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Effect of long storage and soil type on the actual denitrification and denitrification capacity to N_2O formation

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A b s t r a c t. The actual denitrification to N_2O and denitrification capacity to N_2O after flooding of different soil samples stored for over 25 years in air-dry conditions and fresh, air dried samples were compared in our study. Zero N_2O release was observed from the stored soils but the fresh soil samples had very low actual denitrification to N_2O. NO_3^- addition significantly increased the amount of N_2O (denitrification capacity to N_2O) released after flooding, which depended on the length of storage and type of soils and was much higher in stored soils. Prolonged exposure of the soils to drought conditions caused a greater decrease in the Eh value compared with the fresh soil. The total cumulative release of N_2O from the stored and fresh soils was correlated with the reduced NO_3^- and organic C content in soils enriched with NO_3^-. Some soils showed the capability of N_2O consumption. CO_2 release depended on the length of storage and type of soils under flooding after prolonged drought. On average, CO_2 release was higher from the stored rather than fresh soils. The organic C content in the stored soils was generally lower than in the fresh soils, probably due to the storage effect. The cumulative CO_2 release from the stored soils was well correlated with the organic C while no correlation was observed for the fresh soil samples.

K e y w o r d s: actual denitrification to N_2O, denitrification capacity to N_2O, long- and very short-storage time, soil respiration, archived soil

INTRODUCTION

Biological activity in soil can be represented by several different parameters such as respiration, enzyme activity, ammonification, nitrification, denitrification, and emission of gaseous metabolites as well as oxidation-reduction processes (Bieganowski *et al*., 2013; Włodarczyk *et al.*, 2011).

Soils are subjected to temporal variations in temperature and moisture that can cause changes in physicochemical properties. Soil dry/wet cycles result from natural variations in soil moisture driven by environmental and biophysical processes such as precipitation, evapotranspiration, and drainage. Management factors such as irrigation, tillage and land cover (*ie*. vegetation type) can moderate or accentuate the amplitude of these natural cycles (Oliveira *et al*., 2005).

Under in situ conditions, denitrification rates depend on oxygen availability, soil moisture, soil type, pH, NO_3^- concentration, but also on the availability of labile carbon compounds in soil (Burford and Bremner, 1975; Senbayram *et al*., 2009).

Nitrate (NO_3^-) is a key node in the network of the assimilatory and respiratory nitrogen pathways. For bacteria, it is both a nitrogen source and an electron acceptor (Hayatsu *et al*., 2008). In agriculture and wastewater treatment, NO_3^- respiration by microorganisms is an important process in respect to economics, greenhouse gas emission, and public health. Several microbial processes compete for NO_3^-: denitrification, dissimilatory NO_3^- reduction to ammonium (NH_4^+), and anaerobic ammonium oxidation. Denitrification is a respiratory process in which NO_3^- is reduced stepwise to dinitrogen (N_2) ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$). In bacteria, this process is used as an alternative to oxygen (O_2) respiration under low O_2 or under anoxic conditions (Włodarczyk *et al*., 2005).

Intense agricultural fertilization may lead to increased concentrations of NO_3^- in the groundwater (Almasri and Kaluarachchi, 2004). Furthermore, fertilization increases the atmospheric concentrations of methane and nitrous

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oxide (N_2O) and thus contributes to greenhouse gas emissions and global warming (Hanke and Strous, 2010). As a potent greenhouse gas, N_2O is responsible for about 6% of the current greenhouse effect (IPCC, 2007). Moreover, N_2O has received great attention because of its importance for stratospheric ozone depletion (Ravishankara *et al*., 2009). Globally, agricultural soils account for about 60% of the atmospheric N_2O emissions (Kroze *et al*., 1999).

Soil organic matter (SOM) content and texture are important factors affecting carbon (C) and nitrogen (N) mineralization under constant soil moisture but their effects on organic matter mineralization and associated biogenic gas (CO_2 and N_2O) production during dry/wet cycles is poorly understood (Harrison-Kirk *et al*., 2013). Kraft *et al*. (2011) showed that knowledge of the mechanism of NO_3^- reduction in natural ecosystems is still not clear. Although a fair number of studies on pure cultures have been performed, little is known about how the natural microbial communities of terrestrial and aqueous habitats react to changing NO_3^- concentrations and nitrogen speciation.

Easily available soil organic carbon and type of soil are important factors affecting NO_3^- respiration and C mineralization but their effects on CO_2 and N_2O production during flooding of dry soil is rarely investigated and poorly understood (Włodarczyk *et al*., 2005).

Very important information from the point of view of agricultural practices (loss of N) and the environmental protection (N_2O emission) is the denitrifying capacity of the soil, especially the capacity of soil to produce N_2O.

Burford and Bremner (1975) described denitrification capacity (DC) as a process directly related to the total C content, and also to the water-soluble and mineralizable C in the reaction of NO_3^--treated soil incubated at 20°C for 7 days with a C_2H_2 block (as a the sum of N_2O and N_2 forms). In other words, it is a process occurring in soil characterized by natural availability of organic C and enriched in NO_3^-.

Our studies introduce the concept of actual denitrification leading to N_2O formation without addition of NO_3^- (aD_{N_2O}) and the concept of denitrification capacity leading to N_2O formation with addition of NO_3^- (DC_{N_2O}). The two concepts of aD_{N_2O} and DC_{N_2O} are defined as NO_3^- reduction in conditions of a natural organic C content as a source of C and electrons in denitrification. The difference in determination of these two parameters is incubation with (DC_{N_2O}) or without (aD_{N_2O}) additional NO_3^-. The aD_{N_2O} and DC_{N_2O} were determined without a C_2H_2 block, in contrast to DC described by Burford and Bremner (1975).

In the current study, denitrification of long-term air-dried stored soil samples was compared with fresh air-dried samples collected from the same plots and incubated under flooded and fully controlled conditions. The objective of the experiment was to test the impact of prolonged drought conditions and soil type on the denitrification capacity and actual denitrification leading to N_2O formation. It is hypothesized that prolonged dry conditions will increase the denitrification capacity due to biodegradation of not easily accessible organic carbon caused by long storage time.

MATERIALS AND METHODS

Six soil samples from Ap horizon of Silty loam texture collected approximately 25 years prior to the start of the study from mineral soils used for agriculture in Poland, stored under air-dry conditions in the Soil Bank in the Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland, and fresh soils resampled at the same sites in 2012 (stored under air-dry conditions up to incubation) were used in the study.

For measurement of the actual denitrification aD_{N_2O} and the denitrification capacity DC_{N_2O}, the stored and fresh soils were divided into two parts. 5-g portions of dry soils were placed in 22 cm^3 glass flasks and flooded with 5 ml of distillated water. To determine the aD_{N_2O} and DC_{N_2O}, the soil samples were prepared according to the following variants:

I – soils stored (for 25 years) in the Bank of Soil (S) and fresh air-dried soils collected from the same locations as the stored samples (F) with water addition – the results of soil incubation corresponding to actual denitrification leading to N_2O formation (aD_{N_2O}),

II – soils (S and F) with water and NO_3^- addition – the results of soil incubation corresponding to denitrification capacity leading to N_2O formation – (DC_{N_2O}). NO_3^- was added as KNO_3 at the rate of 3 mg of NO_3^--N per 10 g of dry soil (Šimek *et al*., 2004).

The flasks with the soils were tightly sealed with rubber stoppers and incubated in ambient air. The initial concentration of O_2 in the gas headspace at the beginning of the incubation was 20.9% v/v. Paraffin films were placed over the stoppers to ensure hermetic sealing. The soils were incubated at 20°C for 7 days (Włodarczyk *et al*., 2005).

After 1, 2, and 7 days of incubation, the concentrations of N_2O and CO_2 in the headspace were determined with a gas chromatograph (Shimadzu GC-2014, Japan) equipped with a split-splitless injector with an injection divider in two column types and two types of detectors depending on the analysed gas. 100-µl samples were dosed automatically by an auto-sampler AOC 5000. Helium was used as a carrier gas (column flow rate: 5 ml min^{-1}). The concentration of N_2O was analyzed using an electron capture detector (ECD). Separation of the gas samples was performed on the Supel PLOT-Q™ 30 m x 0.32 mm column (manufacturer Supelco). The concentration of CO_2 was measured on the same column using a flame ionization detector (FID). The column oven temperature was 35°C. All detectors operated at 200°C. The concentrations of N_2O-N and CO_2-C were corrected for gas dissolved in water using the literature values of Bunsen

absorption coefficients. The results obtained were calculated per kg of dry soil. The amount of N_2O (incomplete denitrification) and CO_2 release were determined during the 7 days of incubation at different stages of the cumulative curve of N_2O and CO_2 release. The N_2O and CO_2 release was expressed as the maximum cumulative amount of N_2O-N and CO_2-C mg kg^{-1} soil for 7 days of incubation.

The NO_3^- content in soil was measured in 5 g of air-dry soil suspended in 105 ml of 0.025 N $CaCl_2$. The suspension was shaken for 2 h. The filtered solution of NO_3^- ions was determined using a flow spectrophotometer (FIA-Star 5010 Analyzer FOSS Tecator). Soil aeration conditions were estimated by the redox potential (Eh) as described by Gliński and Stępniewski (1985) (Table 1).

Particle size distribution (PSD) was measured using a laser diffractometer Mastersizer 2000 (Malvern, UK) with a Hydro G dispersion unit. The measuring range was 0.02 µm – 2 mm. The following parameters were set: pump speed – 1750 r.p.m. and the stirrer speed – 700 r.p.m. (Sochan *et al*., 2012). Ultrasonification (maximum power – 35W for 4 min) was used for aggregate dispersion (Ryżak and Bieganowski, 2011). The procedure of decreasing obscuration (to the maximum level of 20%) was used when the obscuration was too high after ultrasonification (Bieganowski *et al*., 2010). Mie theory was used for recalculation of light intensity into PSD with the following indices: soil refraction index 1.52, soil absorption index 0.1, and water refraction index 1.33. The measurements were carried out in 3 replications (1 min each measurement – 30 s of red and 30 s of blue light) for each of the three samplings (for each soil) (Table 1).

Determination of other soil properties included C_{org} (TOC-analyzer); pH was determined in the aqueous suspension of soil (v/v = 1/1) using a pH-meter (PIONeer pH Radiometer Copenhagen).

The results were statistically analyzed. Linear ($y = a + bx$), multiplicative ($y = ax^b$), exponential ($y = e^{a+bx}$), and logarithmic ($y = a\ln x + b$) models were used in regression analysis, and in each case the model with the highest R^2 was selected as the best fit for the experimental data, using Microsoft Office Excel 2007. Statgraphics program was used for analysis of variance.

RESULTS

The basic soils characteristics are presented in Table 1. The Mollic Gleysols (MG), Eutric Cambisols (EC), Haplic Phaeozems (HPh), Haplic Podzols (HP), Rendzic Leptosols (RL), and Distric Fluvisols (DF) were formed of silt loam. The soils used for the laboratory experiment were characterized by a wide spectrum of native C_{org} contents ranging from 1.1 (HP) to 3.82 (MG) and from 1.31 (EC) to 3.79 (MG), for the stored and fresh soils, respectively. The investigated soils were characterized by a wide spectrum of the pH reaction value ranging from slightly acidic 5.68 (EC) to alkaline – 7.25 (RL) and from acidic – 5.48 (DF) to neutral – 7.11 (RL). The native NO_3^- content in the stored soils was

T a b l e 1. Basic properties and particle size distributions of stored (S) and fresh (F) soils of Silt loam

Soil No.[1]	Soil units	Granulometric composition (%) (dia in mm)			C_{org}	NO_3^--N[2]	pH[3]	Eh[4]
		sand	silt	clay	%	mg kg^{-1}		mV
145	Mollic Gleysols	34	59	7	3.82^S 3.79^F	0.81^S 5.86^F	5.75^S 6.70^F	290^S 232^F
553	Eutrick Cambisols	31	63	6	1.37^S 1.31^F	0.28^S 0.13^F	5.68^S 5.53^F	296^S 342^F
601	Haplic Phaeozems	14	79	7	1.00^S 1.33^F	0.31^S 0.91^F	7.12^S 5.68^F	207^S 365^F
633	Haplic Podzols	30	63	7	1.10^S 1.38^F	3.4^S 0.68^F	5.74^S 6.50^F	263^S 299^F
724	Rendzic Leptosols	39	54	7	1.79^S 2.80^F	2.96^S 1.53^F	7.25^S 7.11^F	197^S 285^F
941	Distric Fluvisols	39	55	6	1.58^S 1.98^F	3.42^S 6.99^F	5.72^S 5.48^F	262^S 296^F

[1]Soil No. from the Bank of Soil, [2]endogenous nitrate content, [3]pH value from the 0 day of incubation, [4]Eh value from the 0 day of incubation.

in a range from 0.28 (EC) to 3.42 (DF) while in the fresh samples from 0.13 (EC) to 6.99 (DF). At the initial phase of incubation (0 day) the redox potential value (Eh) ranged from (+197) for RL to (+296) for EC for stored soils while for the fresh soils the range was from (+232) for MG to (+365) for HPh.

The differences between the native NO_3^- reduction in the control stored and fresh soils are shown in Table 2. The native NO_3^- content described as % of its reduction during 7 days of incubation ranged from 0 (MG) to 100% (HP, RL, and DF) for the stored soils and from 40.7 (HPh) to 100% (EC) for the fresh soils. The percent of NO_3^- denitrified to N_2O was zero for the stored soils while for fresh soils it ranged from 0 (MG and RL) to 100% (EC, HPh, HP, and DF) depending on the type of soil.

The differences between the added NO_3^- reduction in the stored and fresh soils are shown in Table 3. The added NO_3^- content described as % of its reduction during 7 days of incubation ranged from 31.1 (HP) to 98.1% (MG) for the stored soils and from 49.0 (HP) to 98.1% (MG) for the fresh soils. The percent of NO_3^- denitrified to N_2O ranged from 16.2 (HPh) to 46.1% (RL) and from 5.4 (HP) to 31.4% (RL), for the stored and fresh soils, respectively, depending on the type of soils and length of storage.

Figure 1 shows the course of two types of denitrification - aD_{N_2O} and DC_{N_2O} of the soil without and with the addition of NO_3^- (respectively) during incubation under flooded conditions. The studied soils differed in terms of the amount of N_2O released during aD_{N_2O}. There was no N_2O release during incubation without additional NO_3^- from the stored soils (Table 2 and Fig. 1 inserts). The fresh soils had very low aD_{N_2O}. The cumulative N_2O release during aD_{N_2O} from the fresh soils ranged from-0 (MG and RL) to 2.84 (HPh) mg N_2O-N kg^{-1} of soil (Table 2 and Fig. 1 inserts).

The NO_3^- addition caused a very intense increase in the amount of N_2O released in both the stored and fresh soils and ranged from 48.8 (HPh) to 139.6 (RL) mg N_2O-N kg^{-1} of soil for the stored soils. For the fresh soils, it ranged from 16.32 (HP) to 95.5 mg (RL) N_2O-N kg^{-1} of soil (Table 3 and Fig. 1). The amount of N_2O released from the stored soils was significantly higher than that of the fresh soils, except for HPh No. 601 (Fig. 1c).

Consumption of N_2O in the headspace was observed in some of the fresh samples both in aD_{N_2O} (Fig. 1 b, c, f - inserts) and DC_{N_2O} (Fig. 1 a, d, e) and in one of the stored soils for DC_{N_2O} (99.6% for MG (Fig. 1 a) after its maximum cumulative amount (between 1 and 3 day of incubation). The percent of N_2O consumption on 7 day of incubation ranged from 0 (HP) to 89.9% (HPh) for aD_{N_2O} in the fresh soils and from 0 (EC, HPh and DF) to 32.6 (HP) for DC_{N_2O} in the fresh soils (Table 2 and 3).

There was a significant positive correlation between the cumulative N_2O release and reduced NO_3^- ($R = 0.94$, $p < 0.01$ and $R = 0.93$, $p < 0.001$ for stored and fresh soils enriched with NO_3^-, respectively).

T a b l e 2. NO_3^- reduction, CO_2 release, N_2O release and consumption in stored (S) and fresh (F) control soils

Soil units (No.)	Length of soils storage	NO_3^-		N_2O – cumulative		CO_2 – cumulative	S/F ratio
		Reduction	Denitrified to N_2O	Maximum release	Consumption	Maximum release	
		%		mg kg^{-1}	%	mg kg^{-1}	
Mollic Gleysols (145)	S	0	0	0	0	529.0	5.1
	F	93.5	0	0	0	103.8	
Eutrick Cambisols (553)	S	46.4	0	0	0	142.4	0.9
	F	100	100	0.38	47.0	155.2	
Haplic Phaeozems (601)	S	54.8	0	0	0	138.6	1.0
	F	40.7	100	2.84	89.9	137.1	
Haplic Podzols (633)	S	100	0	0	0	126.6	2.5
	F	50.0	100	1.45	0	50.8	
Rendzic Leptosols (724)	S	100	0	0	0	80.5	0.5
	F	90.1	0	0	0	160.1	
Distric Fluvisols (941)	S	100	0	0	0	179.9	1.0
	F	62.7	100	2.09	58.0	187.6	

S/F CO_2 ratio – the ratio of CO_2 release from stored to fresh soils.

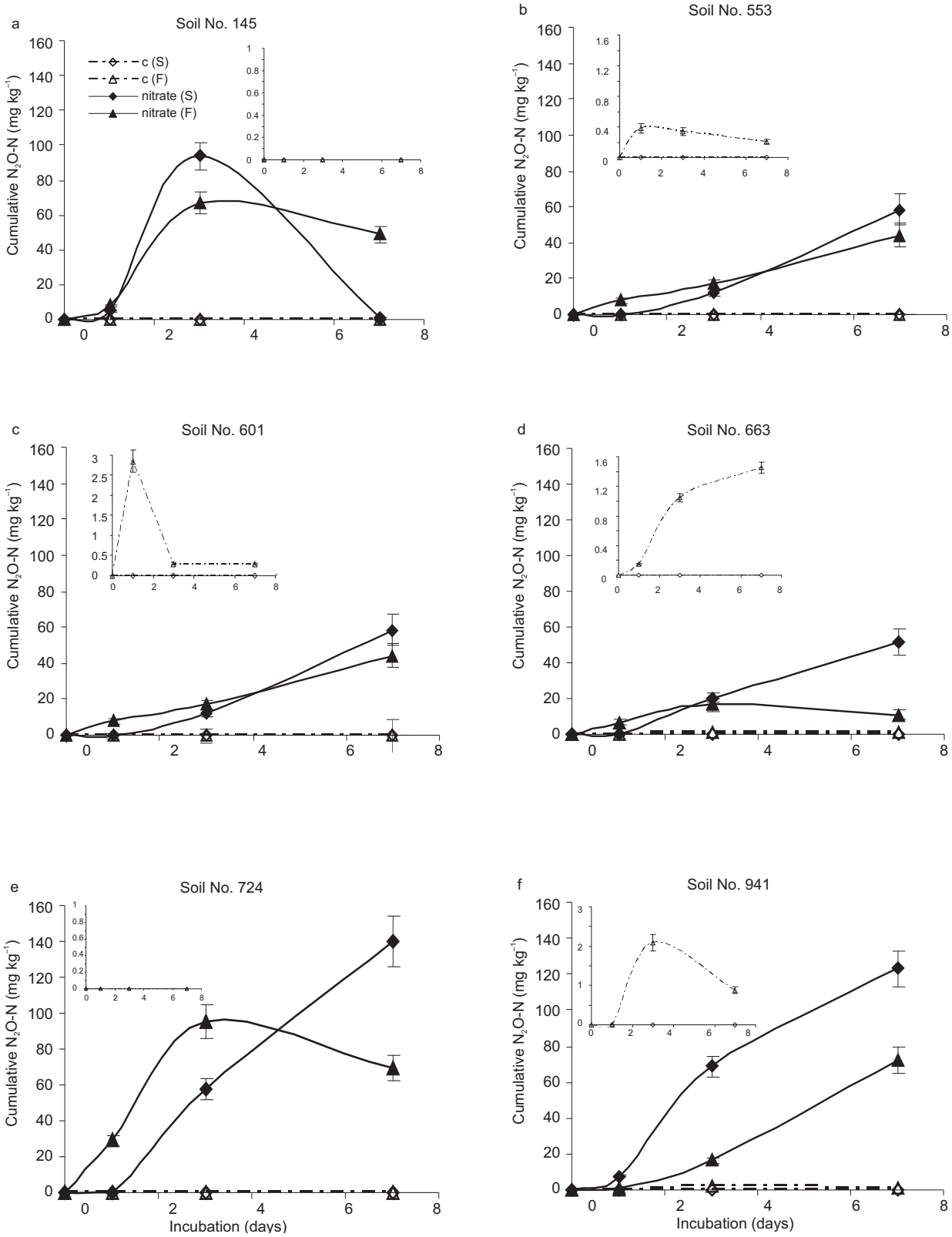

Fig. 1. Cumulative N_2O-N release and consumption from: a – Mollic Gleysols (No. 145), b – Eutric Cambisols (No. 553), c – Haplic Phaeozems (No. 601), d – Haplic Podzols (No. 633), e – Rendzic Leptosols (No. 724), and f – Distric Fluvisols (No. 941) as a function of incubation time (inserts show the curves of the control soils).

T a b l e 3. NO_3^- reduction, CO_2 release, N_2O release and consumption in stored (S) and resampled soils (F) enriched with NO_3^- soil

Soil units (No.)	Length of soils storage	NO_3^-		N_2O – cumulative			CO_2 – cumulative	
		Reduction	Denitrified to N_2O	Maximum release	S/F ratio	Consumption	Maximum release	S/F ratio
		%		mg kg^{-1}		%	mg kg^{-1}	
Mollic Gleysols (145)	S	98.1	31.1	93.6	1.4	99.2	557.5	6.1
	F	98.1	21.9	66.9		27.3	91.2	
Eutrick Cambisols (553)	S	57.3	19.5	59.6	1.3	0	117.0	0.9
	F	51.6	14.7	44.2		0	133,6	
Haplic Phaeozems (601)	S	56.7	16.2	48.8	1.3	0	138.5	1.2
	F	55.6	12.6	37.8		0	115.8	
Haplic Podzols (633)	S	31.1	16.9	51.5	3.2	0	114.9	1.5
	F	49.0	5.4	16.32		32.6	75.1	
Rendzic Leptosols (724)	S	91.9	46.1	139.6	1.5	0	58.7	0.4
	F	97.8	31.4	95.5		27.2	152.3	
Distric Fluvisols (941)	S	64.2	40.5	122.9	1.7	0	200.5	1.0
	F	74.3	23.9	72.2		0	207.5	

S/F N_2O ratio – the ratio of N_2O release from stored to fresh soils, S/F CO_2 ratio – the ratio of CO_2 release from stored to fresh soils.

There was also a close correlation between the cumulative release of N_2O and C_{org} content ($R = 0.93$, $p < 0.01$; and $R = 0.92$, $p < 0.01$ for the stored and fresh soils enriched with NO_3^-, respectively).

Differences in the cumulative CO_2 release between the stored and fresh soils were found during the entire incubation period. The differences were statistically significant except for EC No. 553, HPh No. 601, and DF No. 941 for the control soils. In enriched NO_3^-, no statistically significant differences were found only in two soils (EC No. 553, and DF No. 941). The cumulative CO_2 release from control soils ranged from 80.5 (RL) to 529.0 (MG) mg C kg^{-1} and from 50.8 (HP) to 187.6 (DF) mg C kg^{-1} for the stored and fresh soils, respectively.

The NO_3^- addition to soils slightly changed the respiration activity of the soils compared with the control soils and the amount of released CO_2 ranged from 58.7 (RP) to 557.5 (MG) mg C kg^{-1} for the stored soils and from 75.1 (HP) to 207.5 (DF) mg C kg^{-1} for the fresh soils.

Regression analysis for respiration of soils showed a significant positive relationship between the cumulative CO_2 release and C_{org} ($R = 0.92$, $p < 0.01$) for the control stored soils enriched with NO_3^-. There was no significant relationship for the fresh soil samples.

DISCUSSION

Understanding how dry/wet cycles affect C and N transformations is important in predicting soil organic matter (SOM) dynamics, determining the effects of climate change on greenhouse gas emissions (principally CO_2 and N_2O) from soils (Wu and Brookes, 2005).

However, prolonged dry soil conditions followed by flooding of the soil may cause much larger changes in the dynamics of C and N than very short-term soil dry/wet cycles. A change in the availability of C alters the N transformation associated with biogenic gas CO_2 and N_2O production, especially under conditions of hypoxia. Determination of the aD_{N_2O} and DC_{N_2O} allowed answering two main questions about the influence of long-term storage compared to fresh air-dried soils on:

– actual denitrification (aD_{N_2O}) leading to N_2O formation with a natural content of N and C in flooded soil,
– the capacity of denitrification (DC_{N_2O}) to N_2O formation where the process is not limited by deficiency of NO_3^- after flooding from the standpoint of environmental protection.

This problem increases with the problem of global warming and the prolonged period of drought, followed by heavy rain and flooding. The studied soils showed very low

aD_{N_2O} irrespective of the length of storage after flooding. There was no N_2O release from the long stored soils, but the fresh soils had also very low aD_{N_2O}. In two soils (MG No. 145 and RL No. 724), N_2O was not released from the fresh soils. In the rest of the fresh soils, released N_2O did not exceed 3 mg N_2O-N kg^{-1}. It should be emphasized that there was no emission of N_2O from the stored soil and two fresh soils in the case of the native NO_3^- content. Probably, the low NO_3^- content in these soils was below the threshold concentration for N_2O production. Włodarczyk *et al*. (2004) investigated NO_3^- stability in loess soils under anaerobic conditions and found no denitrification below 25 mg NO_3^--N kg^{-1}. In turn, Senbayrama *et al*. (2012) found that high respiration in treatments with maize straw and sucrose resulted in a transient peak in N_2O emission, declining rapidly towards zero once the NO_3^- concentrations dropped below 20 mg NO_3^--N kg^{-1} dry soil. Therefore, the low content of NO_3^- was a factor clearly limiting the denitrification process, in particular to the form of N_2O. The results led to the conclusion that these conditions were not conducive to growth of heterotrophic bacteria such as denitrifiers. On the other hand, it cannot be excluded that the NO_3^- was denitrified entirely to N_2. Generally, by comparing the NO_3^- content in stored and fresh soils, it can be expected that the N_2O relese will either fall or rise, due to the length of storage of the soil and type of soils.

While comparing the amount of N_2O released from EC No. 533, HPh No. 601, and HP No. 633 of the recently sampled soils with the amount of their native NO_3^- amount, it can be suspected that part of N_2O was derived from the process of nitrification, because the amount of N_2O-N exceeded the amount of N contained in NO_3^-. These soils provide evidence for low nitrification activity under a low natural NO_3^- content. In flooded soils, there is a thin oxygenated layer at the interface between air and water, which may occur at the same time as the processes of nitrification and denitrification (Yu *et al*., 2006). Nitrification and denitrification are the major sources of N_2O emissions from soils (Zhang *et al*., 2011)

In the case of NO_3^- addition, the amount of released N_2O (PD_{N_2O}) significantly increased and depended on the length of storage of soils and type of soils. Much higher N_2O release was observed from the stored soils compared with the fresh samples. The ratio of N_2O released from the stored to fresh soils was always higher than one (S/F N_2O ratio) and ranged from 1.3 (EC and HPh) to 3.2 (HP). It can be expected that the NO_3^- addition to the flooded soil after a long drought led to the development of active denitrifying bacteria under easily accessible C and N. Harrison-Kirk *et al*. (2013) studied CO_2 and N_2O production during sequential dry/wet cycles at laboratory incubation. Following rewetting, the very dry and moderately dry soils produced a short-term C mineralization flush that was, on average, 30 and 15% greater, respectively, than in wet (field capacity) soils. On average, the total N_2O emissions from dry/wet treatments imposed on silt loam and clay loam soils were 33% and 270% greater, respectively, than at the field capacity moisture content, although the effect varied greatly depending on the SOC content. NO_3^- is very mobile in soil; it may rapidly diffuse into soil compartments with low oxygen contents where it may promote biological denitrification. Thus, next to degradable carbon compounds, the NO_3^- concentration in the soil solution is another major factor limiting denitrification (Senbayrama *et al*., 2012).

The substantially higher activity of PD_{N_2O} in the fresh soils than in the stored soils observed after the first three days of incubation in the investigated soils demonstrates a higher adaptive ability of denitrifying bacteria to changes in the availability of C and oxygen (O_2) in the headspace and soil suspension. A rapid change was observed in the respiration type from aerobic to NO_3^- respiration where NO_3^- was used as an alternative electron acceptor instead of oxygen. The results showed that the denitrifying microorganisms in the soil stored in an air-dry state for longer periods need certain time to adapt to the changed conditions of soil moisture. The highest denitrifying activity seen in the stored soil (RL No. 724) was accompanied by the highest decrease in the Eh value (+197 mV), compared with the rest of the stored soils at the beginning of incubation. Considering the influence of the length of storage of the soil and type of soils on the aeration status, it was found that the long storage time under dry conditions caused a greater decrease in the Eh value compared with the fresh soils. The average Eh value for the stored soils was +253 mV and for the fresh ones +303 mV at the beginning of incubation. This means that the Eh value in the stored soils dropped by 50 mV. The biggest difference in Eh between the stored and fresh soils was observed in HPh No. 633 (158 mV) while the smallest one in HP No. 633 (36 mV). The lower Eh value for the stored soils might be due to increa sed availability of C in these soils. Increased availability of C caused higher activity of microorganisms, which led to more rapid oxygen consumption and provided better conditions for denitrification. DeAngelis *et al*. (2010) investigated the acclimation and adaptation of microbial communities to fluctuating environmental conditions. Rapid acclimation to changing conditions suggests the presence of populations with existing physiological capacities for energy generation under a suitable range of redox potential conditions. Soil redox plays a key role in regulating biogeochemical transformations in terrestrial ecosystems (Włodarczyk *et al.*, 2005). The major factors influencing denitrification are oxidized nitrogen compounds (NO_3^- and N_2O), redox potential or O_2 availability, easily degradable carbon, temperature, and soil pH (Peterson *et al*., 2013). Yu *et al*. (2001) found that the N_2O emissions were regulated within a narrow redox potential range of +120 to +250 mV due to the balance of N_2O production and its further reduction to N_2. Therefore, after soil submergence, N_2O is usually produced

first; however, it is absorbed in the soil after its redox potential decreases (Włodarczyk *et al*., 2005). Each microorganism type is adapted to specific Eh conditions and is characterized by its ability to develop within a wider or a narrower Eh-range (Husson, 2013). Generally, much higher N_2O release was observed from the stored than from fresh soils. Furthermore, the cumulative N_2O release from the stored soils was correlated with the reduced NO_3^- and a considerably stronger correlation was found in the case of the fresh soils.

In the present study, there was consumption of released headspace-N_2O, after its maximum cumulative amount, between 1 and 3 day of incubation in some soils. The highest N_2O consumption for aD_{N_2O} was found in HPh No. 601 (89.9%) and for DC_{N_2O} in HP No. 633 (32.6%) in the fresh soil. There was only one case of N_2O consumption in the stored soils (MG No 145 99.2%). These results indicate that some fresh soils reached maximum release of N_2O much faster compared with the stored soils under comparable conditions of incubation. The difference is due to the long period of drought that affects C_{org} bioavailability and the time required for reviving denitrifiers. Włodarczyk *et al*. (2005) reported that, under certain conditions, soils are able to consume N_2O. The boundary value of redox potential for the emission of N_2O was about +250 mV and about 200 mV for consumption thereof under hypoxic conditions. Pastorelli *et al*. (2011) found that the influx of C sources and energy into the oligotrophic soil system is a major driving force in biogeochemical cycles. There are several lines of evidence to support the proposal that severe drought augments dissolved organic carbon (DOC) production and thus controls the observed increases in DOC concentration (Worrall and Burt, 2008). There are some indications that the N_2O-reducing activity of soils is positively correlated with the ratio of available NO_3^- and available organic C in soils (Senbayrama *et al*., 2012).

The aD_{N_2O} and DC_{N_2O} results for the stored and fresh soils indicated that the low natural NO_3^- content was one of the most limiting factor in the process of N_2O release, more than the oxygenation status of the investigated soil because NO_3^- addition resulted in a marked increase in the release of N_2O compared with the non-amended soils. This means that NO_3^- and organic carbon were important factors limiting the denitrification process leading to N_2O release under the experimental conditions. Rivett *et al*. (2008) found that the critical limiting factors for denitrifying bacteria were oxygen and electron donor concentration and availability. Variability in other environmental conditions, such as the NO_3^- concentration, nutrient availability, pH, temperature, presence of toxins, and microbial acclimation appears to be less important, exerting only secondary effects on denitrification rates. In our opinion, there are three critical limiting factors influencing the activity of denitrifying bacteria: concentration and availability of electron donors, anaerobic conditions, and NO_3^- content. In our experiment, NO_3^- (natural and added) very clearly affected N_2O release and depended on the length of storage of soils and type of soils. The studied soils have a very diverse ability to reduce NO_3^-. The NO_3^- reducing was the highest in MG No. 145 (98.1 and 98.1%) for the stored and fresh soils, respectively, and the lowest in HP No. 633 (31.1 and 49.0%) for the stored and fresh soils, respectively. Comparing the effect of the length of the storage of the soils on NO_3^- -reducing activity of soils, it was found that long storage in the case of two soils (EC No. 553 and HPh No. 601) slightly affected the increase in the NO3--reducing activity. In three soils (HP No. 633, RL No. 724 and DF No. 941), this activity significantly decreased, which might be connected with their individual biogeochemistry characteristics. In the case of MG (No. 145), the reducing activity was comparable. Generally, it can be concluded that long storage slightly decreased NO_3^- reduction activity. Dodla *et al*. (2008) found that the capacity of wetland to remove NO_3^- through denitrification was controlled by its physicochemical and biological characteristics.

With regards to the effects of the length of the soil storage on NO_3^--reduction activity to N_2O formation, it was found that long storage of soils increased NO_3^- reduction activity to N_2O formation. We can expect that soils subjected to prolonged drought will produce more N_2O than soils exposed to dry conditions for shorter times.

The cumulative N_2O release from the stored soils was correlated with the reduced NO_3^- and a substantially stronger correlation was found in the case of the fresh soils. There was also a close correlation between the cumulative N_2O released and C_{org} content for the stored and fresh soils enriched with NO_3^- (DC_{N_2O}). There was no correlation between the cumulative N_2O released and CO_2 released for the stored and fresh soils for both aD_{N_2O} and DC_{N_2O}. Under conditions of hypoxia, CO_2 came from both the process of aerobic respiration and anaerobic (NO_3^-) respiration. The activity of both processes may depend inter alia on the oxygenation status of the soil and the availability of oxygen, which is depended on the intensity of both respiration processes, especially in short-term incubation (7 days). Thus, there was no clear relationship between N_2O and CO_2 release. Furthermore, soil microorganisms maintain their microbiological activity for many years from the time of sampling of agricultural soils. This feature in bacterial strains indicates some capacity for memory (Włodarczyk, 2000). Harrison-Kirk *et al*. (2013) have reported that moisture stress history can affect the size of the CO_2 flush following rewetting of dry soil.

The SOM content and texture are important factors affecting carbon (C) and nitrogen (N) mineralization under constant soil moisture but their effects on organic matter mineralization and associated biogenic gas like CO_2 and N_2O production during dry/wet cycles is poorly studied (Harrison-Kirk *et al*., 2013). The tested soil showed a highly

differentiated respiration activity measured as the amount of CO_2 released depending on the length of storage and type of soils under flooding after drought. While comparing the amount of CO_2 released from the stored and fresh soils, it can be assumed that respiration activity was comparable in three soils (EC No. 553, HPh. No. 601, and DF No. 941). In one soil (RL) respiration activity was about half lower in the stored soils while, in two soils (MG and HP), respiration was significantly higher in the stored soils than in the fresh ones. Probably during the prolonged drought in these soils, there was a steady decomposition of organic matter (OM) and accumulation of easily available OM due to the minimum soil moisture during storage and, consequently, deceleration of vital functions of microorganisms. Wetting of dry soil typically results in a flush of C and N mineralization, with elevated rates of CO_2 production persisting for up to 2 weeks following wetting (Beare *et al*., 2009). Worrall and Burt (2008) studied the effect of severe drought on the biogeochemistry of dissolved organic carbon (DOC) production. This study derives five different drought severity indices and compares these to the observed increase in DOC over a 4-year period after each severe drought. Xiang *et al*. (2008) found that drying and rewetting led to a cascade of responses (soluble C release, biomass growth, and enhanced activity) that mobilized and metabolized otherwise unavailable soil carbon, particularly in subsurface soils.

The CO_2 release was the highest in MG No. 145 (529 mg CO_2-C kg^{-1}) for the stored soils and it was 5 times greater than for the same fresh soil (103.8 mg CO_2-C kg^{-1}) under control-flooded conditions. It has long been recognized that rewetting a dry soil causes a pulse of respiration – the 'Birch Effect' (Birch, 1958). More than twice as much CO_2-C was evolved from the long-term stored soils than from the freshly sampled ones (De Nobili *et al*., 2006). The lowest CO_2 release was found in MP No. 633 (40.5 mg CO_2-C kg^{-1}) for the stored soils. The highest CO_2 release was observed in DF No. 941 (187.6 mg CO_2-C kg^{-1}) and the lowest one in HP No. 633 (50.8 mg CO_2-C kg^{-1}) for the fresh soils. Harrison-Kirk *et al*. (2013) studied CO_2 production during sequential dry/wet cycles at laboratory incubation. Following rewetting, the very dry and moderately dry soils produced a short-term C mineralization flush that was, on average, 30% and 15% greater, respectively, than in wet (field capacity) soils. On average, the total CO_2 release from the control soils was slightly higher (199.5 and 132.4 mg CO_2-C kg^{-1} in the stored and fresh soils, respectively) compared with the NO_3^- enriched soils (197.9 and 129.2 mg CO_2-C kg^{-1} in the stored and fresh soils, respectively).

A differentiated rate of respiration activity of the soils, depending on the length of storage and the type of soil, should be noted. A substantially higher rate of CO_2 release at the beginning of incubation was shown in the fresh soils. In most of the fresh soils, the highest rate of CO_2 release was reported after the first day of incubation (EC, HPh, HP, and RL); the other soils (MG and DF) needed three days to reach the highest rate of CO_2 release, while among the stored soils, only two soils (EC and HPh) needed one day to be fully active. The other soils needed three or seven days to adapt to the new conditions of soil moisture. This demonstrates that microorganisms (denitrifying bacteria and other microorganisms) in fresh soils are characterized by a higher adaptive ability to changes in the availability of C and O_2 in the headspace and soil suspension and the faster change in the type of respiration from aerobic to NO_3^- respiration. In turn, in the soil stored in an air-dry state much longer, they need more time to adapt to the changed aeration status of the stored soils. Similarly, in the incubation experiments aimed at analysis the methanogenic potential of long stored soils, the methanogenesis started with time lag (Brzezińska *et al*., 2014).

Comparing the C_{org} content of the stored and fresh soils, we can recognise falling trends due to the storage of the soil. The average C_{org} content for the investigated soils was 1.78 and 2.38 for the stored and fresh soils, respectively.

Generally, the cumulative CO_2 release from the stored soils was correlated with the C_{org}, but there was no correlation in the case of the fresh soils.

CONCLUSIONS

1. The investigations answered two main questions about the influence of long-term storage on the actual denitrification leading to N_2O formation with a natural content of N in the soil and C after flooding and on the denitrification capacity leading to N_2O formation when the process is not limited by deficiency of NO_3 after flooding from the standpoint of environmental protection.

2. The investigated soils showed very low actual denitrification to N_2O irrespectively of the length of storage under flooding. There was no N_2O release from the stored soils but the fresh soils had very low actual denitrification to N_2O.

3. The low native content of NO_3^- was a factor clearly limiting the denitrification process, in particular to the form of N_2O.

4. The amount of N_2O released (denitrification capacity to N_2O) significantly increased after NO_3^- addition and depended on the length of soil storage and type of soils, and it was much higher in the stored soils. The N_2O release was highest in Rendzic Leptosols and Distric Fluvisols for the stored and fresh soils. The lowest N_2O release was found in Haplic Phaeozems for the stored soil and in Haplic Podzols for the fresh soil. Some soils showed the capability of N_2O consumption. The cumulative N_2O release from the stored soils was correlated with the reduced NO_3^- and a remarkably stronger correlation was found in the case of the fresh soils. There was also a close correlation between the cumulative N_2O release and organic C content for the stored and fresh soils enriched with NO_3^- (denitrification capacity to N_2O).

6. The studied soils were characterized by a very diverse ability to reduce NO_3^-. The NO_3^- reduction was the highest in Mollic Gleysols for both the stored and fresh soils and the

lowest one was reported for Haplic Podzols for the stored and fresh soils. The long storage slightly decreased NO_3^- reduction activity and N_2O formation.

7. The tested soil showed a very variable respiration activity measured as the amount of CO_2 released during incubation depending on the length of storage and type of soils under flooding after drought. The respiration activity of soils was comparable in three soils (Eutric Cambisols, Haplic Phaeozems, and Distric Fluvisols) in the stored and fresh soils. In two soils (Mollic Gleysols and Haplic Podzols), respiration was significantly higher in the stored soils than in the fresh ones, while in one soil (Rendzic Leptosols) the respiration activity was about half lower in the stored soils. The CO_2 release was the highest in Mollic Gleysols for the stored soil and 5 times greater than in the same fresh soil. The lowest CO_2 release was found in Haplic Phaeozems for the stored soil. The highest CO_2 release for the fresh soils was observed in Distric Fluvisols and the lowest one in Haplic Podzols.

8. Comparing the organic C content in stored and fresh soils, we can recognize falling trends due to the storage of the soils. The cumulative CO_2 release from the stored soils was correlated with the organic C, but there was no correlation in the case of the fresh soils.

9. The long-time soil storage under air-dry conditions caused a greater decrease in the Eh value compared with the fresh but air-dry soils.

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