Redox potential, nitrate content and pH in flooded Eutric Cambisol during nitrate reduction

T. Włodarczyk, P. Szarlip, M. Brzezińska, U. Kotowska

Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland

Abstract: Topsoils from 16 arable Cambisols developed from sand, loam and silt were used to study soil ability to nitrate reduction under flooding conditions. The strongest drop of redox potential (Eh) was observed during the first day of soil flooding. Decreasing tendency in pH values was observed of alkaline and neutral soils, and an increase in pH of acid and strongly acid soils, accompanied by a fall in Eh values. Redox potential was negatively correlated with the pH values (R^2 = 0.3041; p < 0.001). The fall of NO_3^- varied from 20 to 100% depending on the type of soil and on the time of incubation. With a decrease of nitrate content within the range from 100 to 10 mg NO_3^--N/kg, the value of redox potential decreased from 250 to 190 mV. The highest reduction of nitrates coincided with Eh values within a narrow range between 200 and 210 mV. Statistical analysis of redox potential in the function of the content of nitrates showed a curvilinear relation (R^2 = 0.3823; p < 0.001).

Keywords: soil; redox potential; denitrification; pH

Dissimilative reduction is the process through which some microorganisms use energy generated by electron transport from an organic source to nitrate or to a more reduced nitrogen oxide under anaerobic condition. This metabolic reduction uses cytochromes mostly as electron donors and occurs with liberation of di-nitrogen as the final product. However, some bacteria lack N_2O reductase, and so produce this gas as a terminal product, or lack nitrite reductase, yielding nitrite as the end product (Ingraham 1981).

When dissimilative reduction produces gaseous di-nitrogen or nitrous oxide compounds, the process is called denitrification. Biological denitrification is a respiratory process in which N-oxides (electron acceptors) are enzymatically reduced under anaerobic conditions to nitrous oxide and di-nitrogen for ATP production by organisms that normally use O_2 for respiration (Betlach & Tiedje 1981). However, since reduction, through the metabolic pathway of cytochromes, results in some cases in the production of ammonia or nitrite, some authors prefer the more general name of nitrate respiration for the process. In other cases, the metabolic pathways do not involve membrane-bound enzymes, cytochromes, or electron transport phosphorylations, and the main product is ammonia. This process is called fermentative nitrate reduction (Fenchel & Blackburn 1979).

Nitrous oxide can be produced by denitrification or dissimilatory NO_3^- reduction to NH_4^+ (DNRA), while di-nitrogen is produced only by denitrification. The two processes of NO_3^- reduction are carried out by different bacterial groups. Nitrite is a common intermediate in both processes, high pH favouring NO_2^- accumulation and DNRA. During denitrification the mol fraction of N_2O decreases as pH increases (Stevens *et al.* 1998). Nitrate limitation favours DNRA, while C limitation favours denitrification (Tiedje *et al.* 1982). When NO_3^- is not limiting, competition for C will determine which of the processes for NO_3^- reduction is favoured (Tiedje 1988). Under conditions where NO_3^- concentrations do not limit potential denitrification, the overall rates of both denitrification and nitrification decline with decreasing pH from optima of about pH 7.5 (Müller *et al.* 1980). Such declines in pH occur in particular in urine affected soil.

Just as pH expresses the concentration of H^+ in solution, redox potential is used by chemists to express the tendency of an environment to receive or supply electrons. Aerobic environments usually have a high redox potential because O_2 is available as an electron acceptor. Changes in soil redox potential are related to changes in oxygen levels (Gliński & Stępniewski 1985).

The main purpose of this study was to estimate the potential ability of soils to reduce nitrates where NO_3^- content was a non-limiting factor (100 mg NO_3^--N per kg corresponding to 300 kg NO_3^--N per ha in 20 cm top soil layer) and the relationship between aeration status of the soil reflected by redox potential and nitrate concentration and pH value. The experiments were designed to better recognize the expected interrelations between these factors.

MATERIALS AND METHODS

As test material for the estimation of soil ability to reduce nitrates, topsoils (0–30 cm) from 16 arable Cambisols from Poland were used in the study. The Cambisols were developed from different parent materials such as sand, loam and silt. The soils showed also a high variation in the content of organic matter of endogenous nitrate and pH (Table 1).

The soil samples (5 g of air-dry mass) were placed in 38 cm^3 glass vessels (standard units) and treated with KNO_3 (100 mg NO_3^--N/kg) and distilled water to reach a soil/water ratio of 1:1 (w/w); 0.5 ml of solution (containing 1g NO_3^--N/kg) and distilled water (4.5 ml). The vessels containing suspensions were tightly sealed with rubber stoppers and incubated in an atmosphere diluted with gaseous nitrogen in order to simulate the lower oxygen content in soil air as compared to one. The average concentration of O_2 in the vessels at the start of the incubation was about 10% ± 0.5 (replaced by N_2).

The soils were incubated at 20°C and monitored for O_2 after 1, 2, 3, 5, 7, 10, 14, 21, 28, 32 and 34 days by sampling the air in the vessels. The content of O_2 was determined by gas chromatography, using a Shimadzu GC-14 (Japan) apparatus fitted with a thermal conductivity detector, at 60°C. Gas samples were analysed at 40°C with the use of column packed with a molecular sieve 5A, with He as a carrier gas flowing at a rate of 40 ml/min. Incubation vessels were replicated three times. A set of 30 samples was prepared for each soil. These vessels were opened in three replications after 1, 2, 3, 5, 7, 10, 14, 21, 28, 32 and 34 days of the incubation and used to measure pH, redox potential (Eh) as described by Malicki and Walczak (1983) and Gliński and Stępniewski (1985) and nitrate content. Determination of nitrate in the material studied was performed with the help of the FIA-Star 5010 flow-through spectrophotometric analyser made by Foss Tecator.

Table 1. Basic properties of the soils

Soil No.	Type of soils	Sampling depth (cm)	Particle size distribution (%)			C_{org} (%)	NO_3-N_0 (mg/kg)
			> 0.05 mm	0.05–0.002 mm	< 0.002 mm		
39	soils developed from sand	5–10	95	5	0	0.67	4.53
224		15–25	85	12	3	0.32	6.47
342		10–20	74	21	5	0.44	29.94
434		0–30	81	13	6	0.74	18.49
543		10–25	69	28	3	0.88	9.78
772		0–15	84	15	1	0.49	13.66
110	soils developed from loam	10–15	54	33	13	0.67	10.25
328		0–24	63	23	14	0.77	9.46
351		10–25	71	21	8	0.57	17.88
922		5–20	55	41	4	1.89	5.37
951		5–15	32	60	8	2.85	65.76
113	soils developed from silt	15–20	78	22	0	0.92	58.2
147		15–20	16	49	35	1.95	9.97
672		10–20	28	65	7	0.94	67.42
947		5–20	52	43	5	2.31	32.93
984		10–20	27	67	6	1.24	7.87

The mean value of dependent variables (y) for the determined range of independent variables (x) was used for statistical processing of the data (Statgraphics 5.0). The analysis of regression was used in the next statistical processing of the data. The exponential ($y = e^{a + bx}$) and logarithmic ($y = a\ln x + b$) functions were used in the regression analysis and the model with the highest R^2 was elected as the best fit to the experimental data.

RESULTS AND DISCUSSION

Characterization of studied soils

The studies were conducted on 16 Cambisols selected from the bank of soil samples established at the Institute of Agrophysics, PAS, in Lublin, Poland.

Soils selected for the study originated from Ap horizon with a depth of 0–30 cm (Table 1) and are differed in taxonomical and textural soil units, representing sands, loams and silts.

Air dried soil samples were stored at room temperature within the range from 10 to 20°C.

Principal characteristics of the soils studied are presented in Tables 1 and 2.

The soil material involved in the studies is characterized by varied content of organic matter – from 0.32 to 2.85%, by a wide range of pH values – from 4.14 to 7.53, and by a broad spectrum of content of nitrates – from 4.53 to 67.42 mg NO_3^--N/kg. The soils samples differed in particle size distribution, with sand fraction content ranging from 16 to 95% and clay content ranging from 0 to 35%. Therefore, the soils selected for the study meet the requirement of being representative for Cambisols in Poland.

Dynamics of redox potential changes in the soils

Redox potential values determined after two hours of flooding of soil samples with distilled water with an addition of nitrate (Eh_{O+NO3}), in an amount equivalent to that in samples subjected to incubation, showed a certain variation (Table 2). Among the soils tested, approximately 50% showed Eh_{O+NO3} above 300 mV (within the range from +302 to +332 mV). The initial value of Eh determined in water was similar to that obtained in the soils with the addition of nitrates and varied from +302 to +321 mV. The values of Eh_{O+NO3} of the remaining soils did not drop below +200 mV and varied from +220 to +298 mV, analogous analysis of Eh in water gave values from +229 to +295 mV.

Table 2. Physico-chemical properties of the soils

Type of soils	Soil No.	pH_0#	pH_{final}	Eh_0# (mV)	Eh_{0+NO3}# (mV)	Eh_{final}
Soils developed from sand	39	4.64	4.9	295	292	218
	224	6.32	6.43	256	238	203
	342	7.07	7.5	290	268	204
	434	4.45	5.23	321	331	219
	543	5.99	5.48	295	298	204
	772	4.5	4.58	314	322	215
Soils developed from loam	110	4.38	4.91	284	296	206
	328	6.03	6.99	229	220	193
	351	7.53	6.47	286	286	189
	922	4.45	5.92	295	248	193
	951	4.14	5.64	316	315	204
Soils developed from silt	113	4.5	5.07	313	332	207
	147	6.66	7.38	290	291	105
	672	4.85	5.53	303	303	213
	947	5.29	6.8	302	302	178
	984	4.87	6.49	312	311	172

#the data from the beginning of incubation

Anaerobic conditions caused by soil flooding with nitrate solution and incubation at 20°C for a period longer than 21 days caused a drop in redox potential of the studied soils by 27 to 186 mV (Table 3).

Soils developed from sand and loams showed a similar drop in Eh values, by 32–112 mV and 27–111 mV, respectively, while soils developed from silts were characterized by the greatest drop in Eh values – by 90 to 186 mV (Table 3).

Analysing the rate of decrease of Eh values, caused by the state of anaerobiosis, it was found that the strongest drop in the redox potential took place during the first 24 hours of the experiment, in all the soils studied (Figure 1a,b,c). Subsequent days of the experiment brought only a slight decrease in the values of Eh or a stabilization of the potential, with the exception of soil No. 147 developed from clay, where the value of Eh decreased systematically throughout the period of the experiment and reached the level of 105 mV. Among the soils studied, in three soils developed from loams and in three of silt origin a decrease was observed in the value of redox potential below the level of +200 mV.

Redox potential versus pH of soil

Changes in redox potential in soils caused concurrent changes in the pH value of the soil environment, related with the participation of H^+ ions in oxygen reduction reactions (Ponnamperuma *et al.* 1966; Bohn 1970; Haavisto 1974; Buresh & Patrick 1978).

Table 3. Nitrate consumption and changes in the pH and Eh value at the end of incubation

Type of soils	Soil No.	NO_3^- consumption (%)	ΔEh# (mV)	ΔpH#
Soils developed from sand	39	36.5	–74	+0.21
	224	22.1	–32	–0.90
	342	59.4	–64	+0.12
	434	25	–112	+0.98
	543	86.4	–94	0
	772	31.1	–107	+0.60
Soils developed from loam	110	67.8	–90	–0.19
	328	100	–27	+0.14
	351	100	–103	–1.10
	922	100	–55	+2.09
	951	100	–111	+2.00
Soils developed from silt	113	20.2	–115	+0.12
	147	100	–186	+0.68
	672	60.3	–90	+0.85
	947	100	–124	+1.43
	984	100	–139	+1.78

#the differences between the beginning and the end of the incubation

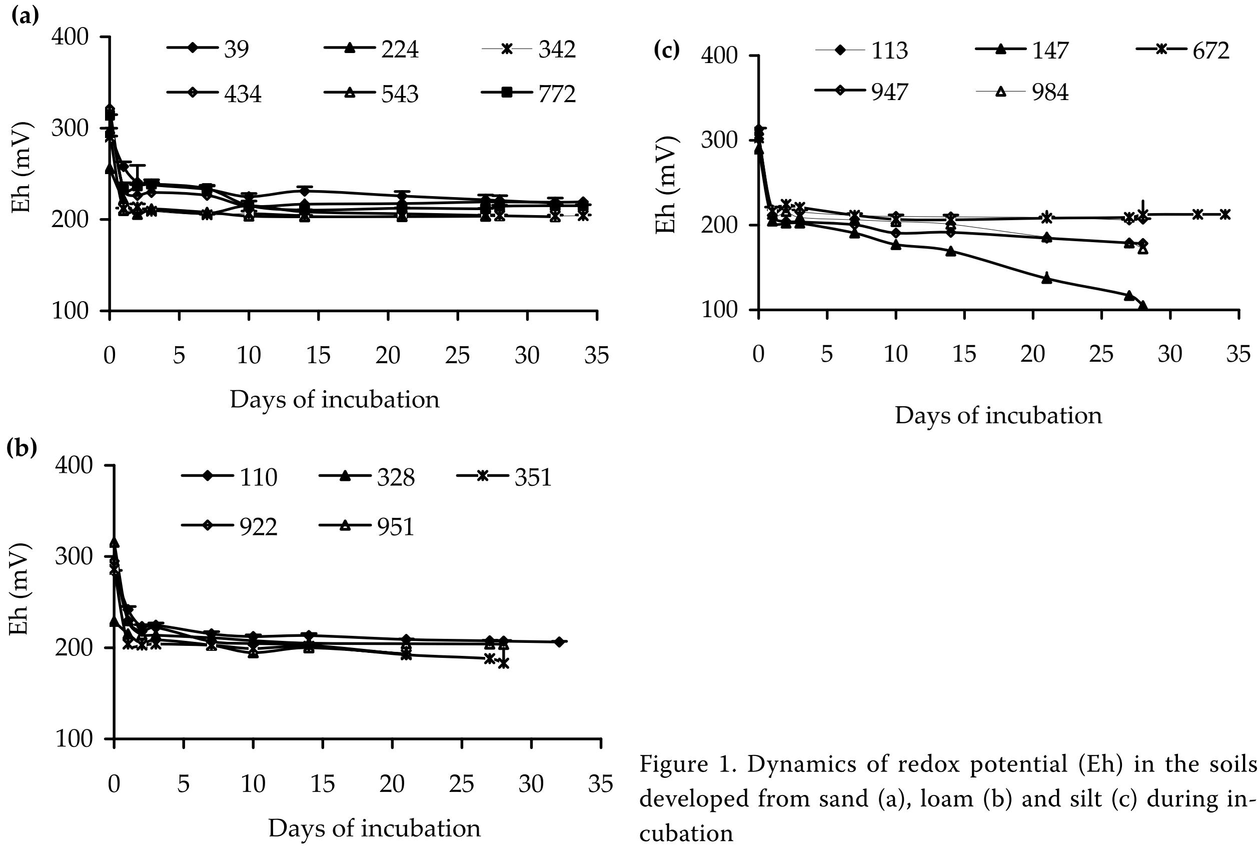

Figure 1. Dynamics of redox potential (Eh) in the soils developed from sand (a), loam (b) and silt (c) during incubation

Analysis of regression of redox potential of the studied Cambisols showed a curvilinear ($y = a\ln(x) + b$), negatively correlated relation with the pH value of the soils ($R^2 = 0.3041$, $p < 0.001$). The range of variation of the reaction of the selected soils permitted the relation to be analysed over a very broad range of pH values, from very strongly acid soils to alkaline soils. Figure 2 presents Eh values of the soils as a function of pH during the period of 34-day incubation. Figure 3, on the other hand, presents the same relation for the initial condition of the soils. The relation is described by an exponential function ($y = e^{a + bx}$), whith R^2=0.3642 ($p < 0.01$). The results obtained remain in conformance with the theoretical Eh which is negatively correlated with pH (Segel 1976).

Studies by Gliński and Stępniewska (1986) show that pH *in situ* under natural conditions is a dynamic value, subject to continual changes, and dependent on the soil moisture and on the oxidation status of the soil.

As follows from Table 4, after 34 days of incubation, in the final phase of the experiment, there were no very strongly acid soils, while there was an

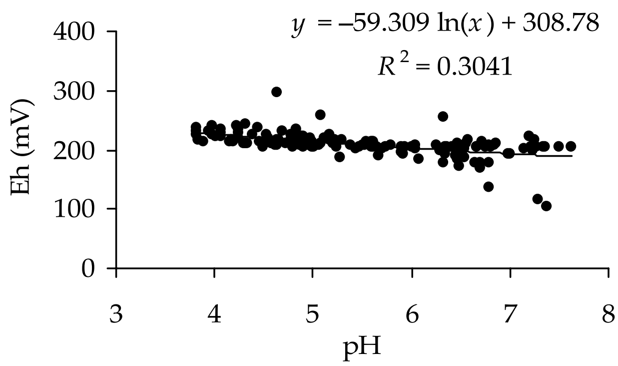

Figure 2. Redox potential (Eh) as a function of pH value in the soils during incubation

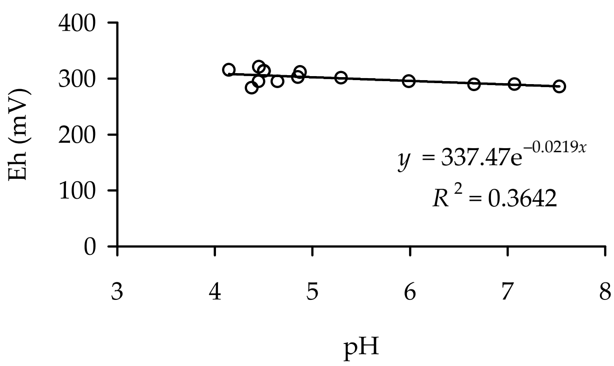

Figure 3. Initial redox potential (Eh) as a function of initial pH value in the soils

Tble 4. Changes in reaction of soils (pH in soil suspension) at the beginning and at the end of incubation

Soil reaction	Number of soil samples	
	before incubation	after incubation
Very acid soils (pH < 4.5)	4	0
Acid soils (pH 4.6–5.5)	6	6
Slightly acid soils (pH 5.5–6.5)	1	6
Neutral reaction soils (pH 6.6–7.2)	2	2
Alkaline reaction soils (pH > 7.2)	3	2

increase in the number of lightly acid soils and a decrease in the number of soils with alkaline reaction. Among the soil developed from sands the maximum increase in the value of pH was approximately one unit at a drop in Eh value by 112 mV (Table 3), while the maximum decrease in pH observed in an alkaline soil was also approximately 1 unit (No. 224), with a slight drop in redox potential – 32 mV (Table 3). The maximum drop in pH for soils developed from loams was observed in the case of an alkaline soil with initial pH of 7.53 – by about 1 unit at ΔEh = 103 mV (No. 351). Maximum increase in the value of pH was recorded in 2 very strongly acid soils – by about 2 units at ΔEh = 55 and 111 mV (No. 922 and No. 951, respectively), which caused that the soils shifted to the group of lightly acid soils. In soils developed from silts no decrease was observed in the value of pH. The increase of pH values (ΔpH) in those soils varied from +0.12 to +1.78 with simultaneous drop in Eh by 115 (No. 113) and 139 mV (No. 984), respectively (Table 3). Similar changes in acidity of studied soils, caused by changes in redox potential, were observed also by other authors. Stępniewska (1988), in her study on the redox properties of mineral soils of Poland, found that maximum changes in soil pH depended on the values at full oxidation and amounted to +1.6 of a unit for acid soils with pH 5 and to –0.5 of a unit in the case of soils with

Table 5. Dynamics of redox potential processes in the soils during first three days of incubation

Type of soils	Soil No.	ΔEh (mV)			ΔNO_3^- (mg/kg/d)			ΔO_2 (%)		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Soils developed from sand	39	34	17	3	0.11	1.58	4.83	1.38	1.28	1.35
	224	16	16	0	0	0.19	7.8	5.97	0.29	0.69
	342	56	0	5	16.01	24.99	2.1	3.51	0.52	3.51
	434	99	0	5	5.51	1.21	0.74	4.82	1.09	0.01
	543	88	0	0	8.61	28.77	16.38	7.07	0.45	0.39
	772	85	0	0	3.31	1.63	5.34	3.59	0.04	0.46
Soils developed from loam	110	54	19	0	6.25	3.67	6.62	3.82	0.60	1.28
	328	15	10	0	5.62	66.62	36.92	3.14	0.52	0.77
	351	82	11	0	16.38	27.3	33.03	8.16	0.54	0.12
	922	18	12	0	1.16	0.89	17.08	5.63	1.22	0.03
	951	85	16	0	14.66	0.74	3.43	6.63	1.53	0.21
Soils developed from silt	113	104	0	4	3.61	3.61	2.66	6.59	1.08	0.87
	147	86	3	0	21.7	88.27	0	4.69	1.20	0.77
	672	86	0	4	8.03	8.13	19.95	4.12	0.10	0.10
	947	91	7	0	18.06	77.51	37.36	4.74	2.61	0.06
	984	98	0	7	4.13	1.4	11.9	8.23	0.23	0.35

pH above 7.5. In one of the acid soils, with pH 5.47, after 40 days of incubation the value of pH was 6.76 with a change in Eh from 371 to 212 mV. Gliński and Stępniewski (1985) state that the processes of reduction in soils are accompanied by a change in their reaction in the direction of neutral, i.e. pH value of acid soils increases and that of alkaline soils decreases, to stabilize after several weeks at the level of pH 6–7.5. Usually, however, those changes do not exceed 2 units of pH. Increase in the pH of acid soils after flooding is attributed by those authors to the acceptance of hydrogen ions by oxygen, mainly as a result of redox reactions taking place in the soil. In the case of nitrate reduction those reactions proceed as follows:

$$NO_3^- + 2H^+ + 2e \rightarrow NO_2^- + H_2O$$

Total reduction of $2NO_3^-$ to N_2 produces $2OH^-$ which caused an increase in the pH of the environment (Sprent 1987). Decrease of the pH of alkaline soil, on the other hand, is related to accumulation of CO_2 under anaerobic conditions (Ponnamperuma 1972).

Redox potential versus nitrate content in soils

As indicated in studies by numerous authors, the exhaustion of oxygen dissolved in water takes place within a few hours after flooding. In particular, there is a decrease of the redox potential of the soil, with the decrease of Eh value below +300 mV. The decrease was caused by the successive activation of redox pairs other than the O_2:H_2O system, for example NO_3^-:NO_2^- (Gliński & Stępniewski 1985).

Under the effect of oxygen content in the soil dropping below 1% (v/v), anaerobic microorganisms begin to dominate in the soil. Gliński and Stępniewski (1985) state that soil flooding with water causes gradual decrease of redox potential until a certain fairly stable level is attained. The rate of the decrease and the value of Eh depend on the intensity of the process of reduction, determined by temperature and by the content of easily decomposable organic substrate, and by the content of oxidized inorganic compounds that are electron acceptors, including nitrates.

Flooding of the soils selected for the study, in which the content of available nitrates ranged from 104.53 to 167.42 mg NO_3^--N/kg (native content +100 mg NO_3^--N/kg), caused a decrease in the content of nitrates in soil suspension during the period of incubation. The soils studied showed varied activity in the reduction of nitrates, where the reduction of NO_3^- varied from 20 to 100% (Table 3). Decrease in the content of nitrates was accompanied by a decrease in the value of redox potential which varied from 15 to 104 mV after the first 24 hours of incubation (Table 5). Statistical analysis of redox potential as a function of nitrate content showed a curvilinear relation which is best described by a logarithmic function with a determination coefficient of R^2 = 0.3823 (p < 0.001). As can be seen in Figure 4, redox potential decreased slightly with decreasing content of nitrates, the decrease being approximately 60 mV. The highest 24-hour reduction of nitrates occurred for a relatively narrow range of Eh values between 200 and 210 mV (Figure 5).

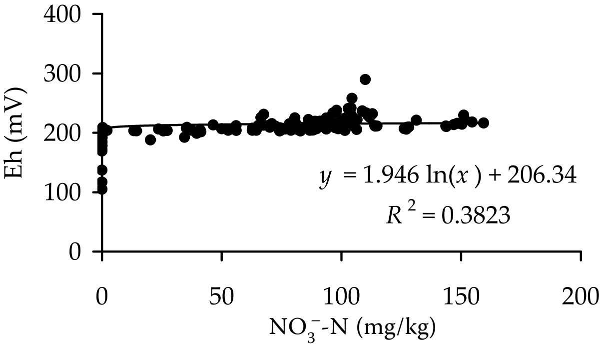

Figure 4. Redox potential (Eh) as a function of nitrate concentration in the soils

Similar values of redox potential were observed by Bailey and Beauchamp (1971) in a loess soil with differing content of nitrates, where Eh was approximately +200 mV. The broadest range of redox potential values in the soils analyzed (from +200 to +340 mV) was recorded at nitrate content levels between 100 and 120 mg NO_3^--N/kg. The limit value of nitrate content for the soils under study appears to be the value of approximately 100 mg NO_3^--N/kg at which the value of Eh decreases in most of the

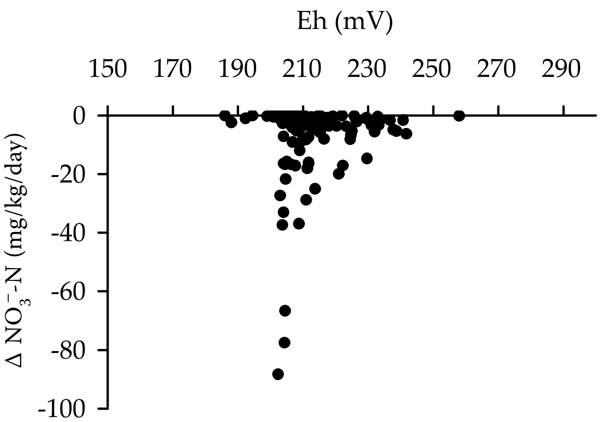

Figure 5. Daily reduction of NO_3^- in the soils as a function of mean Eh values during the period of the experiment

soils. Bailey and Beauchamp (1971), in the study referred to above, observed a substantial drop in the value of Eh after the total depletion of nitrates. The conditions of our study do not confirm such a phenomenon, as in the soils where the content of nitrates dropped to zero the potential did decrease, but not as much as indicated by the authors mentioned above. The range of Eh at zero content of NO_3^- in soil suspension ranged from about +204 to +105 mV (Tables 2 and 3). Table 3 lists the soils in which no content of NO_3^- was recorded in the final phase of the experiment.

As indicated by the results presented in Table 5, the strongest drop in the value of Eh during the analysed initial three days of incubation occurred after the first 24 hours in all of the soils analysed, while the distribution of nitrates in the period under analysis increased with the time in most of the soils. It is to be assumed that a part of the microbes inhabiting the soils studied preferred oxygen, during the first day, as an electron acceptor. On successive days the participation of nitrates in the respiratory processes of the microbes studied increased, with simultaneous depletion of free oxygen. It can, therefore, be stated that it was the decrease in the content of O_2 in soil suspension, clearly observed during the initial 24 hours of incubation (Table 5), that was the cause of the notable drop in the Eh values on the first day of incubation. Soil oxygenation status, usually inversely proportional to the amount of water in soil, is in many studies considered to be the key factor affecting the production of N_2O. That inverse relation between the rate of denitrification and the content of oxygen is described in numerous publications (Bailey & Beauchamp 1971; Focht 1974; Burton & Beauchamp 1985).

CONCLUSIONS

(1) The strongest decrease in redox potential, caused by the state of anaerobiosis, occurred during the initial 24 hours of the experiment in all the soils under study.

(2) Redox potential was negatively correlated with the pH value of the soils studied (for $R^2 = 0.3041$; $p < 0.001$).

(3) In the course of the whole experiment a lowering tendency was observed in the pH values of alkaline and neutral soils, and an increase in the pH of strongly acid and acid soils.

(4) The studied soils showed varied activity in nitrate reduction, where the reduction of NO_3^- varied from 20 to 100% depending on type of soil and on time of incubation.

(5) With a decrease in the content of nitrates within the range from about 100 to approx. 10 mg NO_3^--N/kg, redox potential decreased from 250 to 190 mV. The greatest 24-hour reduction of nitrates coincides with Eh values within the narrow range between 200 and 210 mV.

(6) Statistical analysis of redox potential in the function of nitrate content showed a curvilinear relation which is best described by a logarithmic function with determination coefficient of R^2 = 0.3823 ($p < 0.001$).

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Abstrakt

Włodarczyk T., Szarlip P., Brzezińska M., Kotowska U. (2007): **Oxidačně redukční potenciál, obsah nitrátů a pH zátopové eutrické kambizemě během redukce nitrátů.** Res. Agr. Eng., **53**: 20–28.

Šestnáct vzorků svrchní půdy z orné kambizemě tvořené pískem, jílem a siltem bylo zkoumáno z hlediska schopnosti půdy redukovat nitráty za zátopových podmínek. Největší snížení oxidačně redukčního potenciálu (Eh) bylo pozorováno během prvního dne zátopy půdy. U alkalických a neutrálních půd byla pozorována klesající tendence hodnot pH, u kyselých a silně kyselých půd zvýšení hodnot pH, provázené snížením hodnot Eh. Oxidačně redukční potenciál vykazoval zápornou korelaci s pH hodnotami (R^2 = 0,3041; p < 0,001). Redukce NO_3^- se pohybovala od 20 do 100 % v závislosti na typu půdy a na lhůtě inkubace. Se snížením obsahu nitrátů v rozpětí od 100 do 10 mg NO_3^--N/kg se hodnota oxidačně redukčního potenciálu snížila z 250 na 190 mV. Nejvyšší 24hodinová redukce nitrátů se shodovala s Eh hodnotami v úzkém rozpětí mezi 200 a 210 mV. Statistická analýza oxidačně redukčního potenciálu ve funkci obsahu nitrátů vykazovala křivočarý vztah (R^2 = 0,3823; p < 0,001).

Klíčová slova: půda; oxidačně redukční potenciál; denitrifikace; pH

Corresponding author:

Dr. hab. Teresa Włodarczyk, Instytut Agrofizyki PAN, ul. Doświadczalna 4, P.O. Box 201, 20-290 Lublin 27, Polska
tel.: + 48 81 744 50 61 ext. 134, fax: + 48 81 744 50 67, e-mail: teresa@demeter.ipan.lublin.pl