is both produced and consumed by denitrifying microorganisms.

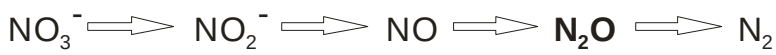

Fig. 1. Denitrification process

The enzymes that are involved in the denitrification process is a sequence: NO_3^- reductase (NAR), NO_2^- reductase (NIR), NO reductase (NOR) and N_2O reductase (N_2OR) (Megonigal *et al.* 2004, Hino *et al.* 2010).

Denitrification is very common among soil microorganisms. Hundreds denitrifiers were isolated from soils, most of them are heterotrophic, facultative anaerobes that belong to a variety of species (Tab. 2). The largest groups are *Bacillus* and *Pseudomonas* genera (Lloyd 1993).

Table 2. Denitrifying microorganisms (Kotełko *et al.* 1979, Lloyd 1993, Paul and Clark 1998, Takaya, 2009)

Genus	Species (examples)	General charcteristics
Achromobacter	*A. liguefaciens, A. fisheri*	Organotroph
Aerobacter	*A. aerogenes*	Organotroph
Agrobacterium	A. tumefaciens	Organotroph
Alcaligenes	*A. eutrophus*	Organotroph
Aspergillus	*A. nidulans*	Eucaryota (fungi)
Azospirillum	*A. brasilense*	Organotroph
Bacillus	*B.* LICHENIFORMIS	Organotroph
Escherichia	*E. coli*	Organotroph
Fusarium	*F. oxysporum*	Eucaryota (fungi)
Halobacterium	*H. denitrificans*	Archebacteria
Micrococcus	*M. denitrificans*	*Paracoccus denitrificans*, formerly
Nitrosomonas	*N. europea, N. eutropha*	Chemolithotroph
Paracoccus	*P. denitrificans*	Chemolithotroph (facultative)
Penicyllium	*Penicillium sp.*	Eucaryota (fungi)
Pseudomonas	*P. denitrificans*, *P. stutzeri, P. aeruginosa*	Organotroph
Rhizobium	*R. meliloti*	Diazotroph
Rhodopseudomonas	*R. sphaeroides*	Photolithotroph
Thiobacillus	*T. denitrificans*	Chemolithotroph (facultative)

Denitrification is the most common form of anaerobic respiration based on nitrogen. Energy is conserved by coupling electron transport phosphorylation to the reduction of nitrogen oxides located outside the cell. Because nitrogen is not assimilated into the cell, the process is dissimilatory. Respiratory denitrification is more energetically favorable than Fe(III) reduction, SO_4^{2-} reduction or methanogenesis, and it tends to be the dominant form of anaerobic carbon metabolism when NO_3^- or NO_2^- are available in poorly aerated soils.

The optimum pH for N_2O emission via denitrification varies with species and age of the organism and nitrate concentration, but most denitrifiers have optimum pH for growth between 6 and 8. Although the process is favoured at slightly alkaline pH, it proceeds up to pH as low as 3.5, and can account for significant N losses in acid soils (Aulakh *et al*. 1992).

Soil acidity through various mechanisms may modulate the emission of N_2O. Increased soil acidity may lower the decomposition rate of soil organic matter (Perrson *et al.* 1989), hence reducing the availability of N substrate for N_2O production. Higher soil acidity directly reduces nitrification and denitrification (Bramley *et al*. 1989). Influence of acidification may severely inhibit N_2O reductase with the result that denitrification yields more N_2O than N_2 (Weier and Gillam, 1986). Another mechanism occurs when decreasing pH reduces the availability of molybdenum that in turn may reduce the synthesis of NO_3^- reductase, a molybdo-protein enzyme. Beside it with decreasing pH, NO_2^- formed by NO_3^- reduction would become toxic and solubilization of aluminium or manganese might cause toxicity effects (Firestone 1982).

The actual mechanism of controlling N_2O emission in acid soils is still unknown. Firestone *et al*. (1980) reported that the influence of soil acidity is exerted through its effect on NO_3 or NO_2 formation. Sitaula *et al*. (1995) reported that N_2O fluxes were significantly reduced at pH 3, increased when the pH increased to 4, but again decreased at pH 5.5 (with no fertilizer application, as well as with the application of 90 kg N ha^{-1}). It is generally accepted that evolution of N_2O relative to N_2 increases with increase in pH (Aulakh *et al*. 1992, Firestone 1982).

Most of denitrifying microorganisms are active also in aerobic conditions. The transition to anaerobic respiration metabolism is implicated by the limited availability of oxygen (Tiedje 1988). Denitrification occurs in micro-spaces (microhabitats) where microbial O_2 demand exceeds the rate of its transport from the atmosphere. Such conditions may occur when the rate of diffusion of O_2 is limited by water filled pores inside the soil aggregates, in areas saturated with water, or in places where oxygen demand is exceptionally high (*hot spots*) due to a local ac-

cumulation of readily available organic matter (Bandibas *et al.* 1994, Pathak 1999). Parkin (1987) showed that a single leaf present in the soil, which constitute only 1% of the soil mass, "supports" up to 85% of the denitrification. Højberg *et al.* (1994) using O_2 and N_2O microsensors demonstrated oxygen consumption that occurred on the surface of soil aggregates simultaneously with production of nitrous oxide. Horn *et al.* (1994) studied the colonization of the artificial aggregates with a diameter of 20 mm by soil microorganisms. Obligatory anaerobes were most numerous in the centre of aggregates, obligatory aerobes on the outer surface, while denitrifying bacteria occupied an area on the border aerobic/anaerobic zone. According to some authors, denitrification occurs even in the driest ecosystems on Earth (Peterjohn 1991).

Although the production of nitrous oxide is mainly connected with the denitrification occurring in anaerobic conditions, there are many reports of N_2O formation by denitrifying microorganisms under aerobic conditions. For example, some species of facultative anaerobic *Pseudomonas* were found to show the ability to denitrife under aerobic conditions. Similarly, the popular enterobacteria *Escherichia coli*, and fungi *Aspergillus* and *Penicylium* can reduce nitrates(III) under aerobic conditions (Yoshida and Alexander 1970, Lloyd *et al.* 1987).

2.2. Nitrification

Nitrification is the process of oxidation of NH_4^+ to NO_2^- and NO_3^- (Fig. 2). Aerobic, chemolitoautotrophic nitrifying bacteria utilize CO_2 as a carbon source. However, some nitrifiers use, to a lesser extent, organic matter (Kotełko *et al.* 1979). Oxidation of NH_3 to NO_3^- is carried out mainly by two distinct groups of bacteria: *Nitrosomonas* and *Nitrobacter* (Koops *et al.* 1991). In the case of *Nitrosomonas*, the oxidation of NH_3 to NO_2^- occurs in two stages: the first is the oxidation of NH_3 to hydroxylamine (NH_2OH), and the second is the oxidation of NH_2OH to NO_2^-. The first step is catalyzed by the enzyme associated with the cell membrane, ammonia monooxygenase. Reaction requires the presence of molecular oxygen, O_2 (Prosser, 1989). Although the majority (approximately 95%) of the total pool of $NH_3 + NH_4^+$ at $pH \leq 8$ is present in the form of NH_4^+, nitrifying microorganisms are referred to as ammonia oxidizers, because at the enzyme level, a form of NH_3^- is used.

In the second stage, of the hydroxylamine is oxidized to NO_2^- by hydroxylamine oxidoreductase, an enzyme located in the periplasmic space (Hooper 1986; Prosser 1989, Muller *et al.* 1995). The oxidation of NH_4^+ has been observed in

heterotrophic fungi, however, bacteria are considered the main source of NO_3^- in most ecosystems.

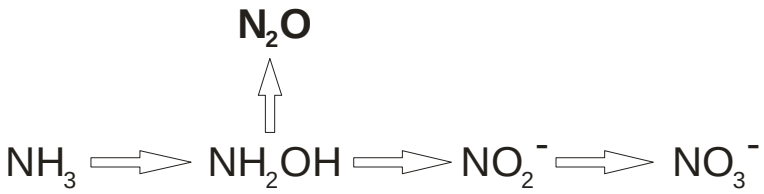

Fig. 2. Nitrification process

In the course of nitrification, N_2O is formed in the first reaction step performed by *Nitrosobacteriaceae.*

The growth of nitrifying bacteria is slow even in favourable conditions. For most species, the growth is optimal at a temperature of 25-30^{o}C, pH 7.5-8.0, and ammonia and nitrate(III) concentrations of 2-10 mM and 2-30 mM, respectively. In such conditions, cell division time is approximately 8 hours for *Nitrosomonas* and 10 hours for *Nitrobacter* (Bock *et al.* 1986). Optimum oxygen concentration is only 3-4 mg O_2 dm^{-3} for the growth medium of these organisms (Prosser 1989). Hynes and Knowles (1984) observed in model studies, that the production of N_2O by *Nitrosomonas europaea* under atmospheric O_2 depends on the pH and a buffer type, with minimum pH of 6.0, and optimum pH of 8.5. A strong increase in the emission of N_2O at pH 8.5 was observed when the inorganic buffer was replaced by an organic one (Hynes and Knowles 1984).

Although field measurements indicate that high N_2O emission rates generally coincide with soil conditions that are conducive to denitrification (anaerobic, good NO_3^- supply), nitrification is often an essential prerequisite for the conversion of urine and N fertilizer inputs into soil NO_3^- (de Klein and Eckard 2008).

2.3. Other processes running with the emission of N_2O

It has been recently shown that other, less known metabolic pathways occur in soil that are associated with the use of nitrates(V) and production N_2O and N_2 (Zehr and Ward 2002).

2.3.1. Denitrification led by nitrifiers (nitrifier denitrification)

Some nitrifying bacteria produce N_2 from NH_4^+ using O_2 or NO_2 (nitrogen dioxide) as oxidants. The process has been named nitrifier denitrification, which indicates that it involves autotrophic NH_3^- oxidizers with an enzyme system similar to that of the heterotrophic denitrifying bacteria (Fig. 3), (Goreau *et al.* 1980, Robertson and Kuenen 1984, Poth and Focht 1985, Wrage *et al.* 2000, Megonigal *et al.* 2004). What is the final product of the route, it depends on the type of microorganism and the presence of available electron acceptors (Lipschultz *et al.* 1981, Hynes and Knowles 1984, Remde and Conrad 1990, Bock *et al.* 1995). All isolated autotrophic microorganisms that oxidize ammonia, and have the ability to aerobic denitrification, belong to the *Nitrosomonas* genus (Ritchie and Nicholas 1972, Anderson and Levine 1986, Kuenen *et al.* 1994, Bock *et al.* 1995).

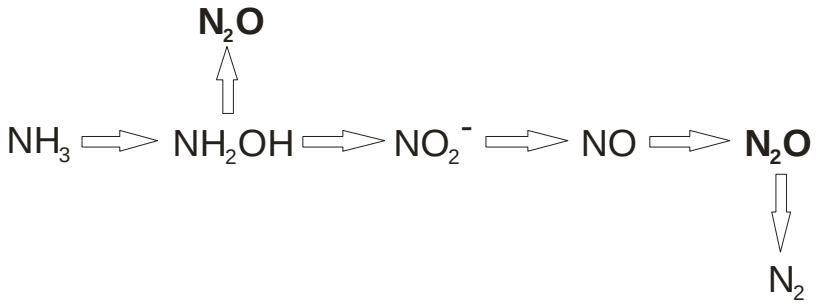

Fig. 3. Nitrifier denitrification

The mechanism of N_2O production in the course of this process remains unclear. Two hypothesis for aerobic denitrification are proposed. The first indicates that N_2O is produced by the ammonia oxidizing microbial activity (Ritchie and Nicholas 1972; Bock *et al.* 1995), while the latter suggests the chemical nature of the reaction (chemodenitrification) in which unstable intermediate products of nitrification are converted (Hynes and Knowles 1984, Stüven *et al.* 1992). The study under aerobic and anaerobic conditions with isotope ^{15}N has confirmed that the main mechanism involved in the production of N_2O was here the enzymatic reduction of NO_2^- (Anderson *et al.* 1993, Jetten *et al.* 1997).

2.3.2. Denitrification dependent on nitrification)

Under natural conditions, nitrate(V) is the end product of chemoautotrophic nitrification. This compound is not used by organisms that carry out this process, while it is attractive for heterotrophic denitrifiers as the terminal electron acceptor. Since nitrification occurs mainly under aerobic conditions, while denitrification occurs mainly under anaerobic conditions, these two processes are spatially "separated". However, if there are sufficiently close to each other, then the NO_3^- transport and utilization can be relatively rapid. Some authors combine these processes and called them as nitrification dependent denitrification (Fig. 4). The process requires the presence of NH_4^+, C_{org} and both aerobic and anaerobic microsites. Some distinct groups of microorganisms are involved in this process (both autotrophic and heterotrophic) and the relative proportions of secreted forms of NO_3^-, NO, N_2O and N_2 may vary considerably. Several factors may cause the reduction of NO_3^- to N_2 will be incomplete, which is reflected in the production of intermediate products (NO and N_2O).

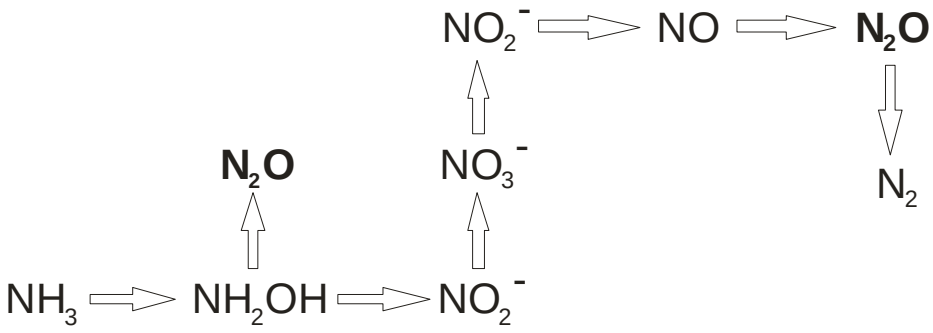

Fig. 4. Coupled nitrification-denitrification

Typical nitrification dependent denitrification takes place in water reservoirs, where nitrification is the main source of NO_3^- for microorganisms performing denitrification process (Seitzinger 1988). A similar situation may be observed in unfertilised soils, where the availability of N forms is heavily dependent on the level of the activity of bacteria assimilating N_2, that catalyze ammonification of organic N compounds and nitrification of ammonium form. However, denitrifica-

tion process is independent of the nitrification, if enough nitrate(V) are supplied to the soil from external sources such as fertilizer, (Megonigal *et al*. 2004).

2.3.3. Dissimilative reduction of nitrates (V) to ammonium (DNRA)

Dissimilative reduction of nitrate to ammonium (Fig. 5) is an anaerobic microbial process in which NO_3^- is converted to NO_2^-, and next to NH_4^+. These reactions are catalyzed by nitrate reductase and nitrite reductase. In this process, N_2O is a by-product. Conditions favourable for DNRA are similar to those of denitrification (Tiedje *et al.* 1982, Zumft 1997).

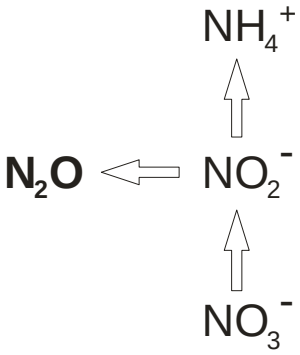

Fig. 5. Dissimilative nitrate reduction to ammonium (DNRA)

It is also assumed that N_2O can be reduced to N_2 not only by denitrifying bacteria, but also by some bacteria that carry out the DNRA process (Samuelsson 1985, Teraguchi and Hollocher 1989, Schumacher and Kornecka 1992).

2.3.4. Non-biological processes

Some reports indicate that an abiotic origin of N_2O in soil is possible. For example, N_2O may be produced by chemical decomposition of NO_2^- (Hooper and Terry 1979). The reaction is favoured by a low pH. The main products are NO and N_2O in small amounts (van Clemput and Baert 1984, Martikainen and De Boer 1993).

According to some authors, chemodenitrification (i.e. chemical decomposition of NH_2OH to N_2O, and chemical reaction between the NH_2OH and NO_2^-) causes loss of inorganic nitrogen that can be observed during growth of Nitrosomonas. europea in aerobic conditions (Stüven *et al*. 1992). However, formation of N_2O by

chemical reaction of NO_2^- and hydroxyl amine does not seem to be important since there was no significant increase in the rate of N_2O production by the addition of NO_2^- or NH_2OH in soils (Bremner *et al*. 1980). Yoshinari (1990) also reported that chemical production of N_2O in soil and other ecosystems is of minor importance as a source of N_2O since the reaction becomes significant only in the presence of relatively high NO_2^- concentration (>1 mM), which is not commonly found in natural environments. In spite of lot of work on the mechanism of N_2O emission, the primary source of observed soil emission is often uncertain. It is generally assumed that a majority of N_2O production occurs in proximity to the surface of soil (Conrad *et al*. 1983). However, Burton and Beauchamp (1994) observed a significant sub-surface N_2O production. They emphasized the need to examine the soil as a three-dimensional body for production, transport and storage of N_2O. Seiler and Conrad (1981) concluded that N_2O produced at depths are likely to be consumed in upper soil layer during upward transport by a diffusive process. This process of N_2O reduction to N_2 during diffusion would be enhanced if the soil were wet, since diffusion coefficient of N_2O is much less than that of N_2 (Letey *et al*. 1980).

Presumably, abiotic N_2O production in most ecosystems is negligible (Webster and Hopkins 1996).

3. N_2O PRODUCTION AND UPTAKE IN LABORATORY EXPERIMENT (SOIL INCUBATION AT DIFFERENT OXYGEN, NITRATE AND ORGANIC CARBON AVAILABILITY)

Although field studies give real information on N_2O emission from soils to the atmosphere, laboratory experiments are very useful because allow to eliminate influence of temperature. Temperature largely fluctuates, and thus strongly effects metabolic activity of soil microorganisms in their natural ecosystems.

This chapter reports the result of the experiment with incubation of 10 topsoils (Tab. 3) of different texture (Cambisol, Luvisol, Phaeozem, Solonetz) under laboratory conditions (Szarlip 2009). Control soils (without addition of N and C substrates), and soils with medium optimal for denitrifiers (containing NO_3^-, glucose and microelements) were incubated under aerobic conditions (wet soils) or under restricted O_2 diffusion (flooded soils) at 20^{o}C. In additional variants, soil headspace was replaced with N_2 to create anaerobic conditions at the start of the incubation.

Table 3. Basic information about soils (Glinski *et al*. 2000)

Soil No	Soil type	Location	Soil horion/ depth (cm)	% content of			N_{og} (%)	OM (%)	pH (H_2O)	$N\text{-}NO_3^-$ (mg kg^{-1})	$N\text{-}NH_4^+$ (mg kg^{-1})
				sand	silt	clay					
27	Luvisol (loamy sand)	Koszalin (Poland)	A1 (15-20)	77.0	21.0	2.0	0.115	1.76	6.5	0.77	36.40
302	Cambisol (loamy sand)	Poznań (Poland)	A1 (0-30)	63.5	33.0	3.5	0.110	0.53	7.7	55.80	36.74
554	Luvisol (loamy silt)	Lublin (Poland)	Ap (0-25)	37.7	58.5	3.8	0.105	1.83	5.9	3.90	25.96
691	Phaeozem (loess)	Opole (Poland)	Ap (0-35)	13.9	79.7	6.4	0.115	1.89	7.2	2.97	61.29
733	Cambisol (loamy sand)	Częstochowa (Poland)	Ap (0-25)	68.0	29.6	2.5	0.100	2.09	6.4	4.73	36.69
794	Phaeozem (loess)	Kielce (Poland)	Ap (10-20)	14.7	79.0	6.3	0.155	1.96	7.6	20.29	31.34
A1	Cambisol (silty loam)	Wieselburg (Austria)	Ap (0-20)	20.0	46.6	33.4	0.155	1.97	7.4	28.77	26.13
S3	Phaeozem (loamy sand)	Makov (Slovakia)	Akp (0-38)	42.6	39.0	18.4	0.230	3.37	8.0	36.72	36.24
C2	Phaeozem (sandy loam)	Tišice (Czech Rep)	Ap (15-20)	60.0	24.0	16.0	nt	3.20	8.5	19.04	35.09
W4	Solonetz (loamy clay)	Karcagpuszta (Hungary)	A (0-20)	20.8	34.0	45.2	0.235	3.56	7.4	15.40	25.04

OM – organic matter; nt – not tested.

Experiment included seven variants.

Control soils – incubation without amendments:

- KT variant – aerobic conditions (wet soil, pF 1.5),
- KZ variant – restricted O_2 diffusion (flooded soil),

Stimulation of denitrification (nitrate and glucose added):

- DT variant – aerobic conditions (wet soil, pF 1.5),
- DZ variant – restricted O_2 diffusion (flooded soil),
- DB variant – anaerobic conditions (N_2 atmosphere, wet soil, pF 1.5).

Stimulation of N_2O uptake (N_2O added):

- PT variant – aerobic conditions (wet soil, pF 1.5),
- PB variant – anaerobic conditions (N_2 atmosphere, wet soil, pF 1.5).

Nitrate was added as KNO_3 (35 mg N^- kg^{-1} $soil^{-1}$). Initial glucose and N_2O concentrations were 1 g kg^{-1} and 1% v/v, respectively. The medium optimal for the growth of denitrifying microorganisms composed of: KNO_3 – 2.0 g, glucose – 10.0 g, $CaCl_2$ – 5.0 g, Winogradski salts – 50 cm^3, distilled water – up to 1000 cm^3) was added in an amount of 0.1 cm^3 per 1 g of soil (Pochon and Tardieux 1962). Winogradski salts contained: K_2HPO_4 – 5.00 g, $MgSO_4 \cdot 7H_2O$ – 2.50 g, NaCl – 2.50 g, $Fe_2(SO_4)_3$ – 0.05 g $MnSO_4$ – 0.05 g, distilled water to 1000 cm^3.

The composition of the air above the soil was determined by gas chromatography analysis using the Shimadzu GC14 chromatograph equipped detectors TCD and ECD. Measurements of soil redox potential (Gliński and Stępniewski 1985) confirmed aerobic conditions of soils incubated at pF 1.5 (Eh in average 543 mV at the end of the incubations).

Tested soils showed high variability of their production and consumption of N_2O.

3.1. Control soils without amendments, aerobic conditions – KT variant

Production of nitrous oxide in control variant under aerobic conditions are presented in Figure 6. Production of N_2O in the most active soil, Pheozem No. 794, began on the third day of incubation. The concentration of nitrous oxide was maintained at a level of 0.6-0.7 mg N_2O-N kg^{-1} for a week and then rapidly increased to a value of 1.96 mg N_2O-N kg^{-1}. In the case of soil No. 302 (Cambisol), N_2O production started only after 10th day of incubation, and N_2O concentration at the end of incubation was 0.68 mg N kg^{-1}.

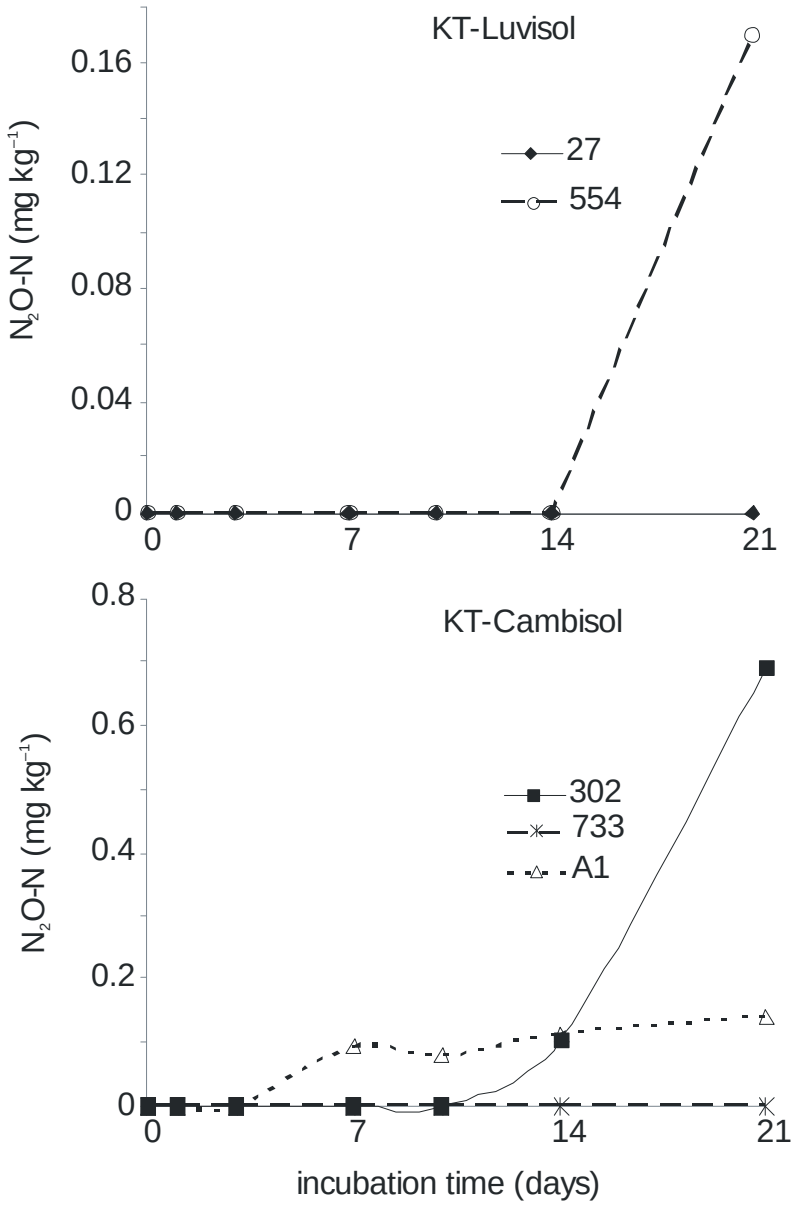

Fig. 6. Changes in the concentration of N_2O in control soils incubated under aerobic condition. Note different scales on the graphs

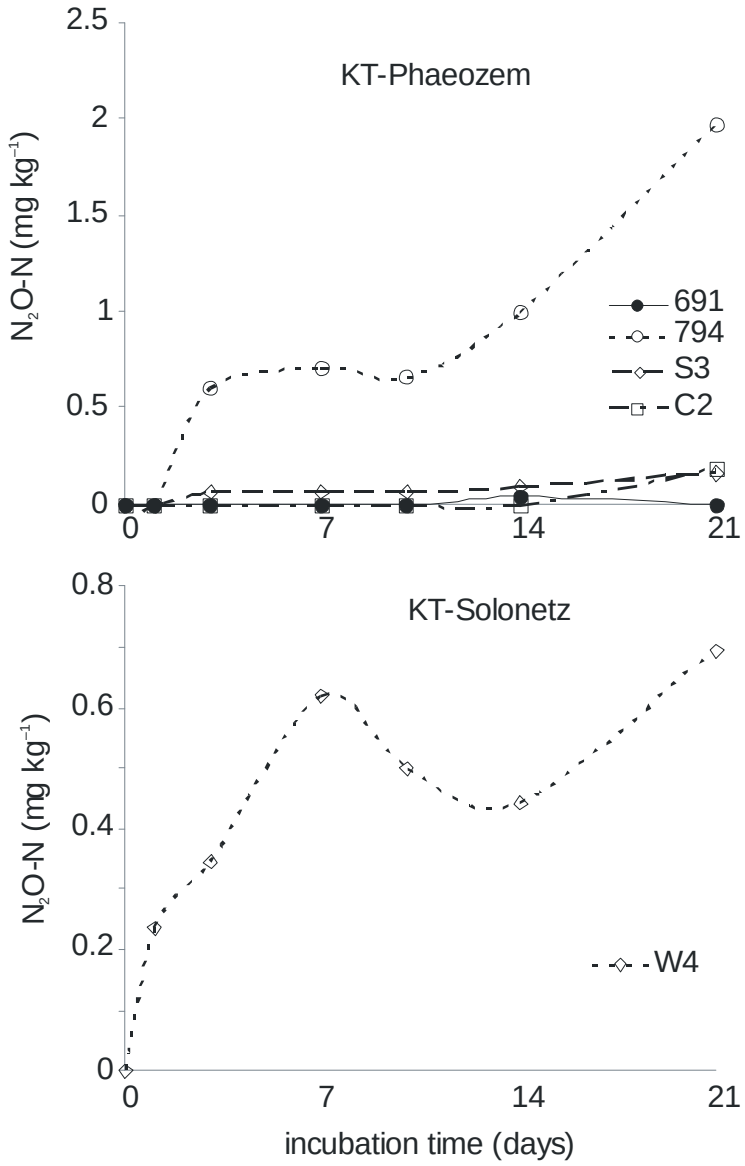

Fig. 6. Cont. Changes in the concentration of N_2O in control soils incubated under aerobic condition. Note different scales on the graphs

In Solonetz soil (No. W4), N_2O oscillated between 0.34-0.69 mg N_2O-N kg^{-1}. Two soils (Luvisol No. 27 and Cambisol No. 733) showed no N_2O production during aerobic incubation without amendments (Tab. 4).

In control aerobic variant, N2O uptake was recorded during incubation of four soils No. 691 and 794 (Phaeozems), A1 (Cambisol), and W4 (Solonetz). The highest N2O production and uptake rate showed soil No. 794, but only soil No. 691 (both Phaeozems) consumed all N2O that was previously produced.

Table 4. Production and uptake of nitrous oxide (N_2O) in control soils incubated under aerobic conditions

KT	Production N_2O				Uptake N_2O			
Soil No.	The highest amount of produced N_2O		The highest production rate		The highest amount of N_2O uptake	The highest uptake rate		% of produced
	mg N_2O-N kg^{-1}	Day	mg N_2O-N $kg^{-1}d^{-1}$	Day	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	0.0	–	–	–	–	–	–	–
302	0.688	21	0.083	14-21	–	–	–	–
554	0.169	21	0.024	14-21	–	–	–	–
691	0.044	14	0.011	10-14	0.044	0.006	14-21	100
733	0.0	–	–	–	–	–	–	–
794	1.957	21	0.301	1-3	0.137	0.017	7-10	7
A1	0.143	21	0.02	3-7	0.021	0.004	7-10	15
C2	0.183	21	0.026	14-21	–	–	–	–
S3	0.164	21	0.034	1-3	–	–	–	–
W4	0.693	21	0.236	0-1	0.021	0.040	7-10	29

3.2. Control soils without amendments, restricted O_2 diffusion – KZ variant

Figure 7 illustrates the dynamics of N_2O in the control soils (without C and N addition) incubated under flooding which limits oxygen availability for soil microorganisms. Under restricted O_2 diffusion, denitrification activity was higher than under aerobic conditions. The most active soil (Pheozem No. 794)

started N_2O production after 1 day lag, and N_2O maximum of 17.2 mg N_2O-N kg^{-1} was reached on the 10 days of incubation. Next, slight uptake of N_2O began, which lasted to the end of incubation. In other soils, N_2O fluctuated between 3 and 7 mg N_2O-N kg^{-1} (soils No. 302 and A1, Cambisols) or in a lower range (other soils). Soil No. 27 (Luvisol) also in this variant did not produced N_2O.

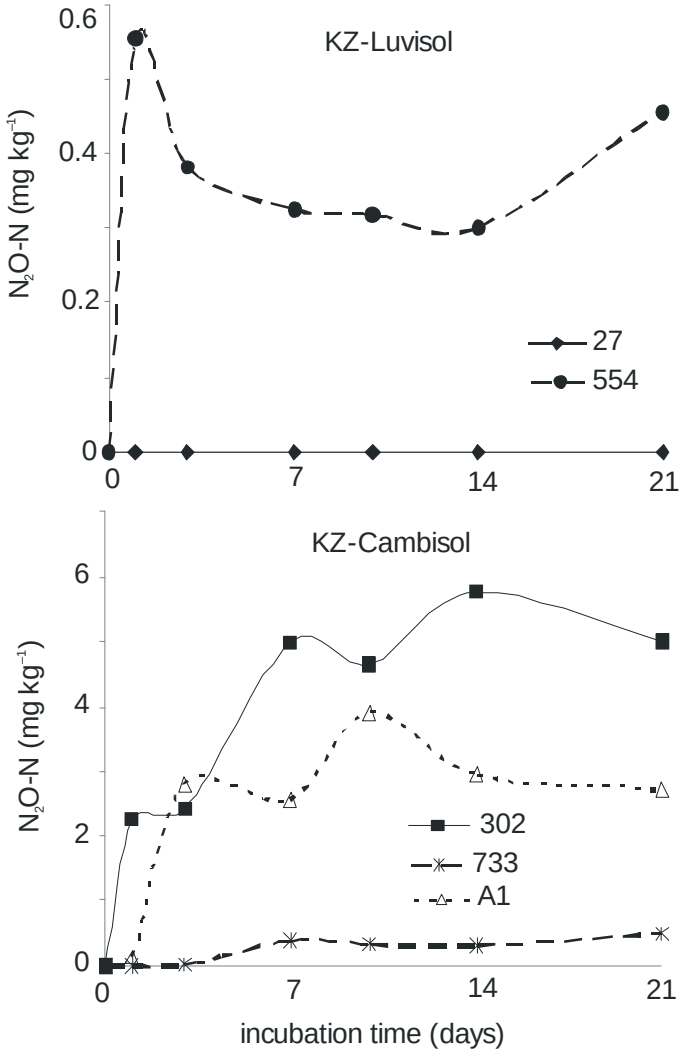

Fig. 7. Changes in the concentration of N_2O in control soils incubated under restricted O_2 diffusion. Note different scales on the graphs

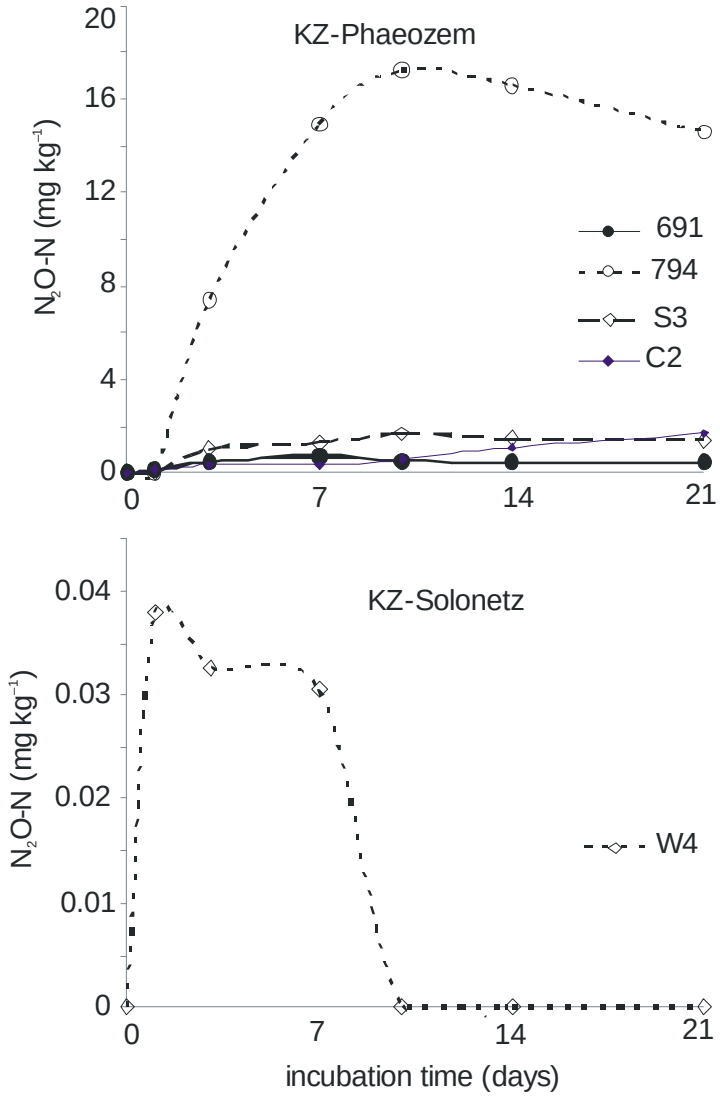

Fig. 7. Cont. Changes in the concentration of N_2O in control soils incubated under restricted O_2 diffusion. Note different scales on the graphs

In general, soils incubated under limited O_2 diffusion showed higher denitrification activity than under aerobic conditions (except of soils No. W4 and 27). The most pronounced increase, about 7-9 fold, was observed in the case of Phaeozems No. 794 and Nos. 302.

N_2O uptake showed all soils with exception of soil Lubisol No. 27. The highest ability to N_2O uptake showed Pheozem No. 794. Only Solonetz soil (No. W4), however, consumed all N_2O formerly produced. In other soils, the amount of N_2O that was taken up accounted for 10-46% of its maximum observed during incubation (Tab. 5).

Table 5. Production and uptake of N_2O in control soils incubated under restricted O_2 diffusion

KZ	N_2O production				N_2O uptake			
Soil No.	The highest amount of produced N_2O		The highest production rate		The highest amount of N_2O uptake	The highest uptake rate		% of emitted
	mg N_2O-N kg^{-1}	Day	mg N_2O-N $kg^{-1}d^{-1}$	Day	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	0.0	–	–	–	–	–	–	–
302	5.763	14	2.275	0-1	0.749	0.113	7-10	13
554	0.553	1	0.553	0-1	0.254	0.085	1-3	46
691	0.678	7	0.161	1-3	0.251	0.045	7-10	37
733	0.501	21	0.088	3-7	0.070	0.017	7-10	14
794	17.24	10	3.696	1-3	2.586	0.283	14-21	15
A1	3.900	10	1.325	1-3	1.170	0.238	10-14	30
C2	1.667	21	0.131	10-14	0.167	0.008	3-7	10
S3	1.684	10	0.516	1-3	0.269	0.057	10-14	16
W4	0.038	1	0.038	0-1	0.038	0.010	7-10	100

3.3. Stimulation of denitrification, aerobic conditions – DT variant

Incubation with addition of nitrate and glucose (medium optimal for the growth of denitrifying microorganisms) allowed to show the potential of tested soils to N_2O production under aerobic conditions with no limitation of the process by insufficient availability of the substrates (Fig. 8). Most active soil in this variant was Phaeozem soil No. 794. Already after three days of incubation, N_2O

in soil headspace reached a high value of 18.14 mg N_2O-N kg^{-1}, and at the end of incubation, N_2O concentrations as 20.22 mg N_2O-N kg^{-1}.

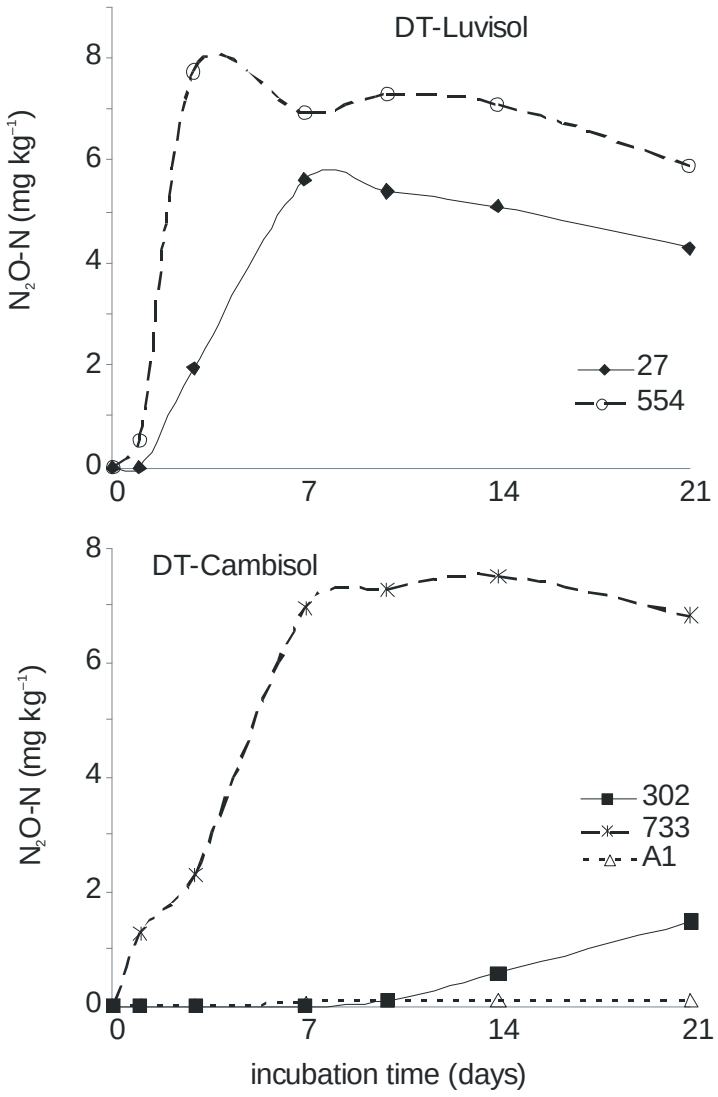

Fig. 8. Changes in the concentration of N_2O in tested soils enriched denitrification substrates incubated under aerobic conditions. Note different scales on the graphs

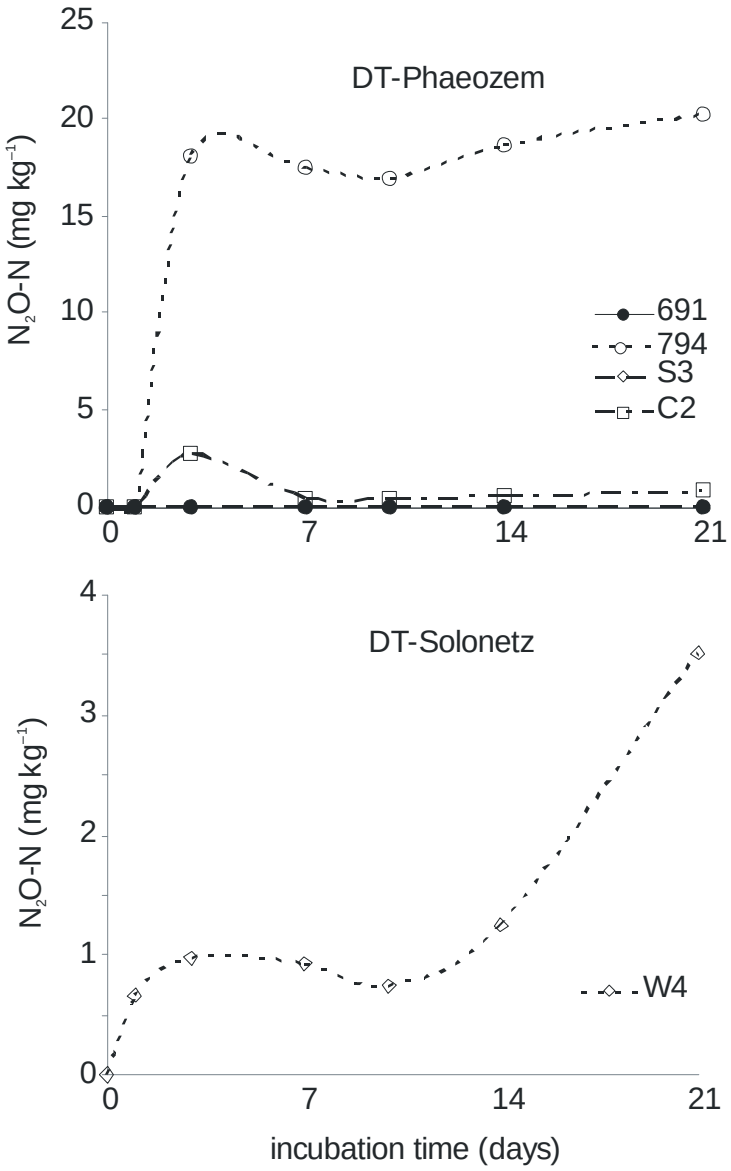

Fig. 8. Cont. Changes in the concentration of N_2O in tested soils enriched denitrification substrates incubated under aerobic conditions. Note different scales on the graphs

In soil No. 27 (Luvisol), the production of N_2O started at the beginning of incubation, and after 7 days reached 5.6 mg N_2O-N kg^{-1} (Tab. 6). Thus, for this soil in previously presented the control variants (KT and KZ), the limiting factor for the production of N_2O was a shortage of denitrification substrates NO_3^- and/or glucose.

The addition of C and N substrates resulted in the stimulation of denitrification also in soil No. 554 (Luvisol). In this case, the amount of evolved N_2O was even higher than in soils No. 302 and W4 (Cambisol and Solonetz, respectively), which belonged to more active soils in control variants.

In DT variant, all soils showed the ability to the production of N_2O. However, in some soils – Phaeozems No. 691 and S3, and Luvisol A1 – the amount of evolved N_2O was even lower than in control KT variant (10 times, by 50% and by 10%, respectively). Soils ability to N_2O sorption ranged between 0 to 100% of its maximum value (Tab. 6).

Table 6. Production and uptake of nitrous oxide in tested soils enriched denitrification substrates incubated under aerobic conditions

DT	N_2O production				N_2O uptake			
Soil No.	The highest amount of produced N_2O		The highest production rate		The highest amount of N_2O uptake	The highest uptake rate		% of emitted
	mg N_2O-N kg^{-1}	Day	mg N_2O-N $kg^{-1}d^{-1}$	Day	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	5.601	7	0.968	1-3	1.344	0.116	14-21	24
302	1.487	21	0.132	14-21	0.0	–	–	–
554	7.739	3	3.608	1-3	1.470	0.209	3-7	19
691	0.004	10	0.002	1-3	0.004	0.001	10-14	100
733	7.526	14	1.290	0-1	0.677	0.099	14-21	9
794	20.22	21	9.069	1-3	1.415	0.184	7-10	7
A1	0.127	14	0.018	3-7	0.032	0.005	14-21	25
C2	2.798	3	1.399	1-3	2.322	0.579	3-7	83
S3	0.059	7	0.016	1-3	0.055	0.009	10-14	93
W4	3.516	21	0.662	0-1	0.844	0.065	7-10	24

3.4. Stimulation of denitrification, restricted O_2 diffusion – DZ variant

All soils incubated with addition of nitrate and glucose under flooded condition showed ability to N_2O formation (Fig. 9). Soil No. 302 (Cambisol) showed other pattern of N_2O changes than other soils. The concentration of this gas reached a high value of 65.2 mg N_2O-N kg^{-1} on the third day of incubation. Then N_2O was consumed. For other soils, maximum N_2O in the headspace was in the range of 0.174-18.5 mg N_2O-N kg^{-1} (Tab. 7).

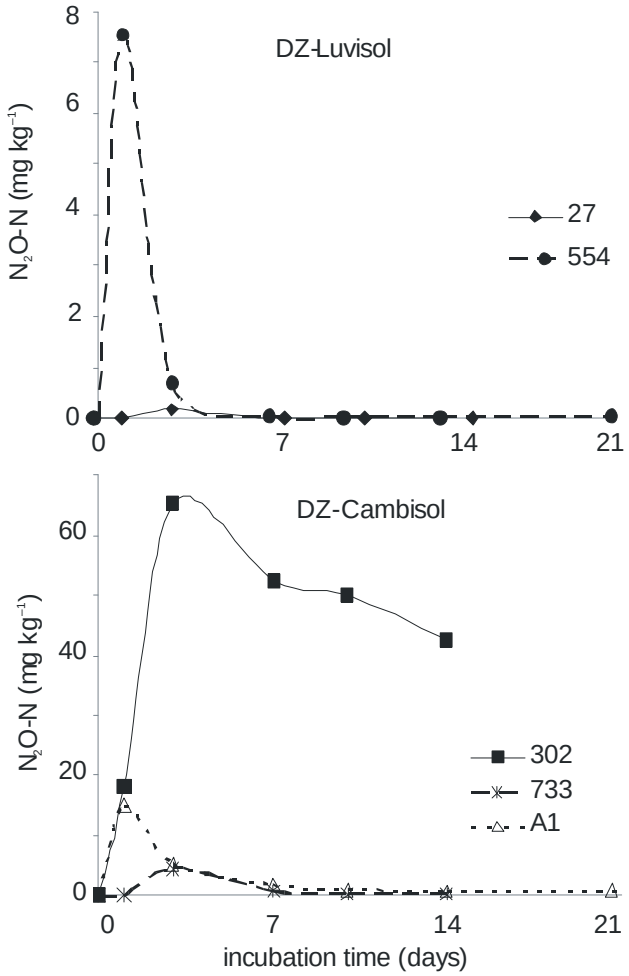

Fig. 9. The changes in N_2O concentrations in tested soils enriched with denitrification substrates incubated under restricted O_2 diffusion. Note different scales on the graphs

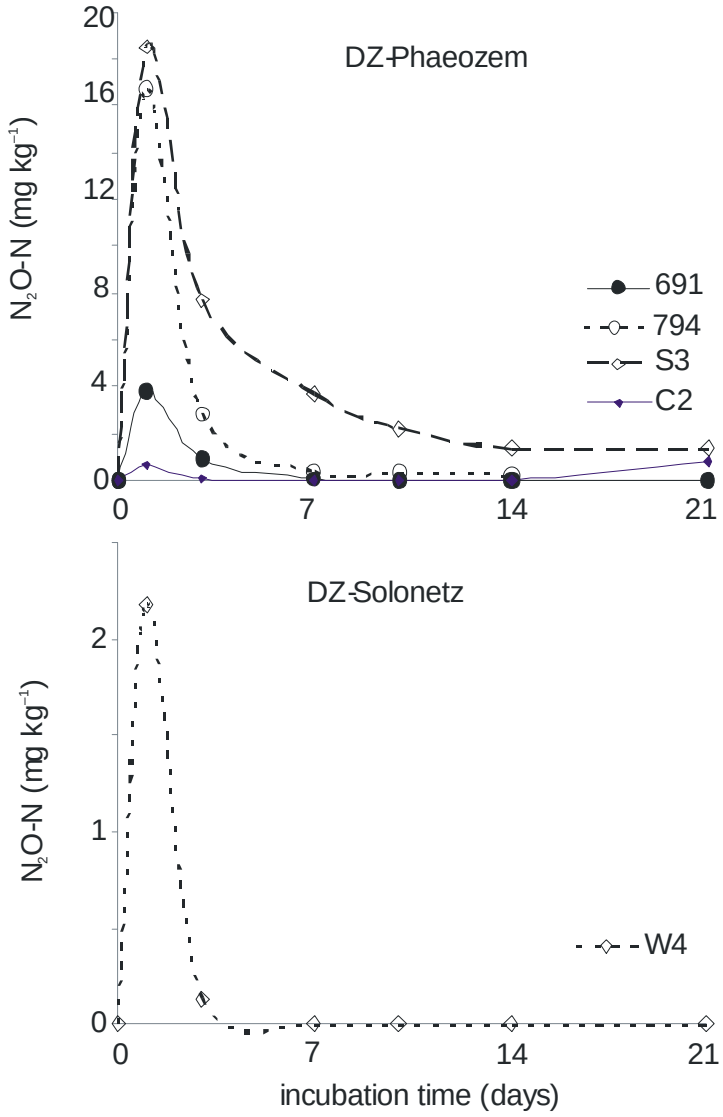

Fig. 9. Cont. The changes in N_2O concentrations in tested soils enriched with denitrification substrates incubated under restricted O_2 diffusion. Note different scales on the graphs

These results confirmed that at limited oxygen concentration, N_2O produced (as an intermediate product of denitrification) underwent further reduction to N_2. In most soils, N_2O uptake occurred within few days after its maximum concentra-

tion. However, in Cambisol No. 302, only 35% of evolved N_2O was consumed. It should be point that soil conditions created in this variant can occur in soils with organic or mineral fertilization after a heavy rain.

Table 7. Production and uptake of nitrous oxide, N_2O in soils enriched denitrification substrates incubated under restricted O_2 diffusion

DZ	N_2O production				N_2O uptake			
Soil No.	The highest amount of produced N_2O		The highest production rate		The highest amount of N_2O uptake	The highest uptake rate		% of produced
	mg N_2O-N kg^{-1}	Day	mg N_2O-N $kg^{-1}d^{-1}$	Day	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	0.174	3	0.087	1-3	0.174	0.044	3-7	100
302	65.241	3	23.591	1-3	22.834	3.216	3-7	35
554	7.547	1	7.547	0-1	7.547	3.428	1-3	100
691	3.812	1	3.812	0-1	3.812	1.444	1-3	100
733	4.457	3	2.228	1-3	4.279	0.962	3-7	96
794	16.707	1	16.707	0-1	13.867	6.949	1-3	83
A1	14.908	1	14.908	0-1	14.312	4.838	1-3	96
C2	0.771	21	0.651	0-1	0.771	0.294	1-3	100
S3	18.541	1	18.541	0-1	17.243	5.421	1-3	93
W4	2.187	1	2.187	0-1	2.187	1.030	1-3	100

As compared to the control variant without substrates addition (KZ), an increase in the production of N_2O in all soils was observed in the range from 3.8 times (Cambisol No. A1) up to 57.8 times (Solonetz No. W4).

3.5. Stimulation of denitrification, anaerobic conditions – DB variant

In this variant, soils were incubated with nitrates(V) and glucose, with soil headspace was replaced by N_2.

The highest concentration of N_2O in this variant was 33.21 mg N_2O-N kg^{-1} that was observed in Phaeozem No. S3 (Fig. 10). As compared to N_2O maximum

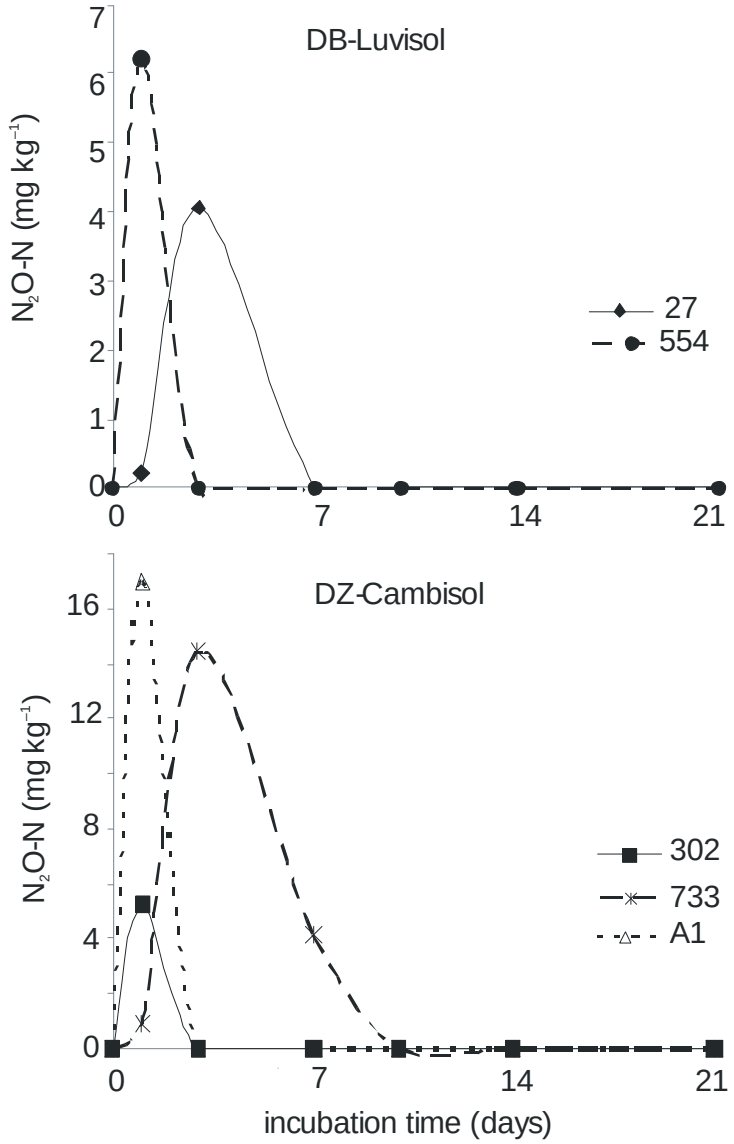

Fig. 10. Changes in N_2O concentration in tested soils enriched with denitrification substrates incubated under anaerobic conditions. Note different scales on the graphs

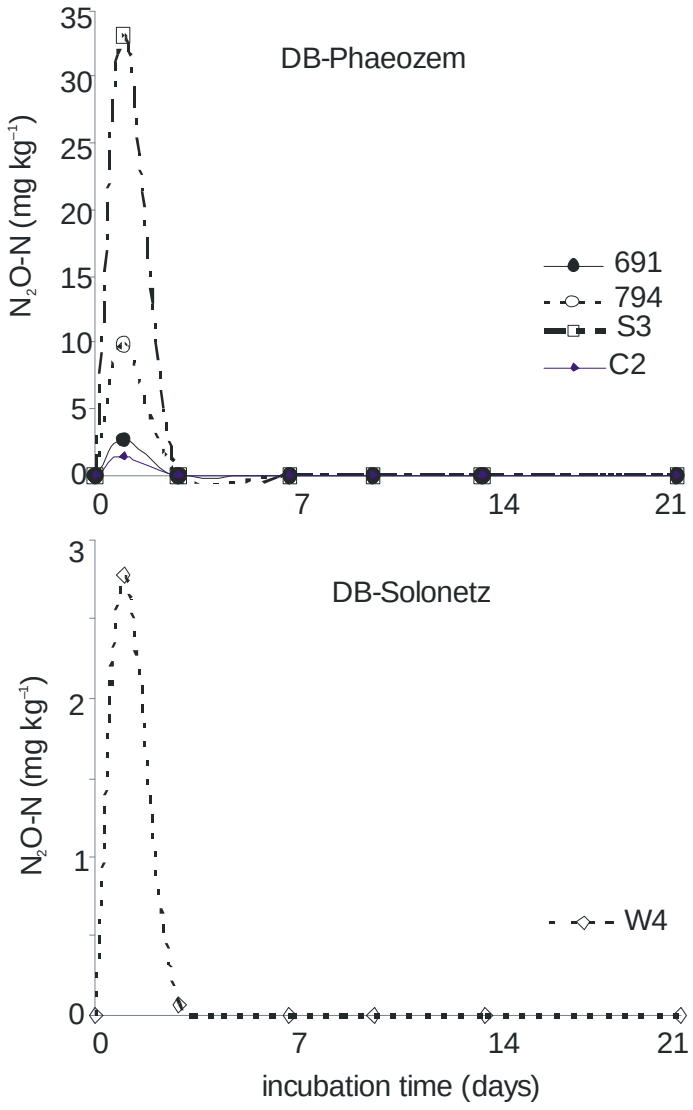

Fig. 10. Cont. Changes in N_2O concentration in tested soils enriched with denitrification substrates incubated under anaerobic conditions. Note different scales on the graphs

recorded in conditions of limited oxygen availability (previously discussed DZ variant), this value was twice lower. Besides, under anaerobic incubation, lower N_2O production was observed also in soils No. 302, 554, 691 and 794 (Cambisol, Luvisol, Phaeozem). In other soils, an increase in the amount of produced N_2O

(up to 23 times in Luvisol No. 27) was observed. In most soils, formation and consumption of nitrous oxide was intensive. All soils consumed 100% of N_2O produced, and most of them – already after 3rd day of incubation (Tab. 8).

Table 8. Production and uptake of nitrous oxide, N_2O in soil samples enriched with denitrification substrates incubated under anaerobic conditions

DB	N_2O production				N_2O uptake			
Soil No.	The highest amount of produced N_2O		The highest production rate		The highest amount of N_2O uptake	The highest uptake rate		% of produced
	mg N_2O-N kg^{-1}	Day	mg N_2O-N $kg^{-1}d^{-1}$	Day	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	4.055	3	1.910	1-3	4.055	1.014	3-7	100
302	5.216	1	5.216	0-1	5.216	2.608	1-3	100
554	6.216	1	6.216	0-1	6.216	3.108	1-3	100
691	2.663	1	2.663	0-1	2.663	1.325	1-3	100
733	14.453	3	6.795	1-3	14.453	2.579	3-7	100
794	9.920	1	9.920	0-1	9.920	4.960	1-3	100
A1	17.008	1	17.008	0-1	17.008	8.504	1-3	100
C2	1.414	1	1.414	0-1	1.414	0.707	1-3	100
S3	33.211	1	33.211	0-1	33.211	16.606	1-3	100
W4	2.772	1	2.772	0-1	2.772	1.353	1-3	100

3.6. Stimulation of N_2O uptake under aerobic conditions – PT variant

The results described above illustrate the capacity of individual soils to production of nitrous oxide and its uptake under different availability of organic carbon, nitrate(V) and oxygen, On this basis, different soils can be compared mainly in terms of N_2O evolution. Addition of nitrous oxide allow to eliminate a restriction on the reaction rate by a too low N_2O concentration.

Under aerobic conditions, the uptake of N_2O was relatively low (Fig. 11). In some soils (soils No. 27, 733, S3), an additional, slight increase of N_2O during the incubation was even observed.

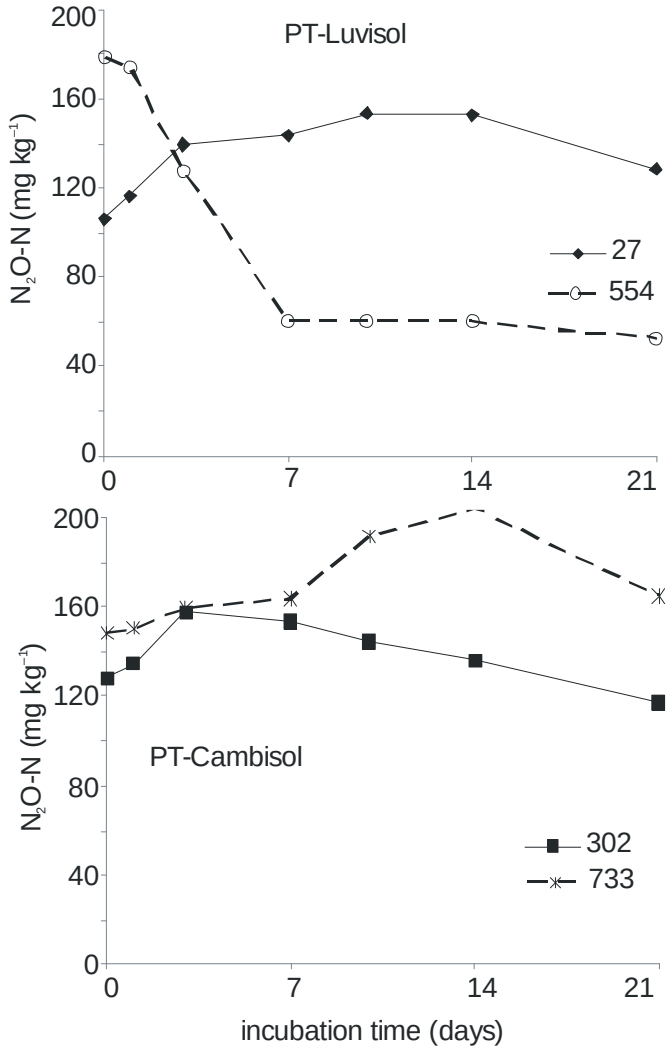

Fig. 11. Uptake of added nitrous oxide under aerobic conditions. Note different scales on the graphs

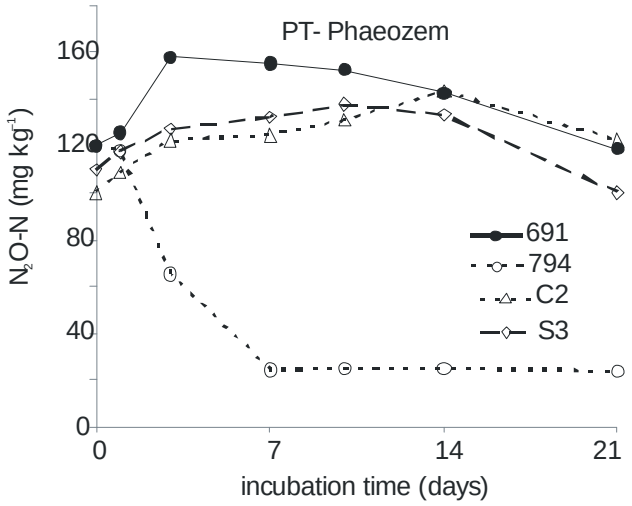

Fig. 11. Cont. Uptake of added nitrous oxide under aerobic conditions. Note different scales on the graphs

Marked decrease in the concentrations of nitrous oxide was noted only in soils No. 554 and 794 (Luvisol and Phaeozem, respectively) – Table 9.

Table 9. Uptake of added nitrous oxide under aerobic conditions

PT	N_2O uptake				
Soil No.	Concentration at the beginning	Maximum amount	The highest uptake rate		% of maximum
	mg N_2O-N kg^{-1}	mg N_2O-N kg^{-1}	mg N_2O-N $kg^{-1}d^{-1}$	Day	
27	106	153.4	3.42	14-21	16
302	128	157.4	2.93	7-10	26
554	179	179.0	23.19	1-3	71
691	120	157.7	3.44	14-21	25
733	148	204.5	5.65	14-21	19
794	120	120.0	26.35	1-3	80
A1	n.t.	n.t.	n.t.	n.t.	n.t.
C2	100	137.5	2.90	14-21	14
S3	110	124.3	4.71	14-21	27
W4	n.t.	n.t.	n.t.	n.t.	n.t.

3.7. Stimulation of N_2O uptake under anaerobic conditions – PB variant

The dynamics of uptake of added nitrous oxide under anaerobic conditions (N_2 atmosphere) has been shown in Figure 12, and key values were given in Table 10. The large uptake of added N_2O was observed in all tested soils. In most soils, this process occurred intensively already at the beginning of incubation, between the first and the third incubation day.

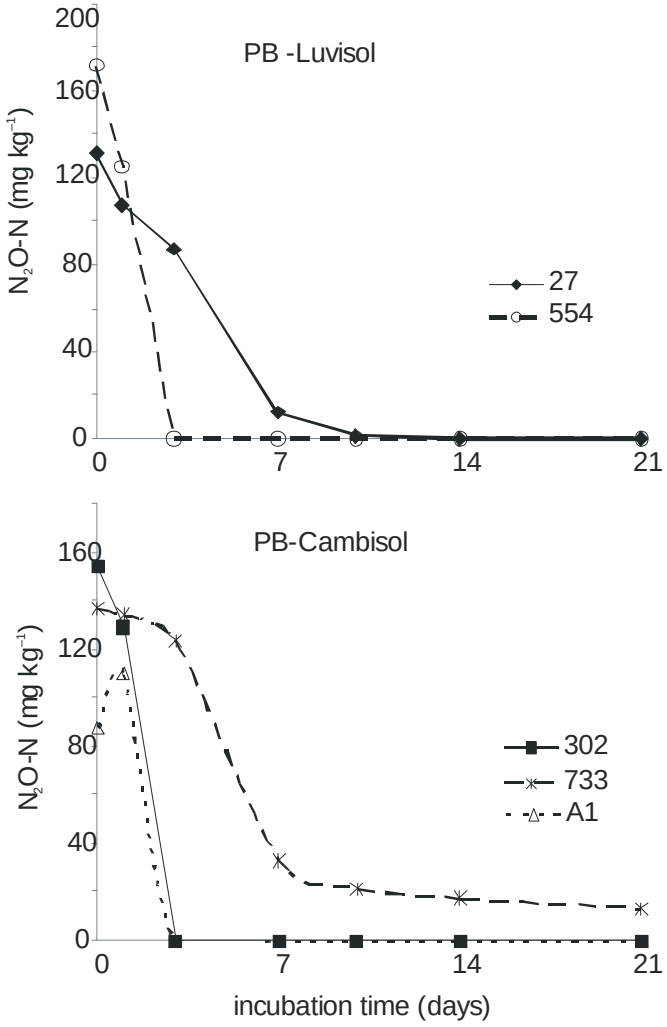

Fig. 12. Uptake of added nitrous oxide under anaerobic conditions. Note different scales on the graphs

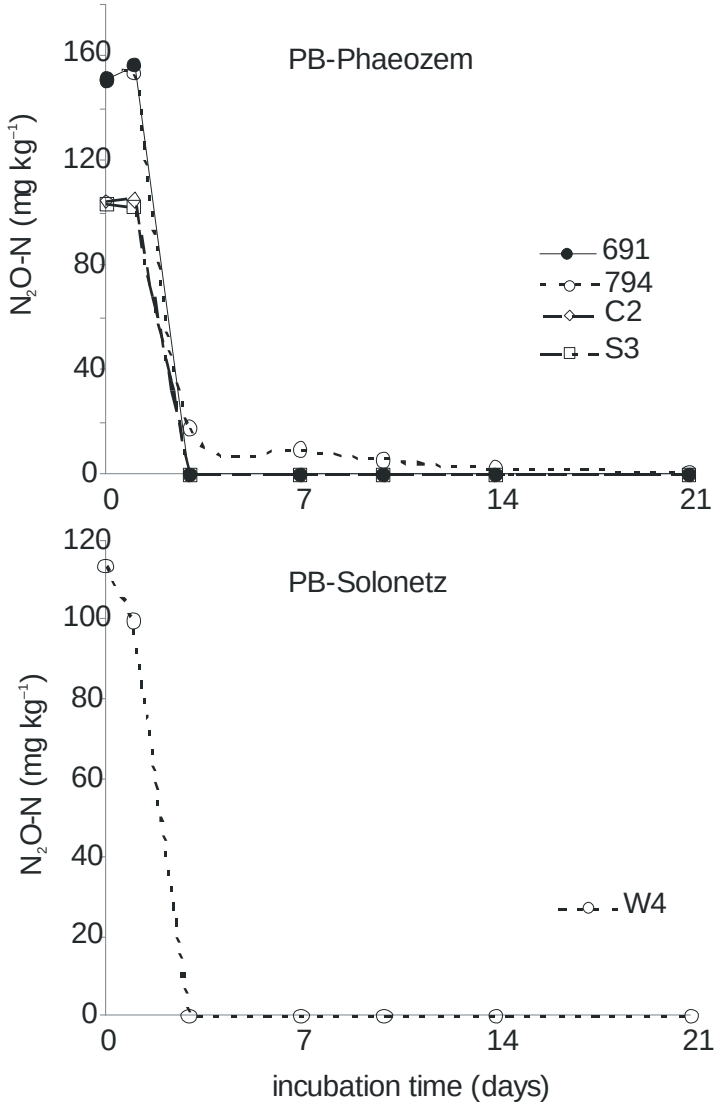

Fig. 12 Cont. Uptake of added nitrous oxide under anaerobic conditions. Note different scales on the graphs

In five soils (Phaeozems No. 691, C2 and S3; Cambisol A1; and Solonetz W4), after three days of incubation, a complete disappearance of nitrous oxide was observed (Figure 12). After three weeks of the incubation, in two soils only (Cambisol No. 733, and Phaeozem No. 794), the consumption of added N_2O was

not total – in result of incubation under anaerobic conditions soils were able to consume of 90-100% of added N_2O.

Table 10. Uptake of added nitrous oxide under anaerobic conditions

PB	N_2O uptake			
Soil No.	Added N_2O	The highest uptake rate		% of maximum
	mg N_2O-N kg^{-1}	mg N_2O-N kg^{-1} d^{-1}	Day	
27	131.15	24.09	0-1	100
302	154.42	64.50	1-3	100
554	171.62	62.56	1-3	100
691	151.11	78.30	1-3	100
733	136.57	22.71	3-7	90
794	150.51	67.78	1-3	99
A1	87.51	55.11	1-3	100
C2	102.92	52.43	1-3	100
S3	104.08	51.01	1-3	100
W4	113.57	49.92	1-3	100

This study confirmed a significant influence of soil conditions on the formation and consumption of nitrous oxide. Multivariate analysis of variance of the results obtained for 7 experimental variants presented above showed, that N_2O concentration in soil headspace significantly depended on soil amendments, inherent soil properties (i.e. tested soil) and oxygen availability, $P<0.001$ (Szarlip 2009).

4. SOIL CONDITIONS FAVORING THE PRODUCTION AND UPTAKE OF N_2O

The experiment reported in Chapter 3 with incubations of 10 soils modified by different availability of oxygen, nitrate and organic carbon showed that all tested soils have potential ability to N_2O production and sorption. Under control conditions (without C and N addition), the maximum N_2O observed in soil head-space was 17.2 mg N_2O-N kg^{-1}. Soil amendment with denitrification substrates, nitrate and glucose (35 mg NO_3^--N kg^{-1} and 1 g kg^{-1}, respectively) resulted in larger

N_2O production, up to 65.2 mg N_2O-N kg^{-1}. In average, 4-fold increase N_2O as compared with control soils was noted. Tested soils varied in their denitrification activity. Both the lowest and highest level (averaged for all variants) showed Phaeozems developed from loess (No. 691 and No. 794, respectively).

Under flooded conditions (restricted O_2 availability), the N_2O concentration was the highest, in average 5.51 mg N_2O-N kg^{-1}. Lower N_2O was under anaerobic conditions (N_2 atmosphere) in average 3.29 mg N_2O-N kg^{-1}, while the lowest – in wet soils (aerobic conditions) – in average 2.56 mg N_2O-N kg^{-1}.

The consumption of N_2O produced during incubation was more efficient in flooded than in wet soils (variants DZ and KZ versus variants DT and KT, respectively, see Table 11). Most soils consumed nearly all N_2O under anaerobiosis, both produced during incubations and added at the start of the experiment (variants DB and PB, respectively), (Szarlip 2009). These results are related to the sensitivity of the enzymes of the denitrification pathway to O_2. It was mentioned above, that this sensitivity is inversely proportional to the degree of substrate oxidation state and increases in the order: NO_3^- reductase < NO_2^-reductase < NO reductase < N_2O reductase (Dendooven and Anderson 1994, McKenney *et al.* 1994, Joye and Hollibaugh 1995).

Table 11. Average concentrations of N_2O and the percentage of N_2O that was consumed in individual variations, explanation in the text (Szarlip 2009)

Soil variant	N_2O, average concentration mg N kg^{-1}	N_2O consumed (% of N_2O maximum)
KT	0.164	18.9
KZ	1.97	28.1
DT	3.29	38.4
DZ	5.51	90.2
DB	2.56	100.0
PT	126.38	34.7
PB	134.34	99.0

Accumulation of the products of incomplete denitrification such as NO and N_2O may result from the large NO_3^- to C_{org}, ratio, or from disturbed balance between different stages of the process. It is also possible, that some microorganisms

do not produce certain enzymes, that leads to the accumulation of by-products (Tiedje 1982). Total reduction of nitrate to N_2 is also favoured by neutral pH (Šimek *et al.* 2002).

Oxygen is considered to be inhibitor for denitrifying enzymes (Knowles 1982) although the critical limit of O_2 varied among different species of denitrifying bacteria. The N_2O yield during nitrification activity is inversely correlated with the concentration of dissolved O_2 (Anderson and Levine 1986). Increased O_2 content enhanced production of N_2O relative to N_2 during denitrification. Under anaerobic conditions, N_2O production was initially found to increase, but this was followed by N_2O consumption in the system and its conversion to N_2 by N_2O reductase (Firestone *et al.* 1980). Letey *et al.*(1981) reported that the soil can act as a N_2O sink under anoxic conditions. They also reported that N_2O emissions were higher in soils with fluctuating redox potential established by alternate wetting and drying cycles.

Hu *et al.* (2010) evaluated the control parameters for N_2O emission in the wastewater treatment process, N_2O emissions were compared in the activated sludge from anoxic–aerobic sequencing batch reactors acclimated under different aeration rates, and fed with synthetic wastewater. Results showed that a higher aeration rate led to a smaller N_2O emission, while reactors acclimated under mild aeration performed the best in terms of nitrogen removal efficiency. Most of the N_2O was produced during the aerobic phase, regardless of the aeration rate. Experiment showed that N_2O production in the anoxic phase was relatively insignificant. This was because the pre-denitrification process used in this study created an optimum circumstance for denitrification, and very little N_2O was produced through conventional denitrification since the N_2O produced was reduced to N_2 immediately by nitrous oxide reductase (N_2OR). The similar result obtained Shiskowski *et al.* (2004). The "DO (Dissolved Oxygen) roof value" differed significantly through the aerobic phase under different aeration rates. During experiment the DO was maintained around 0.2 mg dm^{-3}. Under low DO concentration, the N_2O reductase is more susceptible to oxygen than nitrate and nitrite reductase (Schulthess *et al.* 1994). As a result, the N_2O reduction rate is lower than the reduction rate of nitrate and nitrite. Over 26.1% of removed nitrogen was emitted to the gas phase as N_2O. However, once the DO level gets to the critical value of 1 mg dm^{-3}, the N_2O^-N conversion rate decreased significantly.

Stimulatory effect of nitrate and glucose addition was investigated also in the experiments of other authors. Soil conditions (high moisture, high NO_3^- content and addition of organic C) in study of Bergstermann *et al.* (2011**)** were established

to favour denitrification. The fast increase and high level of N_2O and N_2 fluxes, especially at the beginning of incubation, show the expected effect that nitrate and glucose stimulated the growth and activity of the denitrifier population (Tiedje *et al.* 1983). The impact of the amendment on the time course of (N_2+N_2O) is probably a combined effect of O_2 consumption during C_{org} respiration, high NO_3^- supply and high supply of electron donors for denitrifiers. Decrease of denitrification rates and CO_2 fluxes apparently reflected the ongoing exhaustion of glucose. The N_2 and N_2O fluxes at the end of incubation were thus dominated by denitrification based on soil derived organic C. In the last phase of the experiment, both (pre-wet and pre-dry) treatments had rather similar low gaseous N production. Lack of energy was the likely reason for that because there was still nitrate for denitrification in both treatments. Total denitrification as given by mean (N_2+N_2O) fluxes during the experiment was relatively high (3.67 kg N ha^{-1} d^{-1} for pre-wet and 6.27 kg N ha^{-1} d^{-1} for pre-dry) (Bergstermann *et al.* 2011).

Nitrogenous gas emission from soils varies strongly with soil water content. Soil water can directly and/or indirectly influence denitrification through: (1) provision for suitable conditions for microbial growth and activity; (2) restricting supply of O_2 to microsites by filling soil pores; (3) release of available C and N substrates through wetting and drying cycles; and (4) providing a diffusion medium through which substrates and products are moved to and away from soil microorganisms (Aulakh *et al*. 1992). The water content at which efflux from soils peaks generally increases for the products in the order: NO > N_2O > N_2. (Williams *et al.* 1992). Intensive production of NO is observed at about 20% WFPS (water-filled pore space), N_2O production at the higher soil moisture, about 70% WFPS, whereas N_2 production occurs mainly in soil saturated with water or flooded (Drury *et al.* 1992, Yang and Meixner 1997). In spite, production of N_2O resulting from autotrophic nitrification increases at about 60% WFPS because such air-water conditions favour the aerobic microorganisms, whereas the activity of denitrifiers is relatively low (about 5% of that observed in saturated soil). In soils with low humidity (<50% WFPS), N_2O production decreased, and below 15% WFPS microbial activity associated with the emission of N_2O ceases as a result of water scarcity (Bateman and Baggs 2005; Sapek 2008).

Henault *et al*. 1998 and Freney *et al*. 1979 reported that N_2O emission increased with increase in soil water from air dry to field capacity. When water content is greater than field capacity, N_2O gets reduced to N_2 (Bremner and Blackmer 1979, Freney *et al*. 1978).

Barton *et al.* (1999) observed for agricultural and forest soils, that denitrification was more intensive at higher WFPS in the sandy soils (74-83% WFPS), than in clayey soils (50-74% WFPS). The average WFPS above which the authors observed increased denitrification was 65%. Maljanen *et al.* (2007) observed no N_2O production by Dystric Regosol in the range of 20-40% WFPS, and an increase of N_2O emission with increasing soil moisture to its maximum at 80-90% WFPS. Shelton *et al.* (2000) showed a linear increase in N_2O emissions between 60% and 100% WFPS. Figure 13 shows content of N_2O in soil headspace versus WFPS in 10 mineral soils incubated under different availability of oxygen, nitrate and C source (Chapter 3). Other authors reported the curvilinear nature of the relationship with a maximum emission at around 60% WFPS (Davidson 1991) or 80-85% (Dobbie *et al.* 1999). The relation between soil air-water conditions (expressed as WFPS) and N_2O emission has been determined in numerous experiments. Buchkina *et al.* (2010) showed in the experiment under field conditions, that soil water-filled pore space affects N_2O emission from the soil only if extra nitrogen is applied into the soil in the form of fertilizer and/or manures. Experimental plots receiving no extra nitrogen never emitted much N_2O whatever the soil WFPS. Moreover, N_2O emission from the soil receiving extra nitrogen as fertilizer/manures was never high if soil WFPS was low.

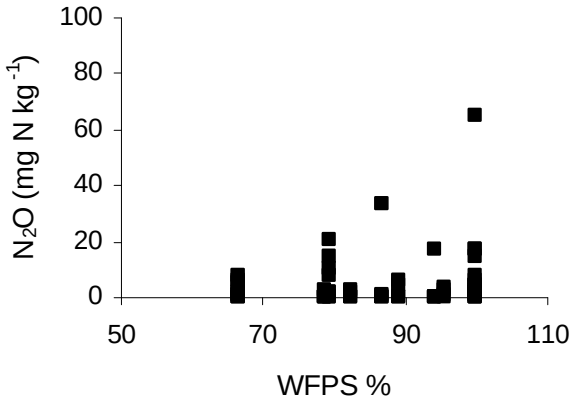

Fig. 13. The relationship between WFPS and N_2O in soil headspace of 10 mineral soils (Luvisol, Cambisol, Phaeozem, Solonetz) incubated under different C and N and O_2 availability (Szarlip 2009)

Vilain *et al.* (2010) tested effect of slope position and land use on N_2O emissions. The authors observed no relationships between contents of NH_4^+ or organic

carbon and N_2O emissions, and showed that the influence of WFPS on N_2O emission rates was also explored and in return clearly evidenced that high N_2O fluxes dominated between 50% and 70% WFPS with a high variability. These results demonstrated a maximum of N_2O fluxes close to 60% WFPS.

Allen *et al.* (2010) investigated an effect of nitrogen fertilizer management and soil waterlogging on nitrous oxide emission from subtropical sugarcane soils in a field experiment. The authors confirmed that heavy rainfall or soil flooding increases the magnitude of N_2O emissions. The authors suggest that N_2O emissions can be reduced by timing N fertilizer application.

The experiment of Jiang *et al.* (2010) on nitrous oxide emissions from Chinese croplands fertilized with a range of slow-release nitrogen compounds (including physically altered – Ca-Mg-P-coated urea, polymer-coated urea and sulfur-coated urea, chemically altered -urea formaldehyde, and biochemically inhibited -urea with dicyandiamide and hydroquinone) observed high N_2O emission at 50-65% WFPS. Similarly, McTaggart and Tsuruta (2003) reported that N_2O emissions from an Andosol was higher at a WFPS of 55% than at 70-80%. This results agree with a previous study at the North China Plain showing that N_2O emission was greatly affected by soil moisture during the maize growing season, and by soil temperature during the wheat growing season (Ding *et al.* 2007, Jiang *et al.* 2010).

Dependency of nitrous oxide formation and uptake on air-water conditions in soil is caused not only by different sensitivity of denitrifying enzymes to oxygen. Apart from this, water in soil effects gas diffusion and solute transport. High water content restricts the diffusion of gases (particularly oxygen, whose diffusion is about 10^4 times slower than in air (Gliński and Stępniewski 1985), while favours diffusion of water soluble compounds. Because nitrifying bacteria require both oxygen and NH_4^+, optimum for the availability of both these substrates occurs when soil is moist but not flooded (Williams *et al.* 1992). However, such situation favours the production of N_2O, as both nitrification and denitrification undergo and produce this gas (Stevens *et al.* 1997). Effect of soil moisture on N_2O emissions is complex, because simultaneously it can be consumed (sorbed) by microorganisms. Higher moisture increases microbial N_2O consumption by limiting gas diffusion into the atmosphere and thereby an increase of its residence time in soil pores (Skiba *et al.* 1997).

Currently it is believed that the biological process that is responsible for N_2O consumption in the soil is its reduction to N_2. The N_2O loss can be observed after its introduction into the soil incubated under anaerobic conditions (Blackmer and Bremner 1976, Teraguchi and Hollocher 1989). Since nitrous oxide is an interme-

diate product of denitrification pathway, it is evolved from microbial cells into the soil air. Thus, it can be used as the only electron acceptor to support the growth of denitrifying bacteria (Koike and Hattori 1975, Bazylinski *et al.* 1986, Zumft and Knoreck 1990, Okereke 1993).

Because N_2O in soil is of microbial origin, the intensity of its formation is controlled by all the factors that affect microbial growth, such as temperature, pH, oxygen, soil moisture, as well as soil type and availability of organic carbon (Paul and Clark 1998). In the field soils, the processes related to N_2O formation depend also on soil management – fertilization, irrigation, agricultural practices, plant cover, the use of chemicals (Włodarczyk 2000, Megonigal *et al.* 2004).

The stimulatory effects of nitrates(V) and organic carbon on the activity of denitrifying microorganisms has been largely documented (Hatano and Lipiec 2004, Megonigal *et al.* 2004, Šimek *et al.* 2004, Włodarczyk *et al.* 2004b, Ullah *et al.* 2005, Brzezińska 2006). Low denitrification activity observed for some soils may probably be just due to the lack of nitrate and/or easy available organic carbon (Petersen *et al.* 2008). Addition of glucose strongly stimulates the cells respiration, which leads to a rapid oxygen depletion (Gliński and Stępniewski 1985). Even in well-aerated soils, microspaces of hypoxia may develop, when oxygen uptake is faster than its diffusion from the adjacent soil pores. Under such conditions, facultative microorganisms use the nitrates as the terminal acceptor of electrons that originate from oxidation of organic substrates.

Addition of nitrate(V) – without organic compounds – has also been shown to accelerate the process of denitrification. However, Włodarczyk *et al.* (2002a, 2004a, 2004b) observed that even within the same soil type (Eutric Cambisols, Haplic Phaeozem), soils greatly differ in the amount of produced N_2O. Some of tested soils showed no response to the NO_3^- addition, while others – accelerated the denitrification activity, and in the range 50-500 mg $N\text{-}NO_3^- kg^{-1}$ showed a typical Michaelis-Menten kinetics. Percentage of nitrates converted to N_2O increased linearly up to 43% with nitrate concentration in the range from 25 to 100 mg NO_3^--N kg^{-1}, but linearly decreased at higher nitrate concentrations reaching practically zero at about 600 mg NO_3^--N kg^{-1}. Nitrous oxide absorption occurred only at nitrate concentrations up to 100 mg NO_3^--N kg^{-1} (Włodarczyk *et al.* 2004b). The bacterial K_M values for N_2O range from 0.5 to 100 μM, and values in soil are even higher (Firestone 1982). However, it is obvious that the K_M values are large compared with the concentration of atmospheric N_2O, which is equivalent to an aqueous concentration of about 8 nM (Conrad 1996).

Studies of other authors indicate that the presence of higher amounts of nitrates(V) may prevent the sorption of N_2O due to the preferential use of NO_3^- as electron acceptor (Wever *et al.* 2002, Petersen *et al.* 2008). It was also observed that the production of N_2 in soil enriched with nitrates(V) does not reach such high values as in the soil without the NO_3^- addition (which might argue for N_2O reductase inhibition by NO_3^-). Ryden (1981) studies indicate that some soils have the capacity to absorb N_2O only when the concentration of NO_3^- is lower than 1 mg kg^{-1}. Thus, the presence of higher amounts of nitrates(V) not only directly affects the amount of evolved N_2O in result of NO_3^- reduction, but also may have indirect effect by the regulation of the last step of denitrification: the reduction of N_2O to N_2. The ratio between carbon substrate and nitrate(V) is also important in this regard. The presence of simple sugars strongly modifies the activity of N_2O reductase. In result of a high glucose addition, the ratio N_2O:N_2 may temporarily rise up to 30 times (Wever *et al.* 2002).

Biological activity of soil is strongly modified not only by environmental factors and soil management, but also by inherent soil the properties (Glinski and Stępniewski 1985, Conrad, 1996, Koper and Piotrowska 2003, Megonigal *et al.* 2004, Wolińska 2010). Soils show a great diversity of microbial abundance and biochemical activity. Strong impact on the level of this activity is the soil mechanical composition.

The importance of soil structure in determining the intensity of N_2O production results, among others from the impact of these soil components on soil porosity, water content that regulate diffusion of both, gases and soluble compounds involved in the process. At a given soil water content, the small pores found in clayey soils are more likely to be blocked than the relatively large pores found in loam and sand soils (Megonigal *et al.* 2004). Bollmann and Conrad (1998) reported that for soils with the same soil water content, higher N_2O emission was found in the fine silt soil than in the coarse silt soil. Based on incubation of 13 Calcaric Regosols developed from different parent materials, Włodarczyk *et al.* (2005a) observed N_2O evolution that reached 13-44% of the initial nitrate-N content – denitrification was the highest in silty soils and lowest in the sandy soils and was negatively correlated with the >0.05 mm fraction but positively with the 0.05-0.002 mm fraction. Moreover, N_2O reduction to N_2 started earlier in finely (*e.g.* loam) than in coarsely textured (*e.g.* sand) soils.

The process of N_2O consumption in soil depends on soil properties and predomination of nitrogen form (nitrate, nitrite or ammonium) present in the soil. Włodarczyk *et al.* (2005b) observed, that a loamy soil amended with N_2O and nitrate (160 and

100 mg N kg^{-1}, respectively) produced additionally 65.7 mg N_2O-N kg^{-1} during 7 days of incubation, whereby N_2O consumption was observed (totally 142 mg N_2O-N kg^{-1}). In sandy soil amended with nitrate and N_2O, nitrous oxide production was much lower and reached only the value of 19.1 mg N_2O-N kg^{-1} during the first 3 days of incubation, after that period only a small N_2O consumption was observed (7.8 mg N_2O-N kg^{-1}). Nitrite inhibited N_2O production and consumption, whereas NH_4^+ effect on N_2O consumption was low in both tested soils.

Some authors pointed that the factors limiting microbial metabolism may be these soil parameters, which were not analyzed in a given experiment. For example, in the study reported in Chapter 3, Solonetz soil (No. W4) was characterized by a high OM content (3.56%) and relatively high contents of total nitrogen, nitrate and clay fraction (0.235%, 15.4 mg NO_3^--N kg^{-1} and 45.2%, respectively). Despite these properties, the denitrification activity in this soil was low. In spite, in the case of Luvisol No. 27, the reason for a low denitrification activity was probably a little nitrate and clay content (0.77 NO_3^--N mg kg^{-1} and 2%, respectively). This soil released up to 5.6 mg N_2O-N kg^{-1} only after soil enrichment with C and N. In this case, the limiting factor was probably a shortage of nitrate(V), while the organic matter content was moderate (1.76%). There was no correlation for 10 tested soils between the basic soils properties (such as OM, pH and granulometric composition) and denitrification activity rate (i.e. rate of both production and sorption of N_2O, as well as the highest N_2O concentration), (Szarlip 2009). Similarly Bandibas *et al.* (1994) found no significant relationship between the OM, NO_3^- and NH_4^+ contents. Nevertheless, many studies confirmed close relationship between the amount of produced N_2O and organic matter content (Glinski and Stępniewski 1985, Hergoualch *et al*. 2007).

Effect of soil properties on N_2O transformation was also observed for a peaty-muck soil (Eutric Histosol) and a brown soil developed from sand (Eutric Cambisol) during anaerobic incubation with KNO_3 or N_2O addition (Włodarczyk *et al.* 2002b). The organic soil showed about 4 times higher denitrification activity (as measured by N_2O emission and NO_3 depletion) than mineral soil (Fig. 14). In turn, the brown sandy soil was characterized by better capacity for nitrous oxide sorption and more intensive respiration activity as compared with peaty-muck soil.

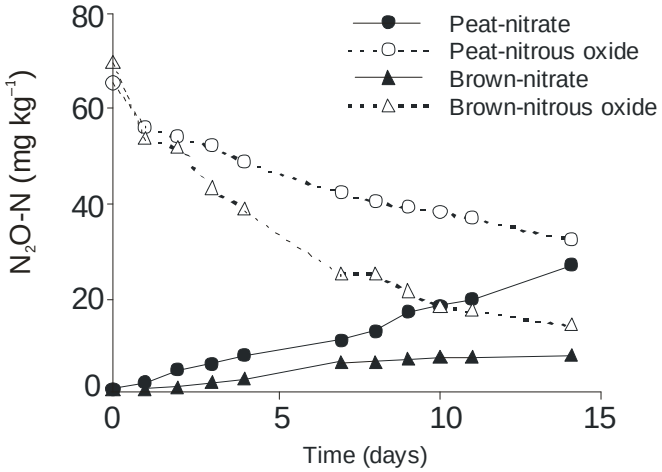

Fig. 14. Nitrous oxide kinetics in peaty-muck soil and brown sandy soil amended with nitrate or nitrous oxide (Włodarczyk *et al.* 2002b)

The laboratory experiment with 14 Cambisols (developed from sand, silt, loess, loam or clay) under flooding showed high variability of tested soils in their denitrification activity (Fig. 15) (Włodarczyk *et al.* 2003). The total amount of N_2O evolved ranged from 3 to 91% of the initial nitrate-N content, and was positively correlated with the organic carbon (C_{org}) content and carbon dioxide evolved (Fig. 16). Tested soils were characterised by a very wide range of redox potential measured for the maximal cumulative N_2O emission (from +417 to +233 mV). The beginning of N_2O emission was observed above 400 mV for light textured soils, while below 400 mV for heavy textured soils.

In the laboratory experiments, Włodarczyk (2000) measured N_2O emission and absorption in 16 soils (Eutric Cambisols) developed from different parent material. Soil samples were amended with NO_3^--N and incubated under lowered oxygen content in the headspace (10% v/v) at the beginning of incubation.

Experiments were designed to investigate the influence of variables such as oxidation-reduction conditions, pH, organic matter content and granulometric composition on soil denitrification activity. Results showed that tested soils were emitters (cumulative production of N_2O ranged from 11.4 to 66.5 mg N_2O-N kg^{-1} of soil) as well as reducers (daily sink of N_2O ranged from 1.3 to 10.5 mg N_2O-N d^{-1} kg^{-1} of soil). The range of reduction of N_2O under investigation conditions was from 10 to 100%, depending on the kind of soil and time of incubation. Production and reduction of N_2O were nonlinearly related to redox potential ($P<0.001$).

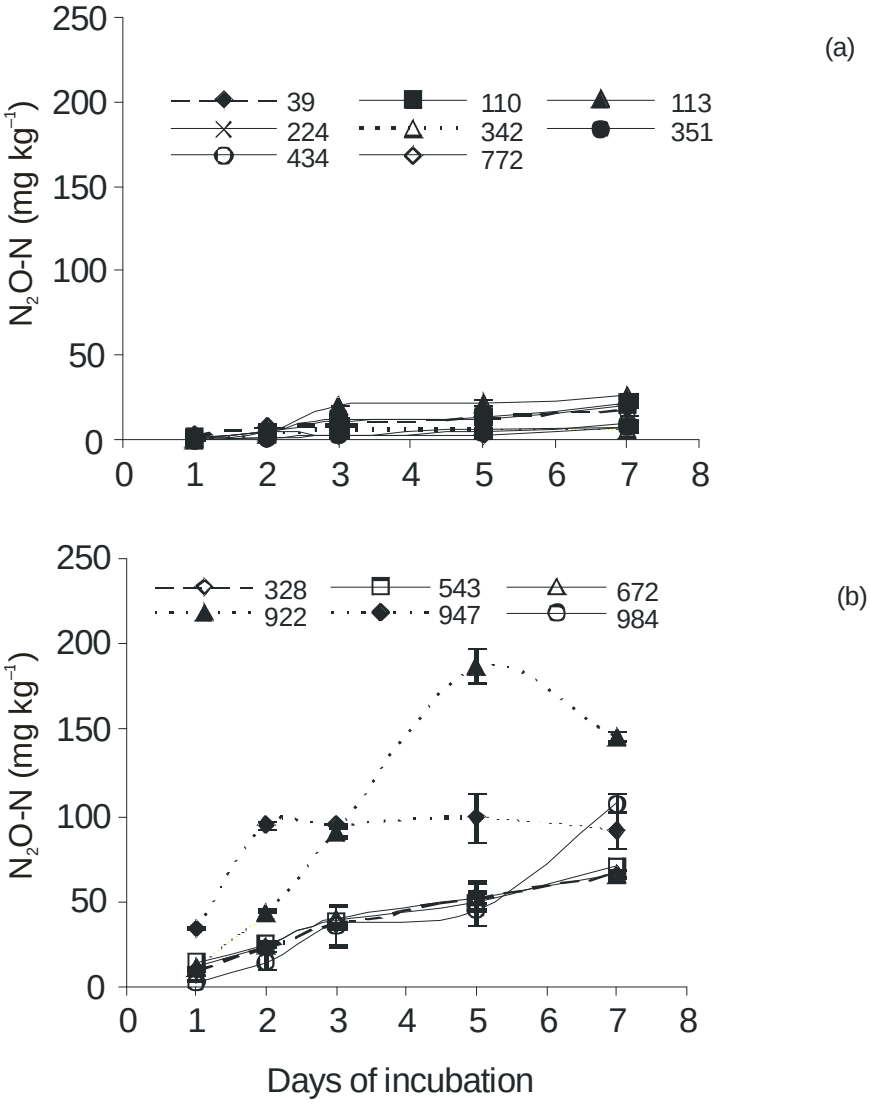

Fig. 15. The course of cumulative nitrous oxide content (mean values with standard deviations) in the headspace during the incubation of the two soil groups: 8 soils with lower activity (a), and 6 soils with higher activity (b). A discontinuous line denotes soil where N_2O absorption was observed (Włodarczyk *et al*. 2003)

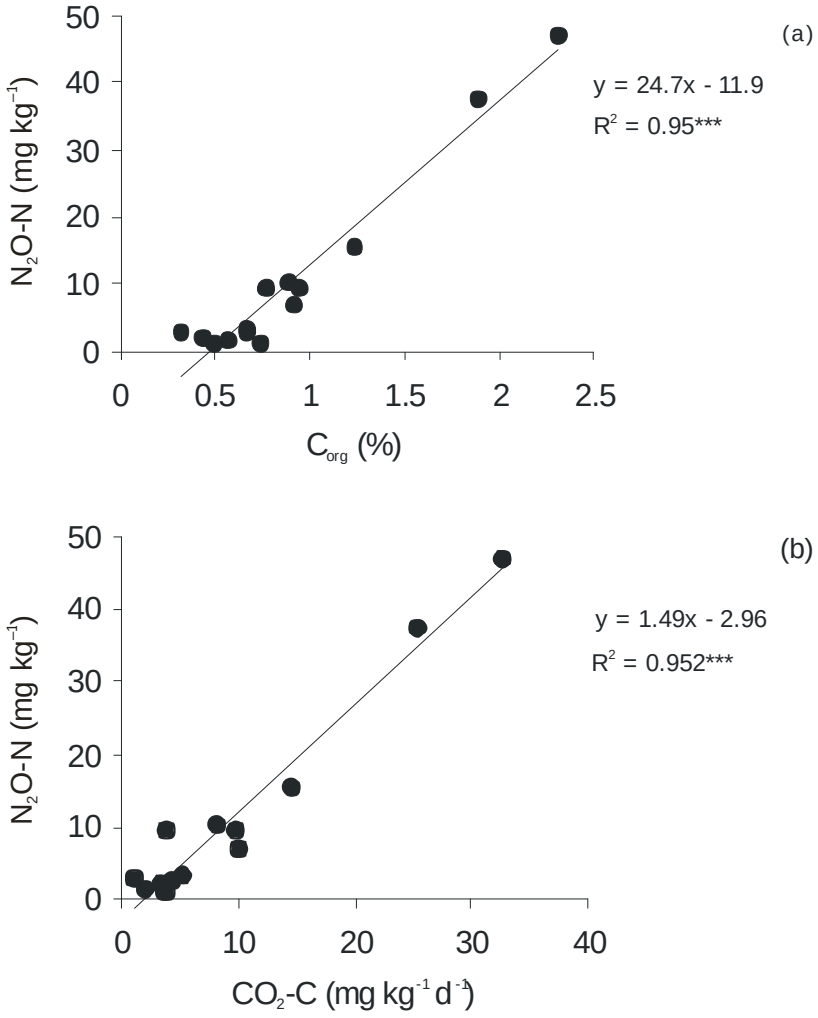

Fig. 16. Diurnal N_2O emission versus C_{org} (a) and CO_2 emission rate (b) (Włodarczyk *et al.* 2003)

The boundary value of redox potential for emission of nitrous oxide was 250 mV, and for absorption of N_2O was about 200 mV (Fig. 17 and Fig 18). Under investigated conditions the maximum emission of N_2O was observed at pH range between 4.5-6.0, but maximum absorption of nitrous oxide occurred at pH from 5.5 to about 7. Absorption of N_2O occurred simultaneously with the reduction of nitrate and after depletion of NO_3^- during the course of the experiment.

Denitrification rate and sink of nitrous oxide showed high correlation with mineralization of organic matter (P<0.001), (Włodarczyk 2000).

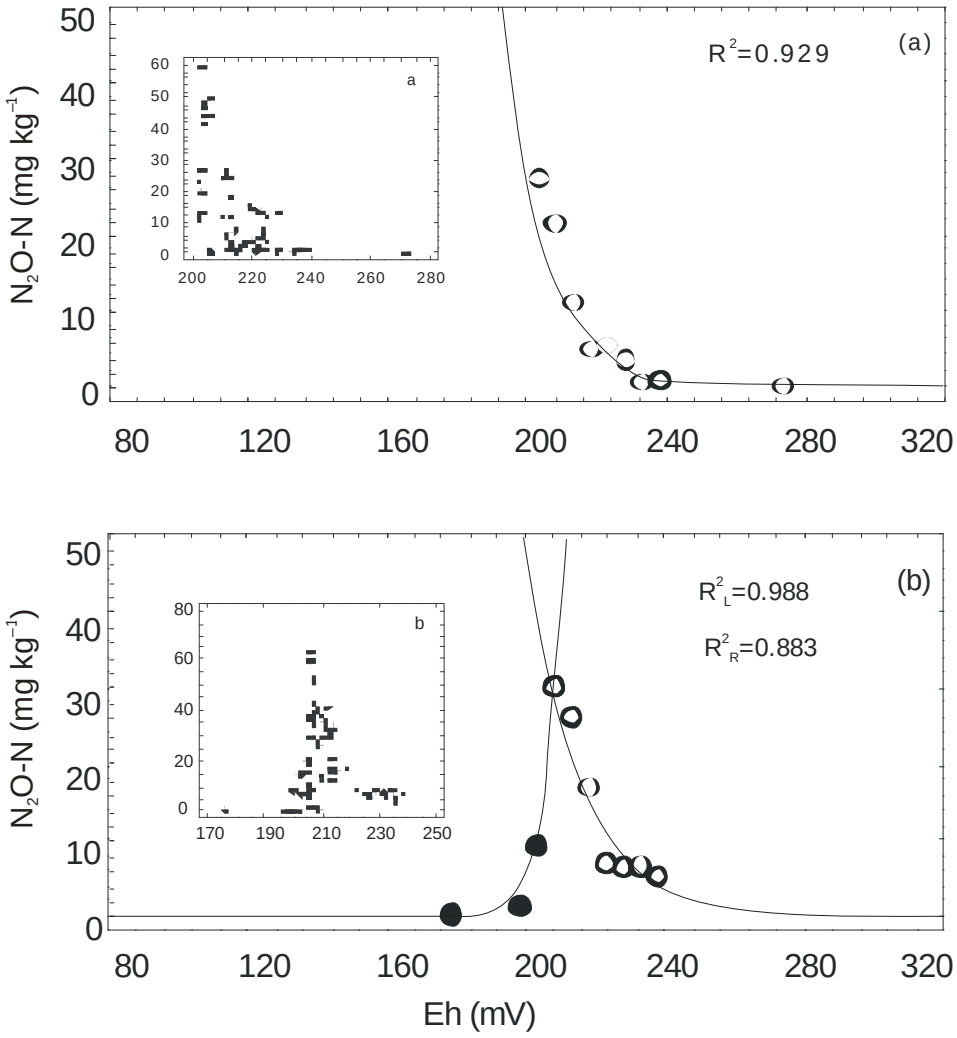

Fig. 17. Equilibrium content of N_2O in the phase of emission (R – right side of figures) and absorption (L – left side of figures) in the soil headspace from the second day (a) and seventh day (b) of the incubation as a function of Eh **(**y = mean values for the determined ranges of x value). Insertion shows single data from all soils (Włodarczyk 2000)

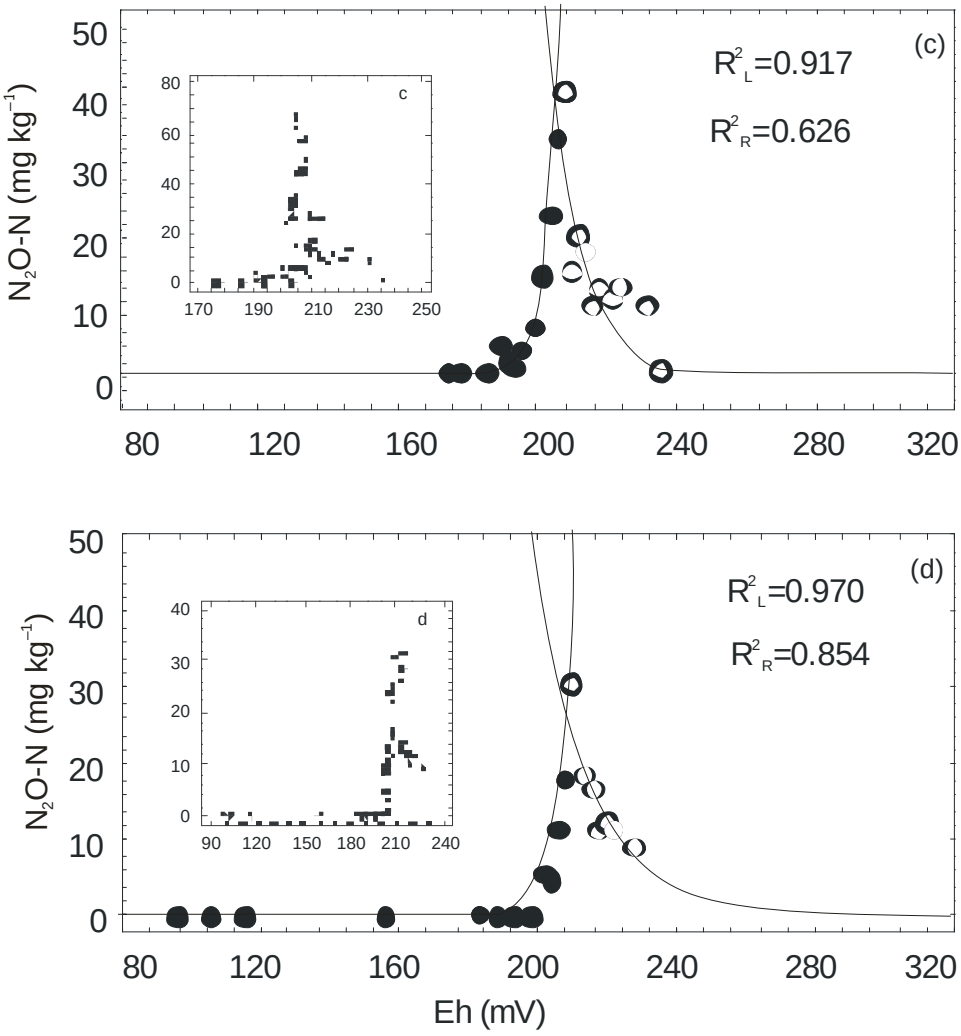

Fig. 17 Cont. Equilibrium content of N_2O in the phase of emission (R – right side of figures) and absorption (L – left side of figures) in the soil headspace from the tenth day (c) and a final day (d) of the incubation as a function of Eh **(**y = mean values for the determined ranges of x value). Insertion shows single data from all soils (Włodarczyk 2000)

Similar relationship for nitrous oxide production and sorption was observed for organic soils enriched with glucose (Brzezińska 2006). N_2O was present in soil headspace at Eh <400 mV, and maximum of N_2O was observed at Eh about 200 mV, below this value N_2O was consumed (Fig. 18). During the incubation of the same organic soils without glucose amendment, a small amounts of nitrous oxide of 5-10 mg N_2O-N kg^{-1} were recorded after prolonged incubation (40 days).

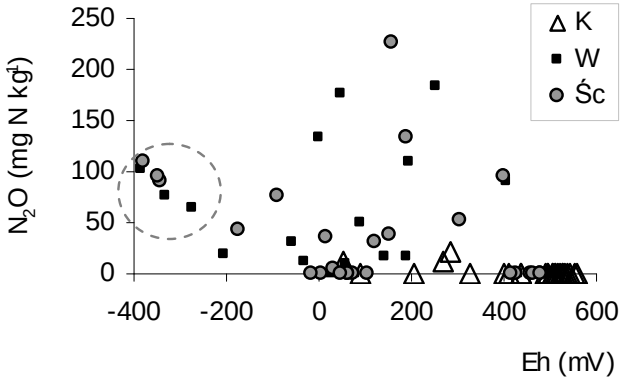

Fig. 18. Equilibrium content of N_2O versus Eh in organic soil amended with glucose (6 mg g^{-1}). Soil samples were incubated at 60% WHC, and flooded with water or municipal wastewater (K, and W or Śc, respectively). Results from 1st and 3rd day of incubation encircled (Brzezińska 2006)

5. EFFECT OF SOIL MANAGEMENT ON THE EMISSION OF N_2O FROM THE SOIL

Soil management changes soil physical status, C and N content, as well as soil microbial biomass and activity (Gajda 2010, Josa *et al.* 2010, Turski 2010). Recent literature reviews indicate that N_2O emission is usually much higher and more variable from arable soils than from natural ecosystems. Besides, it is higher from fertilized grasslands than from forests (Bouwman 1990, Badr and Probert 1992, Hatano and Lipiec 2004). N_2O emission from natural ecosystems is less than 1 kg N ha^{-1} $year^{-1}$ in temperate climate, and less than 2 kg N ha^{-1} $year^{-1}$ in the tropics, while that from the cropped fertilized soil is more than 3 kg N ha^{-1} $year^{-1}$ (Bouwman 1990, Granli and Bøckman 1994). The use of N fertilizers may cause 2-7 ford increase in N_2O emission (Skiba *et al.* 1994). Generally, after the addition of nitrogen sources under field conditions, an increased N_2O emission lasts

up to about 6 weeks. After this time, emission decreases and fluctuates around its natural level, regardless of previously applied fertilizer (Mosier *et al.* 1989). N_2O emissions is generally higher from injected fertilizers as compared to surface broadcast fertilizers, and is lower for nitrate-based fertilizers than for anhydrous ammonia. Other authors believe that the kind of fertilizer does not affect the amount of produced N_2O, but the emission intensity varies over time and space, and results from the interaction between biological, chemical and physical soil properties (Bouwman 1990). According to Mosier *et al.* (1989) soil management and increased rainfall have a greater influence on N_2O emission than the type of nitrogen fertilizer. However, management strategies that increase fertilizer N use efficiency will reduce N_2O emission (Parkin and Hatfield 2010).

It has been observed that legumes effect the production of N_2O. This plants are likely to participate in this process in many ways (Galbally *et al.* 1992). Atmospheric nitrogen (N_2) fixed by the bacteria undergo ammonification, nitrification and denitrification, just like N fertilizers, and becomes a source of nitrous oxide emitted. In addition, symbiotic Rhizobia may contribute to N_2O production. Even 2-3 fold increase in N_2O emissions following the introduction of legume plants on pasture was observed (O`Hara and Daniel 1985).

Soil tillage system and fertilization strongly influences N_2O emission from agricultural soils. Stalenga and Kawalec (2007) estimated that the total nitrous oxide emission increases in the order: organic < integrated < conventional crop production system. The replacement of the conventional system by integrated system (with synthetic N fertilizers application in both systems) resulted in significant reduction of N_2O emission (Tab. 12).

Table 12. Emission of N_2O (in kg ha^{-1}) in different crop production systems (1996-2005), (after Stalenga and Kawalec 2007)

Source of N_2O emission	Crop production system		
	Organic	Conventional	Integrated
Nitrogen synthetic fertilizers	–	1.78	0.89
Manure/compost management	0.32	–	0.40
N_2-fixing crops	0.20	–	0.14
In total	0.52	1.78	1.43

According to Keller *et al.* (1986), N_2O emission increased significantly when tropical forests in central Brazil were converted to agricultural land, while Luizao *et al.* (1989) reports that the soil of pasture land produces three times more nitrous oxide than the adjacent forest soil. Moreover, according to Bowden and Bormann (1986) N_2O in the soil of grubbed land can be transported by ground water, and emitted to the atmosphere in another place.

The increase in N_2O emissions from nitrogen fertilizer used is closely connected with the soil irrigation (Hatano and Lipiec 2004). Under specific conditions of rice fields cultivated in temperate and tropical regions, the loss of nitrogen in the form of N_2O was less than 0.1% of introduced fertilizer, if its applied to the soil after flooding (Simpson *et al.* 1984; Mosier *et al.* 1989).

Denitrification is an important process in soils irrigated with wastewater as it removes nitrate from the soil before it leaches to groundwater (Kotowska and Wlodarczyk 2005, Stępniewska *et al.* 2001). Barton *et al.* (2000) investigated the factors limiting the denitrifying population in a forested land-based wastewater treatment systems irrigated with wastewater, by studying the individual and combined effects of soil aeration, water content, nitrate and carbon on denitrification enzyme activity. The size of the soil denitrifying population appeared to be limited by soil aeration, and limiting oxygen availability increased the denitrifying population above that observed in the field. Furthermore, we found that wastewater irrigation altered the short-term response of denitrifiers to anaerobic soil conditions. Under low oxygen conditions, denitrifiers in the wastewater-irrigated soils produced enzymes sooner and at a greater rate than soils without a history of wastewater irrigation. We propose that the size of the denitrifying population cannot be expected to be large in free-draining, coarsely textured soils even when provided with additional nitrogen and water inputs.

Nosalewicz and Stępniewska (2005) performed a field experiment to study the emission of nitrous oxide form organic soils (peat-muck and mineral-muck) planted with poplar, willow and grasses and irrigated with municipal wastewater. Emitted nitrous oxide reached a maximum of approximately 60 mg N_2O-N m^{-2} h^{-1}. The concentration of N_2O increased with depth in wastewater irrigated soils up to 208 ppm at 70 cm of the depth. The concentration of N_2O in the control soil profiles, which have never received wastewater, did not exceed 0.5 ppm (Nosalewicz and Stępniewska 2005, Nosalewicz *et al.* 2005).

6. REFERENCES

Allen D.E., Kingston G., Rennenberg H., Dalal R.C., Schmidt S., 2010. Effect of nitrogen fertilizer management and waterlogging on nitrousnoxide emission from subtropical sugarcane soils. Agriculture, Ecosystems and Environment, 136, 209-217.

Anderson I. C., Levine J. S., 1986. Relative rates of oxide and nitrous oxide production by nitrifiers, denitrifiers, and nitrate respires. Appl. Envinron. Microbiol., 51, 938-945.

Anderson I. C., Poth M., Homstead J., 1993. A comparison of NO and N_2O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis.* Appl. Envinron. Microbiol., 59, 3525-3533.

Aulakh M. S., Doran J. W. Mosier A. R., 1992. Soil denitrification: significance, measurement, and effects of management. Adv. Soil Sci., 18, 2-57.

Badr O., Probert S. D., 1992. Sources of atmospheric nitrous oxide. Appl. Energy, 42, 129-176.

Bandibas J., Vermoesen A., De Groot C.J., van Clemput O., 1994. The effect of different moisture regimes and soil characteristics on nitrous oxide emission and consumption by different soils. Soil Sci., 158, 2, 106-114.

Barton L., McLay C.D.A., Schipper L.A., Smith C.T., 1999. Annual denitrification rates in agricultural and forest soils: a review. Austral. J. Soil Res., 37, 1073-1093.

Barton L., Schipper L.A., Smith C.T., McLay C.D.A., 2000. Denitrification enzyme activity is limited by soil aeration in a wastewater-irrigated forest soil. Biol Fertil Soils, 32, 385-389.

Bateman E.J., Baggs E. M., 2005. Contribution of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol. Fertil. Soils, 41, 397-388.

Bazylinski D.A., Soohoo C.K., Hollocher T.C., 1986. Growth of *Pseudomonas aeruginosa* on nitrous oxide. Appl. Envinron. Microbiol., 51, 1239-1246.

Bergstermann A., Cárdenas L., Bol R., Gilliam L., Goulding K., Meijide A., Scholefield D., Vallejo A., Well R., 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biology & Biochemistry, 43, 240-250.

Blackmer A.M., Bremner J.M., 1976. Potential of soil as a sink for atmospheric nitrous oxide. Geophys. Res. Lett., 3, 739-742.

Bock E., Koops H. P., Harms H., 1986. Cell biology of nitrifying bacteria. w Prosser J., I. (ed). Nitrification. Special Publication of The Society for General Microbiology, vol 20 Reading, Uk: IRL Press, 17-38.

Bock E., Schmidt I., Stuven R., Zart D., 1995. Nitrogen loss caused by nitrifying *Nitrosomonas* cells using ammonia or hydrogen as electron donors and nitrate as electron acceptor. Arch Microbiol., 163, 16-20.

Bollmann A., Conrad R., 1998. Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Global Change Biol., 4, 387-396.

Bouwman A.F., 1990. Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. Bouwmann A.F. (ed) Soils and greenhouse effect. John Wiley & Sons, Ltd., Chichester, United Kingdom, 61-127.

Bowden W.B., Bormann F. H., 1986. Transport and loss of nitrous oxide in soil water after forest clear-cutting. Science, 233, 867-869.

Bramley R.G.V., White R. E., 1989. The effect of pH, liming, soil moisture and temperature on the activity of nitrifiers in a soil under pasture. Aust. J. Soil Res., 27, 711-724.

Bremner J.M., Blackmer A. M., 1979. Effects of acetylene and soil water content on emission of nitrous oxide from soil. Nature, 280, 380-381.

Bremner J.M., Robbins S.G., Blackmer A.M., 1980. Seasonal variability in emissions of nitrous oxide from soil. Geophys. Res. Lett., 7, 641-644.

Brzezińska M., 2006. Impact of treated wastewater on biological activity and accompanying processes in organic soils (field and model experiments). (in Polish, English summary). Monograph, Acta Agrophysica, 131.

Buchkina N.P., Balashov E. V., Rizhiya E.Y., Smith K.A., 2010. Nitrous oxide emissions from a light-textured arable soil of North-Western Russia: effects of crops, fertilizers, manures and climate parameters. Nutr. Cycl. Agroecosyst., 87, 429-442.

Burton D. L., Beauchamp E. G., 1994. Profile nitrous oxide and carbon dioxide concentrations in a soil subject to freezing. Soil. Sci. Soc. Am. J., 58, 115-122.

Conrad R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). Microbiological Reviews, 60, 609-640.

Conrad R., Seiler W. Bunse G., 1983. Factors Influencing the Loss of Fertilizer Nitrogen Into the Atmosphere as N_2O. J. Geophys. Res., 88, 6709-6718.

Davidson E. A., 1991. Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. Rogers J.E and Whitman W. B. (eds.) Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. American Society for Microbiology, Washington, D. C., 219-235.

de Klein C.A.M., Eckard R.J., 2008. Targeted technologies for nitrous oxide abatement from animal agriculture.Aust. J. Exp. Agric., 48, 14-20.

Dendooven L., Anderson J. M., 1994. Dynamics of reduction enzymes involved in the denitrification process in pasture soil. Soil Biol. Biochem., 26, 1501-1506.

Ding W.X., Cai Y., Cai Z.C., Yagi K., Zheng X.H., 2007. Nitrous oxide emissions froman intensively cultivated maize-wheat rotation soil in the North China Plain. Sci Total Environ., 373, 501-511.

Dobbie K.E., McTaggart I.P., Smith K.A., 1999. Nitrous oxide emission from intensive agricultural systems: variation between crops and seasons; key driving variables, and mean emission factors. J. Geophys. Research, 104, 26891-26899.

Drury C.F., McKenney D.J., Findlay W. I., 1992. Nitric oxide and itrous oxide production from soil: water and oxygen effects. Soil Sci. Soc. Am. J., 56, 766-770.

Ferguson S. J., 1994. Denitrification and its control. Antonine Leeuwenhoek, 66, 89-110.

Firestone M. K., 1982. Biological denitrification. Agron. Monogr., 22, 289-326.

Firestone M. K., Firestone R. B., Tiedje J. M., 1980. Nitrous oxide from soil denitrification: factors controlling its biological production. Science, 208, 749-750.

Firestone, M. K., 1982. Biological denitrification. in Nitrogen in Agricultural Soils (ed. Stevensen, F. J.), Am Soc. Agron. Madison, Wisconsin, 289-326.

Flückiger J., Dällenbach A., Blunier T., Stauffer B., Stocker T.F., Raynaud D., Barnola J.M., 1999. Variation in atmospheric N_2O concentrations during abrupt climatic changes. Science, 285, 227-230.

Freney J.R., Denmead O.T., Simpson J.R., 1979. Nitrous oxide emission from soils at low moisture contents. Soil Biol. Biochem., 11, 167-173.

Freney J. R., Denmead O. T., Simpson J. R., 1978. Soil as a source or sink for atmospheric nitrous oxide. Nature, 273, 530-532.

Gajda A.M., 2010. Microbial activity and particulate organic matter content in soils with different tillage system use. Int. Agrophysics, 24, 129-137.

Galbally I.E., Fraser B.J., Meyer C.P., Griffith D.W.T., 1992. Biosphere-atmosphere exchange of trace gases over Australia. Gifford R.M. and Barson M.M. (eds) Australia`s Renewable Resources Sustainability and Global Change. Bureau of Rural Resources Proceedings, AGPS: Canbera, Australia, 14, 117-149.

Gliński J., Stępniewski W., 1985. Soil Aeration and its Role for Plants. CRC Press, Boca Raton, Florida.

Gliński J., Stępniewski W., Stępniewska Z., Ostrowski J., Włodarczyk T., Brzezińska M., 2000. Agrometeorological aspects of aeration status of arable soils. Agroekologiczne aspekty warunków tlenowych gleb ornych. Monography, Acta Agrophysica, 32.

Goreau T.J., Kaplan W.A., Wofsy S.C., McElroy M.B., Valois F.W., Watson S.W., 1980. Production of NO_2 and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol., 40, 526-532.

Granli T., Bøckman O.C., 1994. Nitrous oxide from agriculture. Norw. J. Agric. Sci. Suppl., 12, 7-128.

Hatano R., Lipiec J., 2004. Effects of land use and cultural practices on greenhouse gas fluxes in soil. Monograph 6, Acta Agrophysica, 109.

Henault C., Devis X., Page S., Justes E., Reau R., Germon J.C., 1998. Nitrous oxide emissions under different soil and land management conditions., Biol. Fert. Soils, 26, 199-207.

Hergoualc'h K., Skiba U., Harmand J.-M., Oliver R., 2007. Processes responsible for the nitrous oxide emission from a Costa Rican Andosol under a coffee agroforestry plantation Biol. Fertil. Soils., 43, 787-795.

Hino T., Matsumoto Y., Nagano S., Sugimoto H., Fukumori Y., Murata T., Iwata S., Shiro Y., 2010. Structural basis of biological N_2O generation by bacterial nitric oxide reductase. Science, 330, 1666-1668.

Højberg O., Revsbech N.P., Tiedje J.M., 1994. Denitrification in soil aggregates analyzed with microsensors for nitrous oxide and oxygen. Soil Sci. Soc. Am. J., 58, 1691-1698.

Hooper A.B., Terry K.R., 1979. Hydroxylamine oxidoreductase of *Nitrosomonas*: production of nitric oxide from hydroxylamine. Biochem. Biophys. Acta, 571, 12-20.

Hooper A. B., 1986. Biochemistry of the nitrifying lithoautotrophic bacteria. In: Schlegel H. and G., Bowien B. (eds) Autotrophic bacteria. Madison, WI: Science Tech. Publishers. 239-265.

Horn R., Stępniewski W., Włodarczyk T., Walenzik G., Eckhardt F.E.W., 1994. Denitrification rate and microbial distribution within homogenous model soil aggregates. Int. Agrophysics, 8, 65-74.

Hu Z., Zhang L., Li S., Xie H., Wang J., Zhang T., Li Y., Zhang H., 2010. Effect of aeration rate on the emission of N2O in anoxic-aerobic sequencing batch reactors (A/O SBRs). Journal of Bioscience and Bioengineering, 109, 5, 487-491,

Hynes R.K., Knowles R., 1984. Production of nitrous oxide by *Nitrosomonas europaea*: effect of acetylene, pH and oxygen. Can. J. Microbiol., 30, 1397-1404.

IPCC, 2001. Climate Change, The Scientific Basis Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change (IPCC).Cambridge University Press, Cambridge.

Jetten M.S.M., Logemann S., Muyzwe G., Robertson L.A., de Vries S., van Loosdrecht M.C.M., Kuenen J.G., 1997. Novel principle in the microbial conversion of nitrogen compounds. Antonine van Leeuwenhoek, 71, 75-93.

Jiang J., Hu Z., Sun W., Huang Y., 2010. Nitrous oxide emissions from Chinese cropland fertilized with a range ofslow-release nitrogen compounds. Agriculture, Ecosystems and Environment,135, 216-225.

Josa R., Ginovart M., Solé A., 2010. Effects of two tillage techniques on soil macroporosity in subhumid environment. Int. Agrophysics, 24, 139-147.

Joye S.B., Hollibaugh J.T., 1995. Influence of sulphide inhibition of nitrification on nitrogen regeneration in sediments. Science, 270, 623-625.

Keller M., Kaplan W.A., Wofsky S.C., 1986. Emissions of N_2O, CH_4 and CO_2 from tropical forest soils. J. Geophys. Res., 91, 11791-11802.

Khalil M.A.K., Rasmussen R.A., 1992. The global sources of nitrous oxide. J. Geophys. Res., 97, 14651-14660.

Koike I., Hattori A., 1975. Energy yield of denitrification: an estimate from growth yield in continous cultures of *Pseudomonas denitrificans* under nitrate-, nitrite- and nitrous oxide limited conditions. J. Gen. Microbiol., 88, 11-19.

Koops H.P., Böttcher B., Möller U.C., Stehr G., 1991. Classification of eight new species of ammonia oxidising bacteria. J. Gen. Microbiol., 137, 1689-1699.

Koper J., Piotrowska A., 2003. Application of biochemical index to define soil fertility depending on varied organic and mineral fertilization. http://www.ejpau.media.pl/series/volume6/issue1/agronomy/art-06.html, EJPAU, Agronomy, 6.

Kotełko K., Sedlaczek L., Lachowicz T.M., 1979. Bacterial Biology (Biologia Bakterii). PWN, Warszawa.

Kotowska U., Włodarczyk T., 2005. Transformation of mineral forms of nitrogen in soil irrigated with sewage (in Polish, English summary). Monograph, Acta Agrophysica, 119.

Kuenen J.G., Lesley A., Robertson L. A., 1994. Combined nitrification-denitrification processes. FEMS Microbiology Reviews, 15, 109-117.

Letey J., Hadas A., Valoras N., Focht D.D., 1980. Effect of preincubation treatments on the ratio of N_2O/N_2 evolution. J. Environ. Qual., 9, 232-235.

Letey J., Valoras N., Focht D. D., Ryden J. C., 1981. Nitrous oxide production and reduction during denitrification as affected by redox potential. Soil Sci. Soc. Am. J., 45, 727-730.

Lipschultz F., Zafiriou O.C., Wofsy S.C., McElroy M.B., Valois F.W., Watson S.W., 1981. Production of NO and N_2O by soil nitrifying bacteria. Nature, 294, 641-643.

Lloyd D., Boddy L., Davies K.J.P., 1987. Persistence of bacterial denitrification capacity under aerobic conditions: the rule rather than exception. FEMS Microbiol. Ecol., 45, 185-190.

Lloyd D., 1993. Aerobic denitrification in soils and sediments: from fallacies to facts. TREE, 8, 10, 352-356.

Luizao F., Matson P., Livingston G., Luizao R., Vitousek P., 1989. Nitrous oxide flux following tropical land clearing. Global Biogeochem. Cycles, 3, 281-285.

Maljanen M., Martikkala M., Koponen H.T., Virkajärvi P., Martikainen P.J., 2007. Fluxes of nitrous oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. Soil Biol. Biochem., 39, 914-920.

Martikainen P., De Boer W., 1993. Nitrous oxide production and nitrification in acidic soil from Dutch coniferous forest. Soil Biol. Biochem., 25, 343-347.

McKenney D.J., Drury C.F., Findlay W.I., Mutus B., McDonnel T., Gajda C., 1994. Kinetics of denitrification by *Pseudomonas fluorescens*- oxygen effects. Soil. Biol. Biochem., 26, 901-908.

McTaggart I.P., Tsuruta H., 2003. The influence of controlled release fertilizers and the form of applied fertilizer nitrogen on nitrous oxide emissions from an andosol. Nutr. Cycl. Agro-ecosyst., 67, 47-54.

Megonigal J.P., Hines M.E., Visscher P.T., 2004. Anaerobic metabolism: Linkages to trace gases and aerobic processes. In: Biochemistry, Schlesinger W.H. (ed). Elsevier-Pergamon, Oxford, UK, 317-424.

Mosier A.R., Chapman S.L., Freney J.R., 1989. Determination of dinitrogen emission and retention in floodwater and porewater of a lowland rice fertilized with ^{15}N-urea. Fert. Res., 19, 127-136.

Muller E.B., Stouthamer A.H., Verseveld van H.W., 1995. Simultaneous NH_3 oxidation and N_2 production at reduced O_2 tensions by sewage sludge subcultured with chemolitotrophic medium. Biodegradation, 6, 339-349.

Nosalewicz M., Stępniewska Z., 2005. Evolution of gases from soil irrigated with purificated wastewater. In: Monitoring and Modelling the Properties of Soil as Porous Medium. Skierucha W., and Walczak R.T. (eds). IA PAS, Lublin, 101-108.

Nosalewicz M., Stępniewska Z., Baranowski P., 2005. N_2O emission from the surface of Eutric Histosol irrigated with munuicipal wastewater (after the second step of purification). Polish Journal of Soil Science, XXXVIII/2, 111-118.

O`Hara G.W., Daniel R.M., 1985. Rhizobial denitrification: a review. Soil Biol. Biochem., 17, 1-9.

Okereke G.U., 1993. Growth yield of denitrifiers using nitrous oxide as a terminal electron acceptor. World J. Microbiol. Biotechnol., 9, 59-62.

Parkin T B., 1987. Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J., 51, 1194-1199.

Pathak H., 1999. Emission of nitrous oxide from soil. Current science, 77, 3, 359-369.

Paul E.A., Clark F.E., 1998. Soil Microbiology and Biochemistry, Academic Press, California, USA.

Perrson T., Lundkvist H., Wiren A., Hyvonen R., Wesser B., 1989. Effects of acidification and liming on carbon and nitrogen mineralization and soil organisms in mor humus. Water, Air Soil Pollut., 45, 77-96.

Peterjohn W.T., 1991. Denitrification: enzyme content and activity in desert soils. Soil Biol. Biochem., 23, 845-855.

Petersen S.O., Schjoning P., Thomsen I.K., Christensen B.T., 2008. Nitrous oxide evolution from structually intact soil as influenced by tillage and soil water content. Soil Biol. Biochem., 40, 967-977.

Pochon J., Tardieux P., 1962. Techniques d`analyse en microbiologie du sol. Edition de la Tourelle, St. Monde, 63-64.

Poth M., Focht D.D., 1985. ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europea*: an examination of nitrifier denitrification. Appl. Environ. Microbiol., 49, 1134-1141.

Prinn R.G., 1994. The interactive atmosphere – global atmospheric-biospheric chemistry. Ambio., 23, 50-61.

Prosser J. I., 1989. Autotrophic nitrification in bacteria. Advanc. Microbiol. Physiol., 30, 125-181.

Remde A., Conrad R., 1991. Metabolism of nitric oxide inn soil and denitrifying bacteria. FEMS Microbiol Ecol., 85, 81-93.

Remde A., Conrad R., 1990. Production of nitric oxide in *Nitrosomonas europaea* by reduction of ntrite. Arch. Microbiol., 154, 187-191.

Ritchie G.A.F., Nicholas D.J.D., 1972. Identification of nitrous oxide produced by oxidative and reductive processes in *Nitrosomonas europaea*. Bochem. J., 126, 1181-1191.

Robertson L.A., Kuenen J.G., 1984. Aerobic denitrification –old wine in new bottles? Antonine van Leeuwenhoek, 50, 525-544.

Ryden J.C., 1981. Nitrous oxide exchange between a grassland soil and the atmosphere. Nature, 292, 235-237.

Samuelsson M.O., 1985. Dissimilatory nitrate reduction to nitrite, nitrous oxide, and ammonium by *Pseudomonas putrefaciens*. Appl. Environ. Microbiol., 50, 812-815.

Sapek A., 2008. Emission of nitrous oxides (NOx) from agricultural soils and natural ecosystems. (Emisja tlenków azotu (NOx) z gleb uprawnych i ekosystemów naturalnych do atmosfery). Woda-Środowisko-Obszary Wiejskie, 8, 283-304.

Schulthess R.V., Wild D., Gujer W., 1994. Nitric and nitrous oxides from denitrifying activated sludge at low oxygen concentration, Wat. Sci. Tech., 30, 123-132.

Schumacher W., Korneck P.M.H., 1992. Anaerobic energy metabolism of the sulfur-reducing bacterium „*Spirillum*" 5175 during dissimilatory nitrate reduction to ammonia. Arch. Micro-biol., 157, 464-470.

Seiler W., Conrad R., 1981. Field measurement of natural and fertilizer-induced N_20 release rates from soils. J. Air Pollut. Cont. Assoc., 31, 767-772.

Seitzinger S. P., 1988. Denitrification in freschwater and coastal marine ecosystems: ecological and geochemical significance. Limnol. Oceanogr., 33, 702-724.

Shelton D. R., Sadeghi A. M., McCarty G. W., 2000. Effect of soil water content on denitrification during cover crop decomposition. Soil Sci., 165, 365-371.

Shiskowski D. M., Simm R. A., Mavinic D. S., 2004. An experimental procedure for identifying the aerobic-phase biological source of nitrous oxide in anoxic-aerobic wastewater treatment systems, J. Environ. En. Sci., 3, 549-553.

Šimek M., Elhottová D., Klimeš F., Hopkins D.W., 2004. Emissions of N_2O and CO_2, denitrification measurements and soil properties in red clover and ryegrass stands. Soil Biol. Biochem., 36, 9-21.

Šimek M., Jíšová L., Hopkins D.W., 2002. What is the so-called optimum pH for denitryfication in soil? Soil Biol. & Biochem., 34, 1227-1234.

Simpson J.R., Freney J.R., Wetselaar R., Muirhead W.A., Leuning R., Denmead O.T., 1984. Transformations and losses of urea nitrogen after application to flooded rice. Aust. J. Agric. Res., 35, 189-200.

Sitaula B.K., Bakken L.R., Gunnar A., 1995. N-fertilization and soil acidification effects on N_2O and CO_2 emission from temperate pine forest soil. Soil Biol. Biochem., 27, 1401-1408.

Skiba U., Fowler D., Smith K.A., 1997. Nitric oxide emissions from agricultural soils in temperate and tropical climates: sources, controls and mitigation options. Nutr. Cycl. Agroecosyst., 48, 139-153.

Skiba U., Fowler D., Smith K., 1994. Emissions of NO and N_2O from soils. Environ. Monit. Assess., 31, 153-158.

Smith P., Martino D., Cai Z., Gwary D., Janzen H., Kumar P., McCarl B., Ogle S., O'Mara F., Rice C., Scholes B., Sirotenko O., 2007. Agriculture. In: Metz B., Davidson O.R., Bosch P.R., Dave R., Meyer L.A. (eds.), Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom, 498–540.

Stalenga J., Kawalec A., 2007. Evaluation of the effect of different crop production systems on nitrous oxide emission and soil organic matter balance (in Polish). Acta Agrophysica, 150, 73-75.

Stępniewska Z., Pasztelan M., Stępniewski W., Brzezińska M., 2001. Effect of water content in peat-muck soil irrigated with municipal wastewater on N_2O i CO_2 emission. Acta Agrophysica, 53, 165-170.

Stevens R.J., Laughlin R.J., Burns L.C., Arah J.R.M., 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biol. Biochem., 29, 139-151.

Stüven R., Vollmer M., Bock E., 1992. The impact of organic matter on nitric oxide formation by *Nitrosomonas europaea*. Arch. Microbiol., 158, 439-443.

Szarlip P., 2009. Release and uptake of nitrous oxide in some mineral soils. PhD thesis, Institute of Agrophysics, Polish Academy of Sciences, Lublin.

Takaya N., Catalan-Sakairi A.M.B., Sakaguchi Y., Kato I., Zhemin Z., Shoun H., 2003. Aerobic denitrifying bacteria that produce low levels of nitrous oxide. Appl. Environ. Microbiol., 69, 3152-3157.

Takaya N., 2009. Response to hypoxia, reduction of electron acceptors, and subsequent survival by filamentous fungi. Biosci. Biotechn. Biochem., 73, 1-8.

Teraguchi S., Hollocher T.C., 1989. Purification and some characteristics of a cytochrome c-containingnitrous oxide reductase from *Wiolinella succinogenes*. J. Biol. Chem., 264, 1972-1979.

Tiedje J.M., 1988. Ecology of denitrificaton and dissimilatory nitrate reduction to ammonia. In: Zehnder A.J.B. (ed) Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York, 179-244.

Tiedje J.M., Sextone S., Mirold D.D., 1982. Denitrification: ecological niches, competition and survival. Antonie Van Leeuwenhoeck, 48, 261-284.

Tiedje J.M., Sexstone A.J., Myrold D.D., Robinson J.A., 1983. Denitrification: ecological niches, competition and survival. Antonie von Leeuwenhoek, 48, 569-583.

Turski M., 2010. Effect of soil management of aggregated stability in loess soil (in Polish). Acta Agrophysica, 15, 197-203.

Ullah S., Breitenbeck G.A., Faulkner S.P., 2005. Denitrification and N_2O emission from forested and cultivated alluvial clay soil. Biogeochemistry, 73, 499-513.

Van Clemput O., Baert L., 1984. Nitrite: a key compound in N loss processes under acid conditions? Plant Soil, 76, 233-241.

Vilain G., Garnier J., Tallec G., Cellier P., 2010. Effect of slope position and land use on nitrous oxide (N_2O) emissions (Seine Basin, France). Agricultural and Forest Meteorology, 150, 1192-1202.

Webster E.A., Hopkins D. W., 1996. Contributions from different microbial processes to N_2O emission from soil under different moisture regimes. Biol. Fertil. Soils, 22, 331-335.

Weier K.L., Gillam J. W., 1986. Efect of acidity on nitrogen mineralisation and nitrification in Atlantic coastal plain soils. Soil Sci. Soc. Am. J., 50, 1210-1214.

Wever H.De., Mussen S., Merckx R., 2002. Dynamics of trace gas production following compost and NO_3^- amendments to soil at different initial TOC/NO_3^- ratios. Soil Biol. Biochem., 34, 1583-1591.

Williams E.J. Hutchinson G.L., Fehsenfeld F.C., 1992. NO_x and N_2O emission from soil. Global Biogeochem. Cycles, 6, 351-388.

Włodarczyk T., 2000. N_2O emissions and absorption against a background of CO_2 in Eutric Cambisol under different oxidation-reduction conditions (in Polish). Acta Agrophysica, Monograph, 28.

Włodarczyk T., Brzezińska M., Kotowska U., Gliński J., 2004a. Nitrous oxide reduction and dehydrogenase activity in Cambisols under flooding conditions. W: Eurosoil. Freiburg, Germany, September, 356, 4-12.

Włodarczyk T., Stępniewska Z., Brzezińska M., 2003. Denitrification, organic matter and redox potential transformation in Cambisols. Int. Agrophysics, 17, 219-227.

Włodarczyk T., Stępniewski W., Brzezińska M., 2002a. Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from Cambisols under flooding conditions. Biol. Fertil. Soils, 36, 200-206.

Włodarczyk T., Stępniewski W., Brzezińska M., 2005. Nitrous oxide production and consumption in Calcaric Regosols as related to soil redox and texture. Int. Agrophysics, 19, 263-271.

Włodarczyk T., Stępniewski W., Brzezińska M., Kotowska U., 2002b. N_2O emission and sorption in relation to soil dehydrogenase activity and redox potential. Int. Agrophysics, 16, 249-252.

Włodarczyk T., Stępniewski W., Brzezińska M., Stępniewska Z., 2004b. Nitrate stability in loess soils under anaerobic conditions – laboratory studies. J. Plant Nutr. Soil Sci., 167, 693-700.

Włodarczyk T., Szarlip P., Brzezińska M., 2005. Nitrous oxide consumption and dehydrogenase activity in Calcaric Regosols. Polish J. Soil Sci., 2, XXXVIII, 97-110.

Wolińska A., 2010. Dehydrogenase activity of soil microorganisms and oxygen availability during reoxidation proccess of selected mineral soils (in Polish). Acta Agrophysica, Monograph, 180.

Wrage N., Dixon L., Estavillo J.M., Nawalany P., Oenema O., 2000. The influence of soil pH and nitrogen fertilizer application on N_2O production by nitrifier denitrification: Some preliminary results. In: Effects of Liming and Nitrogen Fertilizer Application on Soil Acidity and Gaseous Nitrogen Oxide Emissions in Grassland Systems. Oenema O. and Sapek, A., (eds), Falenty IMUZ, Falenty.

Yang W.X., Meixner F. X., 1997. Laboratory studies on the release of nitric oxide from sub-tropical grassland soils: the effect of soil temperature and moisture. In Gaseous Nitrogen Emission from Grasslands. Jarwis S.C. and Pain B.F. (eds). CAB International, Wallingford, UK, 67-71.

Ye R.W., Averill B. A., Tiedje J. M., 1994. Denitrification – production and consumption of nitric oxide. Appl. Environ. Microbiol., 60, 1053-1058.

Yoshida T., Alexander M., 1970. Nitrous oxide formation by *Nitrosomonas europaea* and heterotrophic microorganisms. Soil Sci. Soc. Am. Proc., 34, 880-892.

Yoshinari T., 1970. Emissions of N_2O from various environments. in Denitrification in Soil and Sediment. Revsbech and Sorenson (eds.), Plenum, New York, 129-149.

Zaliwski A.Z., Purchała L., 2007. Estimation of nitrous oxide and methane emission from agriculture in the regional breakdown in 2005 (in Polish). Acta Agrophysica, Monograph, 4, 150, 76-83.

Zehr J.B., Ward B.B., 2002. Nitrogen cycling in the ocean: new perspectives on processes and paradigm. Appl. Envinron. Microbiol., 68, 1015-1024.

Zumft W.G., Knoreck P.M.H., 1990. Metabolism of nitrous oxide. In: Revsbech N. P. and Soerensen J. (eds). Denitrification in soil and sediment. Plenum Press, New York, 37-55.

Zumft W.G., 1997. Cell biology and molecular basis of denitrification. Microbiol. Molecul. Biol. Rev., 61, 533-616.

Zumft W.G., 1993. The biological role of nitric oxide in bacteria. Arch. Microbiol., 160, 253-264.

7. SUMMARY

Nitrous oxide is a greenhouse gas that is ca.300 times more effective at radiative forcing than CO_2 on a mole basis. Moreover, in the stratosphere, N_2O is transformed by photolysis to NO, which is responsible for stratospheric ozone destruction. The vast majority of N_2O originates from microbes that break down nitrogen compounds in soils and in the oceans. Agricultural soils are the most significant anthropogenic sources of nitrous oxide. Agricultural fertilizers, fossil fuel combustion, biomass burning, and animal waste contribute to N_2O production. Increasing N-inputs into agricultural soils are suspected to be responsible for increasing N_2O emission into the atmosphere. The amounts of N_2O emitted from soils depend on complex interactions between soil properties (especially soil aeration status, temperature and carbon availability, soil texture), type and management of N fertilizer preceding crop, residue management, and other agricultural practices as well as prevailing climatic conditions. Soil is heterogeneous and commonly has both aerobic and anaerobic sites. The oxygen status in soil, which is inversely proportional to the amount of moisture held there, appears in many studies to be one of the key factors influencing nitrous oxide production. Nitrous oxide emission from soils varies strongly with soil water content. Total denitrification fluxes (N_2O plus N_2) are directly proportional to soil NO_3^- concentrations when the other important component, a readily metabolizable organic substrate, is also present and non rate-limiting. When a lack of metabolizable organic matter limits potential denitrification, N_2 plus N_2O fluxes do not increase with increasing NO_3^- concentration. Soil texture is a good predictor of denitrification rates at the landscape scale part because it captures the interaction between water content and soil porosity with respect to gas and solute diffusion path length. Apart from nitrous oxide emission soil can also remove atmospheric N_2O under conditions favorable for N_2O reduction. This is probably only a minor sink on the global scale, but elimination of N_2O in the stratosphere is so slow that even a small soil sink can contribute significantly to diminish of the atmospheric residence time of N_2O. N_2O reduction is the only known process important for N_2O turnover and sink in soil. Understanding of the processes related to nitrous oxide formation and uptake may be useful in predicting of N-fertilizer fate in soil.

Keywords: soil, nitrous oxide, N_2O emission, N_2O sink, denitrification, nitrogen, fertilizers

8. STRESZCZENIE

WPŁYW WARUNKÓW GLEBOWYCH NA WYDZIELANIE I POCHŁANIANIE TLENKU AZOTU(I), N_2O

Podtlenek azotu (tlenek azotu(I), N_2O) jest jednym z tzw. gazów cieplarnianych. Efektywność pochłaniania promieniowania podczerwonego przez cząsteczkę N_2O w porównaniu do cząsteczki CO_2 jest około 300 razy większa. Tlenek azotu(I) w stratosferze ulega fotolizie i jest przekształcany w NO, który jest odpowiedzialny za niszczenie warstwy ozonowej. Zdecydowana większość emitowanego do atmosfery N_2O pochodzi z mikrobiologicznych procesów przemian związków azotu zachodzących w glebach i oceanach. Gleby rolnicze należą do największych antropogenicznych źródeł emisji podtlenku azotu. Nawozy azotowe, spalanie paliw kopalnych, spalanie biomasy i odpadów zwierzęcych to dodatkowe źródła N_2O. Uważa się, że zwiększanie dawek nawozów azotowych jest przyczyną wzrostu emisji N_2O do atmosfery. Wielkość emisji tlenku azotu(I) z gruntów rolnych zależy od złożonych interakcji pomiędzy właściwościami gleby – przede wszystkim stanem natlenienia, temperaturą, dostępnością węgla oraz strukturą gleby. Duże znaczenie ma też typ nawozu azotowego, sposób nawożenia, zabiegi rolnicze oraz warunki klimatyczne. Gleba jest heterogennym środowiskiem trójfazowym, w którym w niewielkiej odległości występują obok siebie przestrzenie dobrze natlenione i obszary obniżonej dostępności tlenu. Stan natlenienia gleby, determinowany przez wilgotność, przez wielu autorów uważany jest za kluczowy czynnik wpływający na emisję N_2O. Całkowita denitryfikacja (N_2O plus N_2) jest proporcjonalna do stężenia NO_3^- w glebie, pod warunkiem, że ilość węgla organicznego jest wystarczająco wysoka i nie ogranicza szybkości procesu. Kiedy zawartość materii organicznej jest niewystarczająca, denitryfikacja potencjalna (wyrażona w emisji N_2O i N_2) nie ulega podwyższeniu wraz ze wzrostem zawartości NO_3^-. Skład granulometryczny gleby ma duży wpływ na aktywność denitryfikacyjną gleb, ponieważ od niego w dużej mierze zależą stosunki wodno-powietrzne i porowatość, a tym samym dyfuzja gazów i substancji rozpuszczonych w roztworze glebowym. Gleba jest również zdolna do pochłaniania N_2O. Redukcja N_2O do N_2 jest jedynym znanym sposobem przekształcania tego gazu w glebie. Ten proces uważany jest za mało istotny w skali globalnej, jednak biorąc pod uwagę niskie tempo rozpadu cząsteczek N_2O w stratosferze, nawet niewielkie pochłanianie tlenku azotu(I) przez gleby może znacznie przyczynić się do

redukcji wpływu N_2O na zmiany klimatyczne. Zrozumienie procesów związanych z tworzenia tlenku azotu(I) i jego pochłanianiem może mieć duże znaczenie w przewidywaniu losu nawozów azotowych w glebie.

Słowa kluczowe: gleba, tlenek azotu(I), wydzielanie N_2O, pochłanianie N_2O, denitryfikacja, nawozy azotowe

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