Abstract. The aim of the study was to determine the effect of acetylene block on the emission of nitrous oxide from soils. The study was conducted on three arable soils of Poland: Haplic Podsol - sandy loam, Eutric Cambisol - loess, and Eutric Cambisol - sandy loam, which differ in the initial concentrations of nitrates: 22, 93 and 52 kg N-NO₃⁻ ha⁻¹, respectively. The study was performed with natural samples and enriched with two doses of nitrates (100 and 200 kg N-NO₃⁻ ha⁻¹) in treatments with and without acetylene block. It was found that N₂O emission without acetylene block was on the level of 0-100% as compared to the emission from samples with acetylene block. The effect of acetylene block was more effective in the soils without nitrate amendment.

Key words: nitrous oxide, acetylene blocking technique, soils

INTRODUCTION

Nitrous oxide is an important greenhouse gas and it also regulates stratospheric ozone. The concentration of this gas in the atmosphere increased from 287 ±1 ppbv in the preindustrial period to 314 ppbv, over the last century. The current rate of increase amounts to about 0.2% per year. Based on this increase, it can be estimated that the emission of N₂O is equal to 7 Tg year⁻¹ (Khalil et al., 2002). It is necessary to keep in mind the lifetime of N₂O which amounts to about 300 times more effective, molecule for molecule, than CO₂ in greenhouse effect formation (IPCC, 2001).

Among other sources, soils are responsible for 70% of the emission of this gas to the atmosphere. It is produced during such microbiological processes in soils as nitrification, denitrification or dissimilatory NO₃⁻ reduction to NH₄⁺ (DNRA) (Stevens et al., 1998). Soils may also absorb a certain amount of this gas (Smith et al., 1983).

Acetylene block is a technique applied to study the emission of N₂O (Balderston et al., 1976; Yoshinari et al., 1977) because it inhibits nitrification at low concentrations (0.01-0.1%) and the denitrification sequence at the N₂O stage at the same percentage level (Regina et al., 1998). It has been shown in experiments using 15 N, that C₂H₂ does not inhibit the rates and sequence prior to the stage of N₂O (Paul and Clark, 1996). It is the simplest and most commonly used technique to quantify denitrification.

Acetylene treatment may not always inhibit the action of N₂O reductase completely. When soil is wet, clayey or compacted, the diffusion of C₂H₂ to all soil microsites of microbial activity is difficult. The blocking of nitrous oxide reductase activity is incomplete when concentrations of NO₃⁻ are low or if there is organic carbon readily available and the C:NO₃⁻ ratio is high. Acetylene can also be metabolized in soil (Malone et al., 1998).

On the other hand, it is observed that acetylene >0.1% catalyzes oxidation of NO to NO₂ which can be further processed (Fig. 1).

Fig. 1. The effect of >0.1% acetylene on the transformation of nitrogen by denitrification and the production of N₂O (Conrad, 1996).
The aim of the study was to determine the effect of acetylene block on the emission of nitrous oxide from three different arable soils amended with two doses of nitrates in relation to soils not amended.

METHODS AND MATERIALS

The soils selected came from the collection of the Institute of Agrophysics, Polish Academy of Sciences, in the Bank of Mineral Soils of Poland (Gliński et al., 1991).

The investigations comprised surface horizons of three soils, which occur commonly in the territory of Poland: Haplic Podsol – sandy loam (A), Eutric Cambisol – loess (B) and Eutric Cambisol – sandy loam (C), which differed in the concentrations of nitrates on the levels of 22, 93 and 52 kg N-NO$_3$ ha$^{-1}$, respectively, before the beginning of the experiment (Table 1).

A potassium nitrate solution (doses adequate to 100 kg N-NO$_3$ ha$^{-1}$ and to 200 kg N-NO$_3$ ha$^{-1}$) was added to air dried samples (5 g) of each of the soils, and only distilled water to control samples at a ratio of 1:1. After closing the flasks by means of aluminium caps with rubber lining, 1% v/v acetylene in the ‘acetylene block’ combination was added (marked with a ‘+’). The flasks were incubated at 25 ±1°C during 20 days. Another set of samples with the same doses of nitrates was incubated without the addition of acetylene.

Samples of the headspace gases were analysed after 3 h, every day during the first ten incubation days, and after 20 days, with a gas chromatograph (Varian GC 3800) equipped with a 63Ni electron capture detector. Each day a sample of gas was taken from a new flask. The following parameters of gas chromatograph analysis were applied: detector temperature = 250°C, column temperature = 35°C, column flow = 5 ml min$^{-1}$, injection volume = 100 µl. Gas samples were injected manually. Each gas chromatograph analysis was triplicated.

RESULTS AND DISCUSSION

The emission of nitrous oxide was diverse between the individual treatments, both in terms of the soils and the nitrate doses (Fig. 2).

The highest emission in the control samples, 3140 ppm (6th day of incubation) and 2060 ppm (3rd day of incubation), with acetylene block and without it, respectively, was observed in the case of the Eutric Cambisol formed from loess (soil B). It follows that the natural concentration of nitrates was the highest in this soil.

Among the treatments with single doses of nitrate, the highest concentration of nitrous oxide appeared also in soil B during 6th day of incubation, and amounted to 3200 ppm and 2190 ppm in the sample with acetylene block and without it, respectively. The highest concentration of N$_2$O in samples of soil A with acetylene addition, amounting to 2290 ppm, appeared on 8th day of incubation, and in samples without acetylene - 1120 ppm and occurred on 9th day. Nitrous oxide emitted from soil C reached the highest concentration of 1100 ppm on 8th day in the sample with acetylene and 1100 ppm on 6th day in the sample without it.

The emission of N$_2$O from samples enriched with double doses of nitrates was the highest from soil B and amounted, on 6th day of incubation, to 3910 ppm and 3150 ppm in the combinations with and without acetylene, respectively. For soil A, the emission was on the level of 2480 ppm (8th day) and 1560 ppm (4th day), and for soil C - 1810 ppm (10th day) and 880 ppm (6th day), from samples with and without acetylene addition, respectively.

The highest positive correlation between the emission with and without acetylene block was observed in the treatment with double nitrate dose in the podzolic soil, and the lowest in the control sample (Figs 3 and 4).

About 82% of all measurements proved that the emission of N$_2$O from samples enriched with acetylene was higher than the emission from samples without its addition. The highest effect of acetylene block was shown in the

<table>
<thead>
<tr>
<th>Soil</th>
<th>Soil unit</th>
<th>Depth (cm)</th>
<th>Stones+ gravel (%)</th>
<th>Grain size distribution (%)</th>
<th>C$_{org}$ (%)</th>
<th>pH in KCl</th>
<th>N-NO$_3$ (g Mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Haplic Podsol – sandy loam</td>
<td>15-20</td>
<td>8</td>
<td>55 15 3 5 0.73 5.98 7.17</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>B</td>
<td>Eutric Cambisol – loess</td>
<td>10-20</td>
<td>0</td>
<td>8 15 9 12 0.98 5.36 23.7</td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>Eutric Cambisol – sandy loam</td>
<td>10-20</td>
<td>6</td>
<td>77 0 2 8 0.53 5.23 16.8</td>
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control samples (Fig. 5a) and in samples of soil A (Haplic Podsol – sandy loam) (Fig. 5b).

In the case of over 17% of the measurements, the emission of N₂O without the application of acetylene block was higher than the emission with the addition of acetylene. The majority in this group is represented by samples of soil C (Eutric Cambisol - sandy loam) and samples with single and double doses of nitrate.

The incomplete effect of acetylene addition can be due to poor penetration of the inhibitor into the soil microsites (Watts and Seitzinger, 2000). Diffusivity of C₂H₂ is extremely low in water-saturated soils (Well et al., 2003). The diffusion coefficient of C₂H₂ in water amounts to 1.10 x 10⁻⁵ cm² s⁻¹ at 0°C (Weast et al., 1987).

According to some authors, 6-77% (average 40%) of the total N₂O produced remains entrapped in the soil. The

**Fig. 2.** The dynamics of N₂O emission with (+) and without acetylene block during the incubation of control soil samples and samples enriched with single (1) and double (2) doses of nitrates (A - Haplic Podsol - sandy loam, B - Eutric Cambisol - loess, C - Eutric Cambisol - sandy loam).
researches were carried out by the comparison of two acetylene inhibition methods – traditional and with additional shaking with water (Mahmood et al., 1999).

If we did not add enough acetylene, the inhibition of nitrous oxide reductase would be incomplete. According to some authors, a concentration of 0.1-1% is enough to block the reduction of N$_2$O (Duxbury and McConnaughey, 1986; Yoshinari, 1977). Others suggest that higher concentrations are needed – 2.5% v/v (Regina et al., 1998), 4% v/v (Livingstone et al., 2000) and 5% v/v (Abbasi and Adams, 2000; Well et al., 2003). Laboratory studies often use 10% v/v acetylene for complete inhibition of N$_2$O reduction (Griffiths et al., 1998; Maljanen, 2003; Strong and Fillery, 2002; Teissier and Torre, 2002; Watts and Seitzinger, 2000), but the addition of so much acetylene could greatly disturb the soil gas profile (McConnaughey and Duxbury, 1986) or even create the risk of explosions (Jordan et al., 1998).

Another probable reason of the incomplete action of the addition of acetylene can be low soil nitrate concentration. If the concentration is below 10 µM (corresponding to 75 nmol N g$^{-1}$ dry weight), acetylene did not inhibit the reduction of N$_2$O to N$_2$ (Watts and Seitzinger, 2000). The low correlation

Fig. 3. Correlation between the emission of N$_2$O with and without acetylene block during the incubation the control soil samples and samples enriched with nitrates (A - Haplic Podsol - sandy loam, B - Eutric Cambisol - loess, C - Eutric Cambisol - sandy loam).
between the emission of N₂O without acetylene block and the emission with acetylene block in samples with natural concentration of nitrates in our studies corresponds with research by Jordan et al. (1998) who did not observe any visible effect of acetylene block on the emission from soil without the addition of nitrates.

As the cause of the small effect of acetylene block, the authors give the low pH of the soils under study, because the N₂O - reductase enzyme is more sensitive to low pH than other reductases (Knowles, 1981).

In the presence of sulphides the effect of acetylene block is incomplete (Adkins and Knowles, 1986).
In comparison to other techniques used to measure the emission of N$_2$O, such as the $^{15}$N gas-flux technique or the N$_2$ flux technique, the acetylene inhibition method is the most popular. According to some authors, there is no significant difference in the rate of emission from samples with or without acetylene from a soil with low pH (Griffiths et al., 1998), or there is only a minor effect, from 5 to 24% increase in the N$_2$O flux from samples with acetylene from organic soils (Maljanen et al., 2003). Researchers who compared the few methods for the study of N$_2$O emission obtained different results. In sediments, the emission of N$_2$O with the use of the acetylene inhibition method was higher than the results obtained by the $^{15}$N gas-flux method, $R^2=0.76$ (Livingstone et al., 2000), however in field plots planted with ryegrass, the flux based on $^{15}$N production from $^{15}$NO$_3^-$ was found to be about 1.5 times greater than the flux measured by the acetylene block method (Arah et al., 1993). According to other workers who studied nitrogen isotope techniques, the C$_2$H$_2$ method underestimates the emission of N$_2$O by about 50% (Seitzinger, 1993) or even by 63-88% (Svensson, 1997). Studies on the acetylene block method provided by Watts (2000) proved that the rates of emission of nitrous oxide were one to two orders of magnitude lower than the rates measured by the N$_2$ flux method in both high-moisture organic soils and relatively dry mineral soils.

CONCLUSIONS

1. Among all the combinations, control and enriched, the emission of nitrous oxide was the highest from the Eutric Cambisol formed from loess than from Haplic Podsol formed from sandy loam and Eutric Cambisol formed from sandy loam. In most cases, the highest concentration of N$_2$O occurred after the 6th or 8th days of incubation.

2. The N$_2$O emission without acetylene block was on the level of 0-100% as compared to the emission from samples with acetylene block. The effect of acetylene block was more visible in the soils without nitrate amendment. Among the soils studied, Haplic Podsol – sandy loam was the most susceptible to the action of acetylene block.

3. Under the conditions of the experiments performed, the application of acetylene block is not the best technique and does not fully meet our requirements.

REFERENCES


