Anhang 1: N₂O-Flüsse im Vorversuch

(KAMMANN et al. 1998)
Seasonal variability and mitigation options for N\textsubscript{2}O emissions from differently managed grasslands

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Abstract

Nitrous oxide emissions were measured from nine plots on an old grassland site near Giessen, Germany. The management regimes of the plots differed in the amount of nitrogen (N) fertilizer applied, in the cutting frequency and in the mean annual ground water table height. Emissions of N\textsubscript{2}O occurred mainly shortly after fertilization and during freeze-thaw periods. Additional field incubations (in jars) provided evidence that during frost periods N\textsubscript{2}O emissions as high as 22,000 ng N\textsubscript{2}O-N kg\textsuperscript{-1} soil h\textsuperscript{-1} originated directly from the frozen topsoil. For the highest fertilized plots the freeze-thaw period accounted for 43 and 52% of the total annual N\textsubscript{2}O emissions. Nitrous oxide emissions tended to increase with increasing N fertilizer application and decreasing water table depth. Furthermore, an increase in the number of cuttings per year reduced N\textsubscript{2}O emissions. The results suggest that the ability of plant roots to take up NO\textsubscript{3}\textsuperscript{-} increases with increased cutting frequencies throughout the vegetation period, therefore reducing the amount of NO\textsubscript{3}\textsuperscript{-} available for denitrifying microorganisms.

Keywords: Nitrous oxide flux; grassland soil; freeze-thaw period; cutting frequency; mitigation option

Introduction

About 70\% of the global anthropogenic N\textsubscript{2}O emissions are estimated to be derived from the agricultural sector (Cole et al., 1997), e.g. from increased fertilizer use and indirectly by enhanced N deposition inputs (Granli and Böckmann, 1994; Castro et al., 1993) or land use changes (Keller et al., 1993). In addition, there is a growing interest in various mitigation options for N\textsubscript{2}O emissions (Cole et al., 1997; Mosier et al., 1997).

Permanent grasslands cover about 30\% of the total area in Germany (Statistisches Bundesamt, 1994). Grassland ecosystems generally high in organic carbon (C) content can often be found in areas which are too wet for arable cropping, thus providing suitable conditions for biological denitrification and N\textsubscript{2}O emission (Firestone and Davidson, 1989; Granli and Böckmann, 1994). Many investigations of N\textsubscript{2}O emissions from grasslands have focused on intensively fertilized grasslands, mostly during periods when soils were unfrozen (e.g. Velthof et al., 1996; Allen et al., 1996), on grazed grassland or organic grassland soils and mires (Augustin et al., 1996). Data for non-grazed, mineral grassland soil, including a complete freeze-thaw period, are scarce. The aim of this study was to assess the annual balances and courses of N\textsubscript{2}O emissions from a long-term extensively managed, non-grazed grassland on mineral soil under various management regimes. The results presented here suggest management practices which could keep the N\textsubscript{2}O emissions at a comparable low level without
reducing the dry matter yield. Prolonged frost periods during December–February are common in this area, which enabled the study of N₂O emissions during frost as well as freeze–thaw periods. It revealed the importance of this period for an annual N₂O emission balance.

Materials and methods

Site description

The experimental site (Environmental Monitoring and Climate Impact Research Station Linden, ca. 4500 m²) is located 50°32'N and 8°41.3'E at an elevation of 172 m a.s.l near Giessen, Germany. The semi-natural non-grazed grassland has been managed extensively as a meadow for at least 50 years, fertilized with 50–80 kg N ha⁻¹ a⁻¹ as calcium ammonium nitrate and mown twice per year. The annual mean precipitation and temperature (last 30 years) are 644 mm and 9.9°C. The vegetation, an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria sub-community, is dominated by 12 grass species, 2 legumes and 15 non-leguminous herbs. The soil is classified as stagnohumic gleysol on loamy-sandy sediments over clay (Grünhage et al., 1996). For soil parameters and soil moisture see Table 1 and Table 2.

In 1993, experimental plots (3 x 3 m each) were installed in 6 blocks as a randomized block design experiment to assess the dry matter yield in dependence of varying N fertilizer amounts applied, varying cutting regimes and varying soil moisture within the experimental site (Grünhage et al., 1996). Within each block, a tube was inserted to ground water table depth and water table depth was monitored each working day. Eight of the nine plots selected in 1996 from this experiment to monitor N₂O emissions belonged to the ‘wettest’ of the 6 blocks, with fertilizer treatments ranging from 0–400 kg N ha⁻¹ a⁻¹. These plots were located in 1 m (min.) to 21 m (max.) distance to each other. One additional plot (No. 2 in Table 2, 40 kg N ha⁻¹ a⁻¹) was located in the ‘driest’ block of experimental plots (i.e. driest part of the whole experimental site) whereas the ‘wet’ 40 kg N plot occupied a little depression within the ‘wettest’ block. It was the only one within the ‘wet’ block which carried sedges (ca. 10% area). Therefore, the ‘dry’ and the ‘wet’ 40 kg N plots (Nos. 2 and 3 in Table 2) represented the ‘extremes’ concerning the soil moisture on the experimental site.

Cutting regimes ranging from 2–6 cuts per year were arranged in such a way that the effect of various cutting frequencies could be investigated under the same fertilizer regime. Fertilizer (calcium ammonium nitrate) was applied in granular form in 1–3 doses (Table 2). Gas flux measurements started in August 1996 with 2 replicates (maxichambers) per plot and were carried out every 2 to 5 days where all plots were sampled simultaneously.

Gas sampling and analytical techniques

Nitrous oxide fluxes were measured using a modified closed chamber method (Hutchinson and Mosier, 1981). Semitransparent maxichambers (Polyethylene, dia. 100 cm; h: 50 cm) equipped with a battery driven ventilator and a small vent for pressure equilibration were used for gas flux measurements. Three gas samples were taken during a cover period (t₁ = 60 min) at times t₂, t₃ and t₄ with 60 ml syringes (Becton/Dickinson® Plastipak) fitted with 3-way stopcocks. At measurement days

Table 1

<table>
<thead>
<tr>
<th>Groundwater table depth* (plot no. 2)</th>
<th>&lt;70 cm (all other plots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H₂O)</td>
<td>5.9</td>
</tr>
<tr>
<td>Organic carbon [%]</td>
<td>3.0 (± 0.02)b</td>
</tr>
<tr>
<td>Organic nitrogen [%]</td>
<td>0.3 (± 0.06)</td>
</tr>
<tr>
<td>Bulk density [g cm⁻³]</td>
<td>1.04 (± 0.06)</td>
</tr>
<tr>
<td>Particle density [g cm⁻³]</td>
<td>2.61 (± 0.011)b</td>
</tr>
<tr>
<td>Soil porosity [%]</td>
<td>60.2 (± 2.4)</td>
</tr>
<tr>
<td>Sand (2000-63 μm) [%]</td>
<td>43.2c</td>
</tr>
<tr>
<td>Silt (63-2 μm) [%]</td>
<td>39.0f</td>
</tr>
<tr>
<td>Clay (&lt;2 μm) [%]</td>
<td>17.8e</td>
</tr>
</tbody>
</table>

*Mean depth during summertime.

b_n = 3.

c_n = 8 mixed.

Table 2

<table>
<thead>
<tr>
<th>Plot no.</th>
<th>1a</th>
<th>2b</th>
<th>3c</th>
<th>4d</th>
<th>5e</th>
<th>6a</th>
<th>7f</th>
<th>8g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer N (kg ha⁻¹ a⁻¹)</td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>80</td>
<td>120</td>
<td>240</td>
<td>400</td>
</tr>
<tr>
<td>Number of cuts per year</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Groundwater table depth (cm)</td>
<td>&lt;70</td>
<td>&gt;120</td>
<td>&lt;70</td>
<td>&lt;70</td>
<td>&lt;70</td>
<td>&lt;70</td>
<td>&lt;70</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Avg. soil mois. (vol.%)</td>
<td>34.8</td>
<td>28.1</td>
<td>53.9</td>
<td>43.8</td>
<td>44.6</td>
<td>43.4</td>
<td>32.1</td>
<td>41.1</td>
</tr>
</tbody>
</table>

Fig. 1. Average N$_2$O fluxes from three selected plots during the investigation period, August 1996–October 1997 (a) and related climatic parameters (b, c); see legend for both in (c). ‘Wet’ and ‘dry’ indicates data from the wettest and driest area of the site. wvc = volumetric water content (vol.%); arrow in (a) indicates the date of the first fertilization (identical for all treatments); arrow in (b) indicates time of re-insertion of TDR-probe after frost action expelled the probe.

(measurements performed between 11:00 and 13:00 hours) the chambers were placed in an U-shaped water filled PE-rings which were inserted into each plot (10 cm deep) 2 month prior to the experimental start.

Jar incubations of frozen soil blocks (2.5 × 2.5 × 5 cm = L × W × H; 3 soil blocks per jar) were performed using preserving jars (Weck 0.75 liter) fitted with a septum for sampling (Müller et al., 1997). Jar incubations were carried out on 18 January 1997 under ambient conditions for 60 min. Every 30 and 60 min a gas sample (40 ml) was taken and replaced by 40 ml of a standard with N$_2$O free synthetic air. The N$_2$O emissions in jars were corrected for the dilution effect of the synthetic air. Soil blocks from the frozen 5 cm were cut out from the soil by means of an electrical power saw. The soil was taken from unfertilized, NO$_3^−$ and NH$_4^+$ fertilized soil (fertilized at a rate of 100 kg N ha$^{-1}$ on 12 December 1996) from a site where the water table at that time was close to the soil surface.

Gas samples were analyzed within 8 hours after sample collection. The gas chromatograph (HP 6890) was equipped with a $^{63}$Ni-electrode capture detector (Mosier and Mack, 1980; oven, valve and detector temperatures were operated at 65, 100 and 280°C) and oxygen-free nitrogen (Messer-Griesheim, N$_2$ 5.0) was used as carrier gas (35 ml min$^{-1}$). Accuracy of the gas chromatographic nitrous oxide data at ambient concentration was ±1% or better. N$_2$O flux rates were calculated using linear regression and the ideal gas law with average chamber temperature (measured each time a sample was taken) and mean air pressure during the cover period. The percentage of N$_2$O-N lost per fertilizer N applied was calculated as [annual N$_2$O-N loss$_{fertilized}$ − annual N$_2$O loss$_{unfertilized}$] / fertilizer N applied.

Soil moisture, soil temperature and N analysis

Soil moisture content (top 16 cm) from each plot was monitored at times of gas measurement with permanently installed TDR sensors (sensor type P2G, F. Imko, Germany). Soil temperatures were measured in the driest and wettest plot in 5, 10 and 20 cm depth with soil temperature sensors (Pt 100 DIN 43760) and together with precipitation and air temperature (2 m height) automatically logged as 30 min averages. The data reported here (Fig. 1) are calculated daily means.

Inorganic N concentrations were analyzed for the top 15 cm in 5 cm increments. The soil samples (40 g) which represented a mixture of 4 sampling sites per plot were extracted for 30 min with 200 ml 1 M KCl within 15 min after soil sampling. Extracts were stored until analysis at −20°C. NO$_3^−$-N, NO$_2^−$-N and NH$_4^+$-N were analyzed colorimetrically according to standard procedures (Mulvaney, 1996). N concentration of plant biomass (dried at 105°C) was determined by standard Kjeldahl technique.
Table 3
Nitrate (first value) and ammonium (second value) concentrations (μg N g⁻¹ dry soil) 6 days and one month after the first fertilizer application (24.4.1997); w = 'wet'; d = 'dry'; other plots intermediate soil moisture.

<table>
<thead>
<tr>
<th>Fertilizer treatment (kg N ha⁻¹ a⁻¹)</th>
<th>0</th>
<th>2</th>
<th>40</th>
<th>2 (w)</th>
<th>80</th>
<th>3</th>
<th>80</th>
<th>3</th>
<th>120</th>
<th>4</th>
<th>240</th>
<th>4</th>
<th>400</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.4.1997 (6 days after fertilization)</td>
<td>0-5 cm</td>
<td>0.19</td>
<td>122.5</td>
<td>2.67</td>
<td>7.61</td>
<td>113.6</td>
<td>2.79</td>
<td>6.48</td>
<td>221.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-10 cm</td>
<td>2.71</td>
<td>94.4</td>
<td>2.30</td>
<td>3.23</td>
<td>49.9</td>
<td>3.72</td>
<td>3.26</td>
<td>97.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-15 cm</td>
<td>0.07</td>
<td>2.83</td>
<td>0.22</td>
<td>0.38</td>
<td>1.03</td>
<td>0.32</td>
<td>0.71</td>
<td>3.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>10-15 cm</td>
<td>0.03</td>
<td>0.67</td>
<td>0.27</td>
<td>0.40</td>
<td>1.37</td>
<td>0.22</td>
<td>0.15</td>
<td>1.19</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.85</td>
<td>1.21</td>
<td>0.85</td>
<td>0.53</td>
<td>0.68</td>
<td>0.43</td>
<td>0.76</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.5.1997 (one month after fertilization)</td>
<td>0-5 cm</td>
<td>0.12</td>
<td>0.23</td>
<td>0.37</td>
<td>0.51</td>
<td>0.81</td>
<td>0.92</td>
<td>359.7</td>
<td>499.9</td>
<td></td>
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<tr>
<td></td>
<td>5-10 cm</td>
<td>0.55</td>
<td>1.30</td>
<td>2.00</td>
<td>2.22</td>
<td>3.00</td>
<td>5.97</td>
<td>181.5</td>
<td>321.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-15 cm</td>
<td>0.07</td>
<td>0.16</td>
<td>0.21</td>
<td>0.34</td>
<td>0.41</td>
<td>0.41</td>
<td>36.2</td>
<td>56.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>10-15 cm</td>
<td>0.18</td>
<td>0.23</td>
<td>0.47</td>
<td>&lt;0.1</td>
<td>0.36</td>
<td>0.38</td>
<td>2.29</td>
<td>1.97</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.18</td>
<td>0.18</td>
<td>0.33</td>
<td>0.29</td>
<td>0.45</td>
<td>0.27</td>
<td>11.6</td>
<td>7.43</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
<td>0.12</td>
<td>0.49</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.17</td>
<td>1.05</td>
<td>0.34</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*The two strongest fertilized plots (240 and 400 kg N ha⁻¹ a⁻¹) had received their second N fertilizer application on 16.5.1997. High amounts of NH₄-N or NO₃-N content after fertilization were most likely due to inhomogenous N fertilizer application in granulate form.

Statistical analysis
The SigmaStat version 2.0 software package (SPSS Inc.) was used for statistical analysis. Statistical analysis of \( N_2O \) emission patterns was carried out using the one way ANOVA on ranks (Kruskal–Wallis-test) because time courses of data (temporal variability on a plot) were not normally distributed, even when log-transformed. Significant differences \((p < 0.05)\) among treatments were analysed with the Dunn's Test or Mann–Whitney rank sum test. To test correlations between climatic parameters and \( N_2O \) fluxes, Pearson product moment correlation was performed.

Results
Seasonality of \( N_2O \) emissions
The investigation period (August 1996–October 1997) can approximately be divided into 3 main \( N_2O \) emission periods: two late summer–autumn periods, the winter period and the spring–summer period following fertilization (Fig. 1a). With temperatures \(<10^°C\), \( N_2O \) emissions declined to zero during autumn 1996 (Fig. 1a,c). A large increase of \( N_2O \) emissions occurred with the onset of a frost period. During thawing, \( N_2O \) fluxes increased even further reaching some of the highest emission rates throughout the year (Fig. 1a,c). For the extensive to medium intensive fertilized plots, approximately half of the annual \( N_2O \) emission occurred within 4–6 weeks after fertilizer applications (Fig. 1a). Subsequent fertilizer applications later on in the season did not cause comparable emission peaks for the \(<240 kg N ha⁻¹ a⁻¹\) applications. However, \( N_2O \) emissions from the two highest fertilized plots remained high until mid-August 1997 (Fig. 1a). Only a weak correlation between soil moisture, soil temperature and \( N_2O \) emission rates was observed \((r = 0.6)\).

Applied inorganic nitrogen (\( NH₄⁺ \) and \( NO₃⁻ \)) up to an amount of 120 kg N ha⁻¹ a⁻¹ remained within the top 5 cm and was immobilized quickly (Müller et al., 1997) (Table 3). Except shortly after fertilization, nitrate values for the plots fertilized up to 120 kg N ha⁻¹ a⁻¹ remained below \(\leq 1 μg N g^{-1} \) soil throughout the investigation period. Only on the highest fertilized plots, higher concentrations (among 6 and 80 μg NO₃⁻ N g⁻¹ soil, at 0–5 cm) have been detected later on in the season and down to greater depth \((>10 μg NO₃⁻ N g^{-1} \) soil, at 10–15 cm).

Differences between treatments
Generally, \( N_2O \) emissions for the two highest fertilized plots were always greater than for the less fertilized plots. However, during August to December 1996, no fertilizer effect could be detected within the group of the extensive to medium intensively managed plots \((<240 kg N ha⁻¹ a⁻¹\), Fig. 2). During the winter period, however, \( N_2O \) flux rates followed the previous fertilizer application rate (Fig. 3).

If data from the whole investigation period are compared, a significant influence of the water table level on \( N_2O \) emissions could be observed within the two 40 kg N ha⁻¹ a⁻¹ fertilized plots. The \( N_2O \) emissions from the 'wet' plot compared to the 'dry' plot were significantly higher (mean \(\pm SD\) 12.0 \(\pm\) 29.5 and 4.2 \(\pm\) 13.3 μg N₂O-N m⁻² h⁻¹, Mann–Whitney–Rank sum test, \(p < 0.001\), \(n = 109\)). Therefore, the annual \( N_2O \) emission sum of the 'dry' plot fertilized with 40 kg N ha⁻¹ a⁻¹ was
Influence of different cutting regimes

Preliminary results (Fig. 2) suggested that there may be an influence of cutting frequencies on $\text{N}_2\text{O}$ emission. Therefore, an additional 80 kg N ha$^{-1}$ a$^{-1}$ treatment plot with two instead of three cuts per year was taken into investigation (from October 1996 onwards, Table 2). Comparing data from the whole investigation period, the increase in cuttings from 2 to 3 led to a significant reduction in $\text{N}_2\text{O}$ emissions from the 80 kg N-treatment ($p = 0.027$, Mann–Whitney rank sum test; Fig. 4, left) of about 29% (Fig. 5). In addition, a further increase in cuttings on an additional plot (cuts were made every time the canopy height exceeded 10 cm, i.e. 6 cuts a$^{-1}$) also reduced $\text{N}_2\text{O}$ emissions significantly ($p = 0.018$; Fig. 4, right).

$\text{N}_2\text{O}$ emissions from frozen soil

$\text{N}_2\text{O}$ emissions from frozen topsoils were studied during a severe frost period (December-January 1996/1997). Daily mean air temperatures as low as $-15^\circ\text{C}$ (Fig. 1c) resulted in a frozen topsoil layer of at least 20 cm until the midst of January. However, as indicated by TDR sensors (Patterson and Smith, 1981), the unfrozen water content of the soil still approximated 20% (Fig. 1b). $\text{N}_2\text{O}$ emissions increased during the onset of freezing by a factor of 10–15 (e.g. to 100 $\mu$g $\text{N}_2\text{O}$–N m$^{-2}$ h$^{-1}$, highest fertilized plot, Fig. 1a). Jar incubations of frozen soil blocks taken from the top 5 cm indicated that $\text{N}_2\text{O}$ was emitted by the apparently frozen topsoil (Fig. 6). Furthermore, $\text{N}_2\text{O}$ emission rates were considerably higher from the soil that had previously been fertilized with $\text{NH}_4^+$ (5-fold, $>2000$ ng $\text{N}_2\text{O}$–N kg$^{-1}$ h$^{-1}$) or $\text{NO}_3^-$ (55-fold, $>22,000$ ng $\text{N}_2\text{O}$–N kg$^{-1}$ h$^{-1}$) compared to unfertilized soil.

only half the value compared to the ‘wet’ plot receiving the same amount of N fertilizer (Fig. 5).

The percentage of $\text{N}_2\text{O}$–N emitted (plots ≥120 kg N, equal moisture) was approximately 1% of the N application rate (Fig. 5). However, ‘wetter’ (1.53%) and ‘drier’ (0.50%) soil conditions or lower cutting frequencies resulted in percentages differing from this value. Dry matter production increased up to a fertilizer rate of 80 kg N ha$^{-1}$ for the plots used in this experiment (not shown). The experimental site has its optimum dry matter yield between a N fertilizer application rate of 80 to 120 kg N ha$^{-1}$ a$^{-1}$ (Grünhage et al., 1996).
Fig. 5. Annual sums of NO\textsubscript{2}\textsubscript{O} emissions (integrated on a day-to-day basis). Each bar: mean of the two maxichambers used per plot; up and down reaching capped lines indicate the upper and lower sums. * % NO\textsubscript{2}\textsubscript{O}-N emitted per annual fertilizer N applied. ** Because of the later start, the period was two weeks shorter for this treatment.

Fig. 6. NO\textsubscript{2} emissions from frozen soil blocks incubated in jars, 18 January 1997 (30-min rates, one hour incubation in total; n = 3 jars + SD). Incubation of unfertilized and previously fertilized soil (100 kg NH\textsubscript{4}-N or NO\textsubscript{3}-N applied on 12 December 1996). Below: amount of mineral N present in the frozen soil.

Discussion

Seasonal and annual NO\textsubscript{2} emission

Two main emission periods dominated the annual NO\textsubscript{2} losses: the period following the first fertilization and the off-season freeze–thaw period. Kaiser and Heinemeyer (1996) found similar NO\textsubscript{2} emission patterns from two arable soils in Germany. They reported NO\textsubscript{2} losses in the range of 1 kg N ha\textsuperscript{-1} a\textsuperscript{-1}. This is almost equal to the results obtained in this study for the medium intensive fertilized plots (80–120 kg N ha\textsuperscript{-1} a\textsuperscript{-1}). However, for the highest fertilized plots (240 and 400 kg N ha\textsuperscript{-1} a\textsuperscript{-1}) the emission period extended longer into the season with NO\textsubscript{2}O flux rates only decreasing with August–September drought (in 1997) or decreasing autumn temperatures (at the end of October 1996). Such an extension of high rates of NO\textsubscript{3} emissions was also obtained by Velthof et al. (1996) in a study of different intensively fertilized grassland soils in the Netherlands that received N applications around 400 kg N ha\textsuperscript{-1} a\textsuperscript{-1}. Furthermore, the annual average NO\textsubscript{2} loss (3.8 kg N ha\textsuperscript{-1} a\textsuperscript{-1}) as well as the NO\textsubscript{2} loss in percentage of N-input (0.9%) obtained from an intensively N fertilized, mown clay soil by Velthof et al. (1996) were almost equal to the values reported here from the 400 kg N ha\textsuperscript{-1} a\textsuperscript{-1} fertilized plot.

The fast immobilization of applied fertilizer N in the top 5 cm of the soil and the low nitrate and ammonium contents throughout the rest of the year on the plots fertilized with ≤120 kg N ha\textsuperscript{-1} a\textsuperscript{-1} point to a nitrate limitation of denitrification in the grassland as described by Mosier et al. (1991) or Neff et al. (1994) for native and extensive fertilized grassland ecosystems. Nitrate limitation may have been the reason for the weak correlation between soil moisture, soil temperature and NO\textsubscript{2}O emission rates.

A higher ground water table significantly increased soil moisture and hence NO\textsubscript{2}O emissions from the plots fertilized with 40 kg N ha\textsuperscript{-1} a\textsuperscript{-1} during the year. This is in line with observations by other researchers (Kliewer and Gilliam, 1995; Velthof et al., 1996). The slightly different soil parameters between the ‘dry’ and the ‘wet’ plot are not considered to be responsible for the difference in NO\textsubscript{2}O emission, because the soil porosity (Table 1) and the pf-curves (not shown) of the soils were almost identical. Furthermore, an organic C content of 3% (= ‘dry’; ‘wet’ = 6.6%) should provide a non-limiting C supply for denitrification (Granli and Bockmann, 1994).

In the IPCC 1995 report (Watson et al., 1996) an annual NO\textsubscript{2}O–N emission sum of 1 kg ha\textsuperscript{-1} is used as a ‘background level’ to assess NO\textsubscript{2}O emission from agricultural soils. Nitrous oxide emissions reported here from unfertilized and extensively fertilized plots (40 kg N ha\textsuperscript{-1} a\textsuperscript{-1}) are with 0.18–0.79 kg NO\textsubscript{2}O–N ha\textsuperscript{-1} a\textsuperscript{-1} below this value.

Influence of cutting regimes

An increase in the cutting frequency from 2 to 3 cuts per year led to a significant reduction in annual NO\textsubscript{2}O emissions (28.8% when fertilized with 80 kg N ha\textsuperscript{-1} a\textsuperscript{-1}). Moreover, an increase to 6 cuts per year (80 kg N ha\textsuperscript{-1} a\textsuperscript{-1}) decreased the NO\textsubscript{2}O emissions even more. The reductions occurred during the vegetation period but not during the freeze–thaw period, thus stressing the influence of plants in the observed pattern.

The roots of growing plants are thought to be able to enhance denitrification and NO\textsubscript{2}O production because they supply easily degradable C (root exudates, decaying roots) to denitrifiers and other soil microorganisms (Smith and Tiedje, 1979; Beck and Christensen, 1987; Christensen et al., 1990). This was indeed found in the
presence of high nitrate concentrations (Smith and Tiedje, 1979; Beck and Christensen, 1987), but Smith and Tiedje (1979) also reported a reduction of denitrification by a factor of five from planted soil when the nitrate concentration was low. It is well known that an increase in the cutting frequency improves the root-to-shoot-ratio in plants, leading to a denser rooted topsoil (e.g. Köppers et al., 1988; Mooney and Winner, 1991). N-limiting conditions also promote N partitioning to the roots (Mooney and Winner, 1991). More frequently cut plants may therefore have a greater ability to compete for the mineral N supplied by mineralization and nitrification, hence withholding it from denitrifiers and reducing N₂O emissions. The low nitrate concentrations in the Linden grassland soil throughout the year, except shortly after fertilization, is in line with this hypothesis. Recent findings that bacterial growth could be reduced by plant-induced N limitation support this hypothesis (Wang and Bakken, 1997a,b).

The observed N₂O emission reduction due to an increase in the cutting frequency (29%) is in line with the proposed reduction scenarios by the IPCC 1995 (Watson et al., 1996) of 20%, if practices to 'match N supply with crop demand' and to 'tighten N flow cycles' are applied. Further research is needed to explore the possible mitigation option 'more cuts per year' for other grassland ecosystems.

N₂O emissions from frozen soil and during thawing

In this study we observed a considerable rise of N₂O emission rates during a frost period. Biological processes were found to be still active at temperatures around 0°C especially under an isolating snow cover (Sommerfeld et al., 1993; van Bochove et al., 1996). Goodroad and Keeney (1984) observed N₂O emissions from a frozen soil surface and interpreted it as a result of diurnal freeze–thaw cycles causing N₂O production from the top mm of the soil. Another suggestion was that emissions may have originated from deeper unfrozen layers (Bremner et al., 1980; Goodroad and Keeney, 1984). However, in this study it was shown that N₂O emissions during frost periods originated mainly from the frozen topsoil. Unfrozen water contents during this time ranged from 19–28 vol.%, which may have supported biological activity. A high unfrozen water content in apparently frozen soil is an effect of silt and clay contents in the soil as well as a salting out effect which lowers the freezing point of the soil water (Edwards and Cresser, 1992). Dorland and Beauchamp (1991) demonstrated that denitrification processes continued in supercooled liquid soil slurries fertilized with NO₃⁻ even at temperatures of −2°C in the presence of sufficient amounts of easily degradable C. The Linden grassland soil is well supplied with easily metabolizable C and therefore, nitrate could have been a limiting factor for denitrification in the frozen (and thawing) soil. When nitrate was present in the soil, very high N₂O emission rates from frozen soil blocks were observed (>22,000 ng N₂O–N kg⁻¹ h⁻¹). In the NH₄-treated soil the nitrate concentration was similar to the unfertilized control. Therefore the 5-fold enhanced N₂O emission from this treatment suggests that nitrification was also active in the frozen soil, but was the rate-limiting step towards denitrification (Fig. 7).

Nitrous oxide emissions strongly increased on all treatment plots from the Linden grassland site during thawing, which is commonly observed and attributed to a release of easily degradable C and N from killed soil microbial biomass, disruption of soil aggregates and favourable soil moisture conditions (e.g. Goodroad and Keeney, 1984; Cates and Keeney, 1987; Flessa et al., 1995; Kaiser and Heinemeyer, 1996). During thawing, single N₂O flux rates as high as 411 µg N₂O–N m⁻² h⁻¹ (400 kg N ha⁻¹ a⁻¹ treatment) were detected while nitrate concentrations were quite low (<7 µg N g⁻¹). N₂O flux rates during thawing were comparable to the results of Cates and Keeney (1987) who reported maximum N₂O emission rates of 208 µg N₂O–N m⁻² h⁻¹ from a 237 kg N ha⁻¹ fertilized maize field during thawing.

The freeze–thaw (and early spring) period accounted for as much as 52% of the N₂O emitted from the highest fertilized grassland soil and for 41% from the 240 kg N ha⁻¹ a⁻¹ fertilized plot. For the reminder of the treatments (extensive to medium intensive fertilized), the percentage values ranged among 17–38% of the annual N₂O losses. This figure compares well with findings by other researchers who report N₂O emissions of 6–46% released during spring thaw or freeze–thaw periods (Bremner et al., 1980; Flessa et al., 1995; Kaiser and Heinemeyer, 1996). The results stress the importance of the intensity and duration of the winter frost periods for annual N₂O losses, especially when soils receive greater amounts of N fertilizer during the plant growing season.

Conclusions

The study presented here deals with N₂O emissions from long-term extensively managed, non-grazed grassland and therefore closes an existing data gap. The annual N₂O losses from the unfertilized and extensively fertilized grassland were quite below the assumed agricultural background value of 1 kg N₂O–N ha⁻¹ a⁻¹(IPCC 1995 report, Watson et al., 1996), demonstrating the potential for low N₂O emissions from a nitrate limited extensively managed grassland ecosystem. An increase in the cutting frequency per year from 2 to 3 reduced the annual N₂O losses by 29%, suggesting a practicable mitigation option. By application of the 80 kg N – 3 cuts treatment, an annual N₂O loss of 0.84 kg N ha⁻¹, less than the 'agricultural background value' of 1 kg N ha⁻¹ (Watson et al., 1996) was achieved which was still in the range of the optimum dry matter yield (Grünhage et al., 1996). This suggests that it may be possible to adapt management regimes in non-grazed grasslands in such a way that
nitrate is kept a limiting factor for N₂O producing denitrifiers, but with no reduction in the biomass yield. However, high fertilizer applications resulted in large N₂O losses comparable to intensive fertilized grasslands, e.g. in the Netherlands. Results indicate that prolonged frost periods may contribute to a large extent to annual N₂O losses (up to 50%), thus stressing the importance of off-season N₂O losses for the annual balance.

References


Anhang 2: Die Bodenluftsonden-Methode

(KAMMANN et al. 2001a: Druckfahne des im Juni 2001 erscheinenden Artikels)
A new sampling technique to monitor concentrations of CH$_4$, N$_2$O and CO$_2$ in air at well-defined depths in soils with varied water potential

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Summary

A new sampling technique for measuring the concentrations of trace gases (CH$_4$, CO$_2$ and N$_2$O) in the soil atmosphere from well-defined depths is described. Probes are constructed from silicone tubing closed with silicone septa on both ends, thereby dividing an inner air space from the outer soil atmosphere without a direct contact. The gas exchanges between the inner and outer atmosphere only by diffusion through the walls of the silicone tube. Tests revealed that the gases N$_2$O, CO$_2$ and CH$_4$ in the enclosed space reached 95% equilibrium with the surrounding atmosphere at 20°C within 7 h or faster. The probe measurements are reproducible: the standard deviation of samples taken from 26 probes stored in the laboratory atmosphere equaled that of a standard gas. The probes can easily be constructed and installed at specified depths in the soil.

The method has the following advantages compared with other methods that use spaces with holes in them for gas exchange: (i) the silicone probe enables trace gases to be sampled in wet soils, including ones that are waterlogged or temporarily saturated; (ii) the sampling itself does not create low pressure and hence does not create mass flow in the soil matrix from undefined depths; and (iii) the probe can be made to take samples of gas of any required size. The silicone probes did not show ageing effects during 18 months of use in the field in a mineral soil under grass. The probes yielded comparable results: three probes inserted at 5 cm depth in a uniformly treated 100-m$^2$ plot provided nearly identical average trace gas concentrations within the measurement period.

Introduction

Our understanding of the biological processes such as nitrification, denitrification, methane oxidation or production that produce and consume trace gases in the soil has greatly improved in the last decade (N$_2$O fluxes reviewed by, e.g., Atalik et al., 1992; Granli & Beckmann, 1994; CH$_4$ fluxes by, e.g., Conrad, 1989; Knowles, 1993). Often surface fluxes were measured in closed chambers, which integrate the heterogeneous biological activities in the vertical column of soil below them. The results raise several additional questions, for example concerning the transport of gases dissolved in soil solution (Heincke & Kaupenjohann, 1999), the development of gradients in the concentrations of trace gases during winter when ice may bar or restrict diffusion to the surface (Burton & Beauchamp, 1994), and the simultaneous production and consumption of trace gases such as N$_2$O or CH$_4$.

Several disparate techniques for sampling the air in the soil have been developed so far. Almost all of the techniques, however, include some kind of openings to the soil matrix for the air exchange between sampler and soil. For example, Dowdell et al. (1972) constructed samplers from porous sintered bronze cups with 5-mm pore size that were used to measure various gases (Dowdell et al., 1972; Dowdell & Smith, 1974; Egginton & Smith, 1986). Goodrow & Keeney (1985) and Cates & Keeney (1987) used vertically installed open polyvinyl chloride pipes into which rubber stoppers were inserted, creating a 20-ml space in each. They sampled about 10 ml of soil air with a capillary tube through the rubber stopper. The multilevel sampling probes of Burton & Beauchamp (1994) and the sampling device of Sotomayor & Rice (1999) both used holes cut into vertically inserted tubes at the desired sampling depth. The technique developed by Fang & Moncrieff (1998) included rectangular gas traps with holes (2.5 cm × 2.5 cm × 5 cm) buried at various depths. The soil air was circulated with a pump between the gas trap and a sampling device at the soil surface previous to sampling.
The holes or openings to the soil matrix have the disadvantage of allowing water to enter when the soil is flooded. This situation frequently occurs in wetlands when the water table is high, or when the soil is saturated following heavy rain. Furthermore, the holes could become blocked by small soil particles after a time. Fang & Moncrieff (1998) reported that about one fifth of the volume of the inner gas trap was filled with soil at the end of the sampling period from January to October. Depending on the analytical techniques amounts of 2–10 ml of gas sample, collected by the methods mentioned above, might not be sufficient for analysis. In all the methods the removal of too much sample would diminish the pressure and cause mass flow from the soil matrix, thereby contaminating the sample during extraction.

The idea of using silicone tubing for sampling air was first tested and reported by Holter (1990) who used short pieces of silicone tubing to sample O₂, CH₄ and CO₂ from dung pats ('silicone rubber diffusion chambers'). Nielsen et al. (1997) sampled nitrogen gases from narrow-bore silicone tubing wrapped around a specially constructed probe, which they used for adding ¹⁵NO₃⁻-labelled solution at various depths in the soil. Jacinthe & Dick (1996) tested the diffusivity of silicone tubing for large N₂O concentrations in preliminary tests and in a column experiment in the laboratory. They found that N₂O diffused quickly through 2.4-mm regular and 4.8-mm reinforced walls of silicone tubing at 22°C (4.4 and 8.3 h, respectively).

In contrast to Jacinthe & Dick (1996), who tested large concentrations of N₂O (30–60 μl l⁻¹), and Holter (1990), who measured CH₄ and CO₂ at large concentrations (9.6% CH₄, 7.2% CO₂), we tested for small near-ambient concentrations of CO₂, N₂O and CH₄. When CH₄ is taken up by methane oxidizers in the soil, concentrations as small as 300 μl l⁻¹ CH₄ may result (Bender & Conrad, 1992; Mosier et al., 1997). In addition, long periods of very small emissions of N₂O are common in, for example, fertilized grasslands (e.g. Mosier et al., 1991; Kammann et al., 1998). During such periods, small (near atmospheric) concentrations of N₂O can be expected at least in the topsoil, as found by Soomayor & Rice (1999). Therefore, the silicone tubing method is of use only if small gradients between the inner and outer atmosphere lead to a quick and complete equilibration, and this had to be confirmed by tests. We found that indeed N₂O, CH₄ and CO₂ diffuse quickly into silicone, hence making it an ideal material for sampling trace gases in soil that is saturated temporarily.

Materials and methods
Construction of the soil air probes from silicone tubing
Probes are constructed from silicone tubing as shown in Figure 1. Each consists of 1.3 m of tubing (Kaiensee, Giessen, Germany; internal diameter 10 mm, wall thickness 3 mm, and yielding about 100 ml gas) closed with silicone septa at both ends. The tubing is rolled into a flat coil in the form of a snail and fixed by wrapping wire mesh around it. At one end, a right-angled 3.17-mm stainless steel tube is inserted to connect the silicone 'snail' in the soil with the soil surface (Figure 1). The end of the steel tube is fitted with a three-way stopcock that enables the soil air to be sampled with 60-ml syringes (both standard medical supply). For the gas-tight fitting of the steel tube to the stopcock, one of the stopcock-caps is carefully drilled with a 3-mm burer in such a way that the inner cone in the cap remains intact. Afterwards, the drilled cap is pushed over the stainless steel tube. When the stopcock is screwed into the drilled cap with the cone still intact, the connection between stopcock and steel tube is gas-tight because the diameter of the steel tube is slightly larger than the hole in the cap. Hence, with a closed stopcock, the air in the probe is disconnected from the outer atmosphere, allowing only diffusion through the silicone walls.

Inserting the silicone probes into the soil
The silicone coils are inserted into the soil as follows. A pit is dug to the maximum depth of interest with one face vertical, into which are cut horizontal slots with a tool made for the purpose. This tool is a steel rod; it has a T-piece as handle, and near its lower end is mounted a horizontal knife blade 20 cm long (see Figure 2). It is driven into the bottom of the pit until the knife blade is at the desired sampling depth, and it is rotated so that the knife cuts a slot. Then it is raised 2–3 cm to cut another slot, thereby freeing a semicircular slice of soil, which is removed to create a cavity. A coil is inserted into the cavity with good contact, both above and below, with the soil. Several cavities can be made in the wall of a single pit. Even if they are at different depths the rod should be moved to a new position for each so that they are not vertically above one another and the soil will not collapse. The pit is then refilled with soil, horizon by horizon, in the same sequence as it was originally.

We tested the probes in a wet meadow where the soil is a Fluvic Gleysol with textures sandy loam over clay. We dug pits to 60 cm and inserted coils at 5, 15, 30 and 50 cm in July 1998. We refilled the pits, as above, and after 2 months we could detect no difference between the restored land and the surrounding meadow. We began to sample on 20 August, and thereafter sampled at intervals of 4–7 days. We also recorded the height of the ground water table in the meadow from tubes inserted into the soil at six locations. For further information on the experimental site see Kammann et al. (1998) and Grünhage et al. (1996).

Diffusion through the silicone tube walls
In addition to the large concentrations of N₂O, as tested by Jacinthe & Dick (1996), we measured the diffusivity of silicone for small concentration gradients of CH₄, N₂O and
Sampling technique for trace gases in the soil air

Figure 2: Insertion of silicone probes into the soil. 1. A steel tool with a right-angled knife blade is driven into the bottom of the pit previously dug into the soil. When the tool is turned, the knife cuts a horizontal slit into the vertical side wall. By cutting a second slit 2–3 cm deeper, a horizontal soil layer ('half cake') can be removed. 2. A coiled silicone probe is inserted into the horizontal cavity. 3. After insertion the pit is refilled with previously removed soil, horizon by horizon, in correct sequence.

CO₂. For this we used 130 cm of silicone tubing (16 mm outer diameter, 3 mm wall thickness), closed with silicone septa at both ends, enclosed in a bag filled with gas (Figure 3). A rubber stopper on the bag, fitted with two glass tube connectors with stopcocks, allowed us to sample the atmosphere in the bag as well as the atmosphere in the silicone tube with 60 ml polyethylene syringes (Beckton/Dickinson® Plastipak). The tightness of the connection between the glass-silicone tube was ensured by withdrawing a gas sample (about 40 ml) from the silicone tube without refilling it with air, thereby creating low pressure. After 5 minutes, the stopcock of the silicone tube connector was opened to the atmosphere, and, if the probe was gas-tight, a fierce fizzing was audible as the air entered. Even a tiny leak in the silicone, made on purpose with a needle to test this, led to pressure equilibration within 5 minutes. Therefore, if there was no sound after 5 minutes when sampling in the field then we knew that the probe was leaking.

Three series of tests were made in which the bag was filled with (i) a standard gas containing 2486 μl l⁻¹ CO₂, 2.49 μl l⁻¹ N₂O and 19.76 μl l⁻¹ CH₄ (in synthetic air; Deutsches Steingägerl GmbH, Mülhausen, Germany) and (ii, iii) synthetic air with 79.5% N₂ and 20.5% O₂ without trace gases (Messer–Griesheim GmbH, Siegen, Germany). The silicone tube contained (i) atmospheric air at the beginning or (ii) either atmospheric air or (iii) the standard gas (for concentrations see above) at the beginning. After various lengths of time one sample was taken from the silicone tube and six samples were taken from the atmosphere in the bag by syringes (Figures 4 and 5). The three concentration combinations tested (i–iii) are displayed in Figure 4, while in Figure 5 the combination (i) was used.

The equilibration was described by calculating the per cent difference between the concentration in the silicone tube and the mean value in the bag, which was set at 100%. This was done because for each measurement the bag had to be evacuated and refilled with (standard) gas, resulting in concentrations that were not identical for every sampling occasion (for range of variation within a test series see Figure 4). To the decline of the relative ‘per cent difference’ with time was fitted an exponential decline function expressed as

$$D_t = D_0 + a \exp(-bt),$$

(1)

where $D_t$ is the per cent difference at time $t$, $D_0$ is the minimum per cent difference the model predicts, and $a$ and $b$ are coefficients. If Equation (1) is solved for $t$ to calculate the equilibration time needed to yield a 95% equilibrium between the atmosphere in the bag and that in the silicone tube then Equation (2) results:

Three-way stopcocks, closed
Silicone tube closed with 2 septa
Bag, filled with test gas

\[ t = -\frac{1}{b} \ln \left( \frac{D_1 - D_0}{a} \right) \] (2)

Analytical technique

The gas samples taken by syringes were analysed for CO₂, N₂O and CH₄ automatically within 1-24 h following their collection, by a gas chromatograph (HP 6890) and the automatic sample system described by Loffield et al. (1997). The chromatograph was equipped with an ECD (⁶⁷Ni-electron capture detector, at 290°C) and an FID (flame ionisation detector, at 230°C). The standard deviations of 10 measurements at ambient concentration were 4 μl l⁻¹ for CO₂, 4 μl l⁻¹ for N₂O and 8 μl l⁻¹ for CH₄ or less than these values (day-to-day variation).

Results and discussion

Diffusion through the silicone tubing material

Nitrous oxide was observed to diffuse quickly into the silicone tubing with the 3-mm thick walls (Figures 4 and 5), even when the concentration gradient for N₂O was much smaller than used by Jacinthe & Dick (1996). As those authors presumed, CO₂ and CH₄ also diffuse well through silicone tubing. After 7 or more hours at 20°C had passed the concentrations in the bag and the tube atmospheres differed less than 5% (see Figure 4). The equilibration did not improve much when a longer time period passed (Figure 5). When \( t \), Equation (2), was calculated with the coefficients obtained from the curve fittings (Figure 5), a 95% equilibration was reached on average after 2.9 h for CO₂, 1.5 h for N₂O and 6.6 h for CH₄. The times Jacinthe & Dick (1996) found for N₂O were slightly larger (4.4 h with 2.4 mm wall thickness). Holter (1990) obtained even shorter diffusion times at 22°C for the larger CO₂ and CH₄ concentrations he used. With silicone tubing of 1 mm wall thickness, he found times of 1.45 h for CH₄ and 0.7 h for CO₂ until 95% equilibration was reached.

However, as mentioned by Holter (1990), the silicone diffusion properties may vary somewhat, depending not only on the temperature and wall thickness but also on the commercial batch of silicone used. Therefore, all of the reported diffusion times serve as guidelines only for the times that have to pass between samplings. Jacinthe & Dick (1996) estimated from their data for N₂O that it took 2.5 times longer to reach equilibrium when the temperature was reduced by 10 degrees (wall thickness of the silicone tube 2.4 mm). In our experiment the equilibration took longest for CH₄ (6.6 h), and the wall thickness of the silicone tubing was larger (3 mm). Therefore, we let 4 days pass in winter when the soil temperature was around 0°C as a minimum time between two samplings to ensure equilibration.

We did not test for diffusion of O₂, but Holter (1990) did, and he reported that O₂ took a little longer than CH₄ and CO₂ to reach 95% equilibrium. We observed smaller O₂ peaks in the FID chromatograms with rising water table in autumn 1998 and no O₂ peaks at all when large CH₄ concentrations (>100 μl l⁻¹) occurred in the grassland soil (results not reported here). Therefore we suggest that the silicone probes might be valuable for measuring O₂ as well in the atmosphere of (saturated) soils.

Reproducibility of the probe measurements and spatial variation in the field

Samples were taken from 26 probes that were kept in the laboratory at slightly above-atmospheric concentrations. The probe measurements are highly reproducible, for the standard deviation almost equals that of measuring a single gas repeatedly: the results were 411 ± 5.9 μl l⁻¹ for CO₂, 382 ± 2.9 μl l⁻¹ for N₂O and 1900 ± 5.5 μl l⁻¹ for CH₄, respectively.

Figure 6 displays the concentrations of gas measured from August 1998 to January 2000 with three probes inserted at 5 cm
depth in 100 m² of meadow to which 40 kg N ha⁻¹ had been added in April 1998 and 1999. The small box plots inserted in each graph indicate the range of concentrations during the period. The lower and upper bounds of the boxes are the 25 and 75 percentiles, the solid and dotted lines within the box mark the median and mean values, respectively, and whiskers below and above the box indicate the 10 and 90 percentiles.

Within the period, the mean concentrations of the three probes were nearly identical with 3950, 3790 and 4290 μl l⁻¹ for CO₂, 1.58, 1.56 and 1.58 μl l⁻¹ for CH₄, and 0.37, 0.37 and 0.39 μl l⁻¹ for N₂O. On a wetter part of the experimental site, where the groundwater table on average was 28 cm closer to the soil surface, but which received the same amount of fertilizer, the concentrations were always much larger, with mean concentrations of 13,460 μl l⁻¹ for CO₂, 3.59 μl l⁻¹ for CH₄, and 0.61 μl l⁻¹ for N₂O during the same period. This indicates that the horizontal area covered by a silicone soil air probe (coil diameter about 20 cm) yields comparable concentration values, at least in fairly homogeneous grassland soil. To adjust the silicone probe method to more inhomogeneous (e.g. ploughed arable) soils one should increase the diameter of the silicone coil by using longer pieces of tubing. This is done in an analogous way when measuring N₂O fluxes at the soil surface with the closed chamber method by using larger cover boxes (Kaiser et al., 1996).

Practical performance of the silicone probes

During the time we have been using the equipment (18 months), only two of the 25 probes inserted failed to keep low pressure. The probes did not suck in air from the atmosphere after a sample was taken, and water was sometimes found in the syringe on sampling, e.g. after heavy rain. The leakage occurred soon after the probes had been inserted into the soil. An analysis of critical manufacturing points revealed that the insertion of the stainless steel tube into the silicone septum was the most likely weak point: when the silicone septum is penetrated by a sharp needle to allow the steel tube to be inserted, it can slowly tear around the tube. A better alternative is to use a hole-puncher (a tool to pierce, e.g., a leather belt) to make a small round hole in the septum, then insert the steel tube, and finally fix the tube by filling the space between the inner septum walls and the steel tube with silicone caulking. Ten probes, a 'second generation', were manufac-
Figure 5 Equilibration of the inner silicone tube atmosphere with the surrounding bag atmosphere with time. The "per cent difference" is the difference between the mean of the atmosphere in the bag and the value measured in the silicone tube, expressed in percentage of the bag mean (±100%). (For the exponential decline function fitted to the data, see text.)

Figure 6 Mean concentrations and standard deviation of CO₂, N₂O, and CH₄ in 5 cm depth of three soil air probes during the measurement period (August 1998–January 2000). The three probes were installed in the soil of a uniformly treated 100 m² of grassland fertilized with 40 kg N ha⁻¹ year⁻¹. The ranges of the data from the whole measurement period are displayed as box plots (see text).

We suggest that a second steel tube with stopcock be inserted into the second silicone septum in a probe. Thus modified, the probe in the soil can be flushed with a tracer gas (e.g. ¹⁵N-labelled N₂O or other gases) so as to measure gas diffusion in the soil.

Advantages of the silicone air probe method

One advantage of the new method is that it can be used in wet, even waterlogged, soil because the silicone excludes H₂O and lets gases such as CO₂, N₂O, and, especially interesting in wet soils, CH₄ diffuse through its walls. A second advantage is that sampling air from the silicone probe does not create low pressure and hence does not create mass flow in the soil matrix from undefined depths. Methods that use holes cut in air samplers (e.g. Burton & Beauchamp, 1994; Fang & Moncrieff,
1998; Sotomayor & Rice, 1999) may generally have this problem, depending on the ratio of the sample size taken to the samplers' inner volume. The authors cited above minimized the problem by adjusting this ratio (taking small air samples). With the silicone probes, the sample is simply taken from the silicone-enclosed space itself, restricting low pressure to the inner probe atmosphere. The volume-to-wall thickness ratio of the silicone probes used here and the air sample size that could be withdrawn with a 60-ml syringe were chosen in such a way that the tube did not collapse from low pressure (Jacinthe & Dick, 1996) while delivering a sufficient amount of sample that fitted our analytical needs (about 40 ml). The amount of sample can be increased by simply using longer pieces of silicone tubing when the probe is constructed. Normal atmospheric pressure can be re-established quickly by allowing atmospheric air (or N2, He or other gases from a gas balloon connected via the three-way stopcock) to enter the silicone probe. A third advantage is that silicone as well as the other materials used for the probes age slowly. A silicone probe, once installed, can be used in long-term field experiments, without problems such as blocking of the sampling holes by soil particles bringing the experiment to a premature end.

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Anhang 3: CH$_4$-Flüsse im Vorversuch

(KAMMANN *et al.* 2001b: Manuskript zur Veröffentlichung akzeptiert)
Methane fluxes from differentially managed grassland study plots: the important role of CH$_4$ oxidation in grassland with a high potential for CH$_4$ production

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"Capsule": In grassland soils, the thin aerobic topsoil horizon had a very high methane oxidation potential.
Abstract

Methane oxidation fluxes were monitored with the closed chamber method in eight treatment plots on a semi-wet grassland site near Giessen, Germany. The management regimes differed in the amount of nitrogen (NH₄NO₃) fertilizer applied and in the height of the in ground water table. No inhibition of CH₄ oxidation occurred, regardless of the amount of annual N fertilizer applied. Instead, the mean CH₄ consumption rates were correlated with the mean soil moisture of the plots. However, the correlation between daily soil water content and corresponding CH₄ oxidation rate was always weak. During drought period (late summer) water stress was observed to restrict CH₄ oxidation rates.

The findings led to the question whether methane production with soil depth might modify the CH₄ fluxes measured at the surface. Therefore, two new methods were applied: i) soil air sampling with silicone probes and ii) anaerobic incubations of soil cores to test for the methane production potential of the grassland soil. The probe measurements revealed that the CH₄ sink capacity of a specific site was related to the vertical length of its CH₄ oxidizing column, i.e. the depth of the CH₄ producing horizon. Anaerobically incubated soil cores produced large amounts of CH₄ comparable to tropical rice paddy soil. Under field conditions, heavy autumnal rain in 1998 led to a dramatic increase of soil CH₄ concentrations up to 51 µl l⁻¹ at a depth of 5 cm. Nevertheless, no CH₄ was released when soil surface CH₄ fluxes were measured simultaneously. The results thus demonstrate the high CH₄ oxidation potential of the thin aerobic topsoil horizon in a non-aquatic ecosystem.

Keywords: Methane oxidation; Methane production potential; Grassland soil; Silicone probes; Vertical soil column

1. Introduction

Methane, the most important greenhouse gas after CO₂ (Schimel and Gulledge, 1998), can be oxidized by aerobic methanotrophic bacteria, contributing about 5.8% to the atmospheric CH₄ sink (Houghton et al., 1996). Methane oxidation has been shown to occur worldwide in various soils, as in temperate (Castro et al., 1995) and tropical forests (Keller et al., 1990), savannas (Seiler et al., 1984), grasslands (Mosier et al., 1991), deserts (Striegl et al., 1992) and tundra and taiga (Whalen and Reeburgh, 1996). In addition, methane oxidizing bacteria alter methane fluxes to the atmosphere from aquatic environments such as rice paddies by consuming up to 80% of the methane produced in the soil, before it escapes to the atmosphere (Conrad and Rothfuss, 1991; Butterbach-Bahl et al., 1997).
Human activities are thought to have reduced the potential soil sink capacity for methane by about 30%, in part as a result of N fertilizer application (Ojima et al., 1993; Hütsch, 1998b). An immediate inhibition following application of NH$_4^+$ has frequently been observed in terrestrial ecosystems (Mosier et al., 1991; Hütsch, 1998a; Hütsch et al., 1994; Powlson et al., 1997). Sometimes the inhibition prevailed even when NH$_4^+$ concentrations in the soil declined to background levels of native unfertilized soils (Mosier et al., 1991) or when fertilizer application was stopped for 3 years (Hütsch et al., 1994). Inhibition of methane-monooxygenase by ammonium (Bédard and Knowles, 1989; Carlsen et al., 1991; Nesbit and Breitenbeck, 1992; Adamsen and King, 1993) could not be the reason for persistent inhibition when NH$_4^+$ concentrations reach background levels (Mosier et al., 1991). Another underlying mechanism might be a shift between the populations of nitrifiers and methane oxidizers, if they compete for the same ecological niches in the soil structure (Bédard and Knowles, 1989; Hütsch, 1996).

In many observations the dominant factor controlling CH$_4$ oxidation is the soil water content (Jones and Nedwell, 1993; Castro et al., 1995; Mosier et al., 1997). Castro et al. (1994) found a linear correlation between soil water content and CH$_4$ oxidation, explaining 78% of the CH$_4$ oxidation rates. Rising soil water contents, covering the soil microbes with a thicker water film, are considered to represent a diffusive barrier for the substrate methane (Koschorreck and Conrad, 1993), because CH$_4$ transport in water is $10^4$ fold slower than in (soil) air. In addition, the low CH$_4$ substrate (i.e. atmospheric) concentration usually limits the process rate (King and Adamsen, 1992). Both diffusive and substrate limitation seem to be the cause for the usually quite low temperature sensitivity of methane oxidation (Born et al., 1990; King and Adamsen, 1992; Crill et al., 1994; Mosier et al., 1993; Jones and Nedwell, 1993).

The results reported here are parts of a preliminary study in the Free Air Carbon dioxide Enrichment experiment (FACE) being performed at the Institute for Plant Ecology, University of Giessen. Among other issues, the FACE-Experiment deals with the effects of rising atmospheric CO$_2$ concentrations on the fluxes of CH$_4$ and N$_2$O in the grassland. Hence, the aim of this preliminary study was to characterize the grassland's potential to oxidize or produce methane under different management regimes. The following questions were addressed: (a) Is there a measurable influence of the soil water content and/or level of the ground water table on the CH$_4$ oxidation rates and how strong is the relationship? (b) Is there an inhibitory effect when different amounts of N fertilizer (NH$_4$NO$_3$) are applied to the grassland and if there is one, does it persist? (c) Are methane emissions common in the semi-wet grassland under investigation and does it have a potential for methane production?
2. Materials and methods

2.1. Site description

The grassland site is located near Giessen, Germany and has been managed as a meadow for at least 50 years, fertilized with 50 to 80 kg N ha$^{-1}$ yr$^{-1}$ as calcium ammonium nitrate and mowed twice per year. The soil is a Fluvic Gleysol with a texture of sandy loam over clay. The annual mean precipitation and temperature are 644 mm and 9.9°C.

In 1993, 6 experimental plots (3 x 3 m each) were installed as a randomized block design experiment to assess the dependence of dry matter yield on varying N fertilizer amounts, cutting frequencies and soil moisture within the experimental site (Grünhage et al., 1996). In 1996, eight locations were selected from this experiment to monitor CH$_4$ fluxes and N$_2$O emissions, (for N$_2$O results see Kammann et al., 1998). Except for one location, all others belonged to the wettest block in the experiment (1 – 21 m from each other). Granular calcium ammonium nitrate fertilizer was applied in 1-3 annual doses (Table 1). The plot with the lowest soil moisture (40 kg N ha$^{-1}$ yr$^{-1}$) was located in the driest block of the experiment. In contrast, the wettest 40 kg N plot was located in a little depression and was the only one containing sedge. Therefore, the driest and the wettest 40 kg N plots represent the extremes of soil moisture, whereas all other plots were intermediate in nature. The parameters of the topsoil horizon (0-15 cm) of the wettest/driest sites were respectively: pH 6.2/5.9, organic C content 6.6%/3.0%, organic N content 0.5%/0.3%, sand 10.0%/43.2%, silt 58.1%/39.1% and clay content 31.9%/17.8%.

Gas flux measurements were started in August 1996 with 2 replicates per plot (maxichambers, see below) and were carried out every 2 to 5 days where all plots were sampled simultaneously. Soil air was sampled every 4 to 7 days from 1st September 1998 to 6th January 2000 following the insertion of silicone probes in mid-July 1998 (see below).

2.2. Methane flux measurements

Methane fluxes were measured using a modified closed chamber method (Hutchinson and Mosier, 1981). Semitransparent maxichambers (Polyethylene, 100 cm diameter, 50 cm height) equipped with a battery driven ventilator and a small vent for pressure equilibration were used for gas flux measurements. Three gas samples were taken during the period of vegetation cover ($t_c = 60$ min) at times $t_0$, $t_c/2$ and $t_c$ with 60 ml syringes (Becton/Dickinson® Plastipak) fitted with three-way-stopcocks. On measurement days the chambers were placed in U-shaped water filled PE-frames. The frames were 10 cm deep.
into the soil and were installed in the plots at each 'chamber location' two months before the measurements started.

Gas samples were analyzed within 1 to 24 hours following sample collection by means of the automatic sample system described by Loftfield et al. (1997). The gas chromatograph (HP 6890) was equipped with an ECD ($^{63}$Ni-electron capture detector) and a FID (flame ionization detector). Accuracy of the gas chromatographic CH$_4$ measurements at ambient concentration was $\pm 8 \mu l l^{-1}$ or better. Methane flux rates were calculated using linear regression and the ideal gas law with average chamber temperature (measured each time a sample was taken) and average air pressure during the cover period.

2.3. Soil air sampling

The method is described in detail by Kammann et al. (2001). Probes were constructed from silicone tubing (inner diameter 10 mm, wall thickness 3 mm, length 130 cm) closed with silicone septa on both ends, thereby dividing an inner air space from the outer soil atmosphere without a direct contact. The exchange of CH$_4$, N$_2$O and CO$_2$ between the inner and outer atmosphere was only by diffusion through the walls of the silicone tube, whereas H$_2$O was excluded. Hence the method allowed air sampling even when a probe was flooded, according to the amount of the gas dissolved in the soil water. For sampling the atmosphere in the silicone probe, a stainless steel tube was inserted in one septum and fitted to a three-way stopcock, which enabled air sampling with 60-ml syringes. Soil air probes were inserted at 5, 15 and 30 cm depth (Table 1) and in the wettest plot (40 kg N ha$^{-1}$ a$^{-1}$) an additional probe was inserted at 50 cm depth. The first soil air sampling (Sept. 1$^{st}$ 1998) was performed 6 weeks after the probes had been inserted in mid-July 1998. Samples were taken every 4 to 7 days to ensure sufficient time for equilibration of the soil air with the inner probe atmosphere.

2.4. Methane production potential: Core sampling and incubation

To test the potential methane production of the grassland soil, 10 soil cores with intact structure were anaerobically incubated at 20°C. The utility of the method was first reported by Wachinger et al. (1999) for monitoring the microscale spatial variability of methane production. On 14 October 1998, soil cores (4 cm high and 100-cm$^3$ volume, excluding the first cm with thick grass roots) were obtained within a 0.25 m$^2$ area using metal soil coring tubes. These wet soil cores were adjusted to field capacity by placing them on ceramic plates with suction applied by a 630 mm water column until they stopped releasing water. Each core was enclosed separately in a gas-tight Poly-oxy-methylene chamber, connected to a N$_2$ gas supply and flushed for two hours. First, the cores were incubated statically;
however, with the onset of methane production, soon its concentrations in the sampled air exceeded the measurement limit of the FID. Therefore, a flow-through method was used, where N₂ (humidified) was pumped continuously through the chamber with a flow rate between 3.3 and 3.5 ml min⁻¹. The out-flowing air was collected through a 50-ml syringe on a daily to weekly basis and analyzed with the FID.

2.5. Soil moisture, soil temperature, N analysis and statistics

The soil moisture content of the top 16 cm from each plot was monitored during the gas measurements with permanently installed TDR sensors (sensor type P2G, Fa. Imko, Germany). Soil temperatures were measured in the driest and wettest plots at 5, 10 and 20 cm depth with soil temperature sensors (Pt 100 DIN 43760), together with precipitation depth and air temperature (2m height). Inorganic N concentrations were analyzed for the top 15 cm in 5 cm increments according to standard procedures (Mulvaney, 1996; Kammann et al., 1998). SigmaStat version 2.0 software package (SPSS Inc.) was used for linear regression and correlation analysis.

3. Results

3.1. Influence of soil moisture on CH₄ flux rates

Methane oxidation dominated the direction of CH₄ fluxes in all treatment plots (August to November 1996 and April to November 1997, see Figure 1). However, single small events of CH₄ emissions were observed during autumn 1996 following rain events. The time courses of the CH₄ oxidation rates were similar on all treatment plots, but the average rates differed in magnitude (Figure 1; Figure 3). The CH₄ oxidation rates (chamber means per treatment and measurement day) were poorly correlated to the corresponding daily soil moisture values or soil temperature at 5 cm depth (Table 2). The soil air CH₄ concentrations at 5 and 15 cm depth measured from September 1998 to January 2000 in the dry soil (CH₄ oxidation) were also poorly and negatively correlated to the corresponding soil moisture values, while the CH₄ production exhibited a poor, but positive relationship (Table 2; Figure 6). For the 40 kg N, dry treatment, there was a stronger correlation between declining CH₄ concentrations at 5 cm depth and rising soil moisture when only values below 50 vol.% were included (r = 0.602, Figure 6).

In August and September 1997, a severe drought occurred. During that period there was a significant correlation (r² = 0.72, p < 0.001, Figure 2) between the declining soil moisture
values and decreasing CH$_4$ oxidation rates. With the first rainfall in October 1997, the CH$_4$
oxidation rates recovered again despite a much lower soil temperature (Figure 1, Figure 2).

3.2. Influence of N fertilizer application on CH$_4$ oxidation rates

There was no inhibition of methane oxidation from the N fertilizer applications (Figure 3). Treatments that received as much as 240 or 400 kg N showed on an average a higher CH$_4$
consumption rate than did the wetter 40 kg N or the 80 kg N treatments. There was no
direct correlation between the amount of N fertilizer applied and the average methane
oxidation rates (Figure 4b). However, the average CH$_4$ oxidation rates of the treatments
were negatively correlated to the soil moisture ($r^2 = 0.71$) (Figure 4a). Highest oxidation
rates occurred mainly on the slightly drier plots even if a great amount of N fertilizer (e.g.
240 kg N treatment) was applied during the vegetation period. In addition, the CH$_4$
oxidation rates did not decrease directly following fertilizer application (Figure 1, 23rd
April 1997), nor did the soil air methane concentrations increase at 5 cm depth or deeper
after fertilization in April 1998 in one of the treatments (not shown in the figure).

3.3. The CH$_4$ production potential of the grassland soil: Anaerobic incubations

The methane production began on Nov. 7th 1998, three weeks after the start (Oct 15th 1998)
of the anaerobic, static incubation of the grassland soil cores. By the end of Nov. 1998, the
N$_2$-flow-through technique was applied. During the anaerobic incubation, 3 different types
of time series of methane production potentials could be observed (Figure 5a and b, note
the different scales). In type 1 (chambers T2, T3, T4 and T7) the production rates rose
constantly until the incubation was terminated on Feb 9th 1999. In type 2 (chambers T8 and
T9), productivity values dropped slowly during the three months of incubation. Chambers
T1, T5 and T6 (type 3) did not produce methane over the whole incubation period.
However, these chambers produced CO$_2$ at rates in the same order of magnitude as the
CH$_4$ producing chambers (not shown in the figure). The spatial variability of the 0.25 m$^2$
area sampled was high and it ranged from zero CH$_4$ production to about 55 µg
CH$_4$-C 100 cm$^{-3}$ h$^{-1}$ (corresponding to 19.6 µg CH$_4$ day$^{-1}$ g$^{-1}$ soil dry weight when the site's bulk
density of 0.9 g cm$^{-3}$ was used for the calculation).

3.4. In-situ CH$_4$ production in the grassland soil

With heavy autumnal rains and rising ground water table in 1998 (Figure 7b), the soil air
CH$_4$ concentrations rose quickly in the wettest treatment plot, beginning at 50 cm depth
(Figure 7a). The rise took place at all depths soon thereafter, reaching values as high as
235-µl l⁻¹ CH₄ at 30-cm depth on the Dec. 1st 1998. The probes at 15, 30 and 50 cm depth were sometimes flooded during the winter when the soil water table was high (Figure 7a, b). This was the cause for the 50-cm values being lower than the 30-cm values (see also Table 3).

On Nov. 17th the CH₄ concentration at 5-cm depth reached a maximum of 51-µl l⁻¹ CH₄ (Figure 7a). Nevertheless, when simultaneous CH₄ flux measurements were performed on the treatment plots with the closed chambers (less than 1 and 1.5 m distance from the probes), no net CH₄ efflux could be detected, and no net CH₄ oxidation from the atmosphere took place.

The probe measurements also revealed that the length of the CH₄ oxidizing vertical soil column was not identical in all treatment plots. The lowest CH₄ concentrations were found in the 40 kg N 'dry' treatment at all depths when compared to the corresponding depths in other treatments (Figure 8; Table 3). The wetter plots comprised periods were the CH₄ concentrations reached values several-fold higher than the ambient atmospheric concentration (Table 3).

4. Discussion

4.1. Influence of different N fertilizer levels on CH₄ oxidation

The mean CH₄ oxidation rates observed during the experimental period ranged from -10.1 µg CH₄-C m⁻² h⁻¹ on the wettest treatment to -19.3 µg C m⁻² h⁻¹ on the driest site, and rates of 25 – 35 µg CH₄-C m⁻² h⁻¹ commonly occurred at the 'dry' plot during summer. The rates are comparable to values observed by other authors; Tate and Striegl (1993) reported average oxidation rates of 20 to 32 µg CH₄-C m⁻² h⁻¹ from native and annual prairie that was burned. Mosier et al. (1991) monitored rates between 15 and 26 µg CH₄-C m⁻² h⁻¹ in highland and lowland prairie and unfertilized meadow. Boeckx et al. (1997) found rates of 25 µg CH₄-C m⁻² h⁻¹ from clay grassland soil (incubation of soil cores).

In many other investigations, the application of NH₄⁺ reduced methane oxidation rates almost immediately (forest soils, Steudler et al., 1989; short-grass steppe, Mosier et al., 1991; landfill cover soil, Boeckx and van Cleemput, 1996; laboratory incubations with different soils, Tlustos et al., 1998 and Hütsch, 1998a). Gulledge et al. (1997) observed a delayed inhibition in the methane oxidation up to 60-70% occurring three years after the first fertilizer application. They attributed the delay to suppression in the population growth of methane oxidizers and to an inhibition of de-novo enzyme synthesis. In the present case, we did not find any relationship between the amount of N fertilizer applied (which consisted of 50% ammonium) annually and the methane oxidation rate. It is unlikely that inhibition may develop even 3 to 4 years after establishing the treatment plots. Gulledge et al. (1997) observed a similar situation during the third year under colder
climatic conditions. Tate and Striegl (1993) and Whalen et al. (1991) also did not observe N-inhibition. There could be different reasons for a lack of N inhibition on the methane oxidation processes. Since the first application of fertilizers by farmers decades ago (50 – 80 kg N ha\(^{-1}\) a\(^{-1}\)), a certain degree of inhibition of CH\(_4\) oxidation might have persisted without recovery, resulting in the measured oxidation rates. Such long-term inhibitions have been previously reported (Hütsch et al., 1994; Goulding et al., 1996; Powlson et al., 1997). Hence, the higher N doses applied on the intensively fertilized plots may have had no additional inhibitory effect. Although this possibility cannot entirely be excluded, it seems unlikely because the measured rates from the plots fertilized with 40 kg N ha\(^{-1}\) a\(^{-1}\) were quite comparable to CH\(_4\) oxidation rates reported from unfertilized grassland soils elsewhere in temperate regions (e.g. Mosier et al., 1991). Moreover, no (short-term) decline in the CH\(_4\) oxidation rates was observed following N applications.

Another possibility might be a spatial separation of processes within the vertical soil profile. Most of the N (ammonium) turnover processes that are proposed to produce inhibitors for methane oxidation (Tlustos et al., 1998; Hütsch, 1998a) are located in the top few centimeters of the soil, while the gross methane oxidation may have taken place in deeper soil layers. Koschorreck and Conrad (1993) and Crill (1991) found the main CH\(_4\) oxidizing activity in the top few centimeters of the Ap horizon. Hütsch (1998c) reported the maximum CH\(_4\) oxidizing activity to occur in the zone nearest to the soil surface in an agricultural soil, unless there were constraints (e.g., ploughing or drilling) preventing that process. However, the top 5 cm of the grassland soil were always the horizon with the strongest decrease in CH\(_4\) concentration, despite the fertilizer treatment level. In addition, no short-term inhibition of rising CH\(_4\) concentrations in the 5 cm layer could be observed following the N fertilizer applications.

A third explanation for the lack of N inhibition could be the N-turnover characteristics of the semi-wet, fertile grassland that immobilizes large amounts of applied NH\(_4^+\) quickly (within 1-2 days, Müller et al., 1997) within the top 5 cm of the soil. Usually the NH\(_4^+\) concentrations were very low (<10 µg N g\(^{-1}\) soil) during the whole year, decreasing to about zero with soil depth in all treatments (Kammann et al., 1998). The mineral N is taken up efficiently by the highly productive plant biomass at the study location (>6000 kg dry mass ha\(^{-1}\) year\(^{-1}\) without N fertilizer application, Grünhage et al., 1996). Therefore, the concentrations of NH\(_4^+\) itself, as well as possible inhibiting intermediates such as NO\(_2^-\) (King and Schnell, 1994; Conrad, 1996) might have decreased so quickly following N application that no inhibitory effects could develop.

4.2. The influence of soil moisture on CH\(_4\) oxidation
Soil moisture has often been described as the dominant independent variable determining methane oxidation rates (Jones and Nedwell, 1993; Castro et al., 1994; Castro et al., 1995; Boeckx and van Cleemput, 1996; Mosier et al., 1997). Increasing soil moisture is considered to slow the diffusive methane (and O2) transport from the atmosphere to the water-film covered microbial population (Whalen and Reeburgh, 1990; Koschorreck and Conrad, 1993; Mosier et al., 1997). For example, Castro et al. (1994) found that 78% of the CH4 oxidation rates in a forest soil could be explained by linear correlation with the soil moisture values. As expected from those findings, there was a significant correlation in our study between the average soil moisture of the treatment plots and the corresponding average CH4 oxidation rates. A wetter treatment plot oxidized on an average less CH4 than a drier one (Figure 1).

However, on a day-to-day basis, there was only a very weak correlation between decreasing CH4 oxidation rates and increasing soil moisture values (Table 2). In contrast, the soil air CH4 concentrations exhibited a (also weak) negative correlation, i.e. decreasing CH4 concentrations when soil moisture increased. Such a finding could only be due to a higher CH4 oxidation rate with increasing moisture in the surrounding soil. Moreover, correlation of the CH4 concentrations at 5 cm depth was strongest when soil moisture values >50 vol.% (i.e. 65-70% WFPS, Water-Filled Pore Space) were excluded. Above a threshold value of 50 vol.% (grey portion in Figure 6a) there was no influence of the soil moisture on the CH4 concentrations (r = 0.09).

Taken together, the results do not support the common perception of a growing soil water film restricting CH4 (and O2) fluxes from the atmosphere to the CH4 oxidizing soil microorganisms. The soil air probe measurements demonstrated that the length of the CH4 oxidizing vertical soil column, in combination with the duration of the CH4 production at greater depths contribute to the magnitude of the CH4 fluxes into the soil. Whalen et al. (1996) and Mosier et al. (1997) described increasing CH4 production as a cause for declining CH4 oxidation rates in ecosystems with frequent CH4 production. In the present study, the grassland soil has a large CH4 production potential, as demonstrated by the anaerobic incubations of samples taken from an area that had not been flooded at least since 1993. The measured CH4 production rates (max. 19.6 µg CH4 g⁻¹ dwt day⁻¹) were comparable to the rates reported by Wassmann et al. (1998) (max. 13.6 µg CH4 g⁻¹ dwt day⁻¹) from tropical rice soil slurries, incubated at a higher temperature (25°C). Methanogenic bacteria in methane producing ecosystems such as bogs, swamps or rice paddies are known to survive O2 conditions unfavorable to CH4 production (Conrad, 1989; Knowles, 1993). Peters and Conrad (1995) were even able to induce CH4 production in desert soil slurries by anaerobic incubation.

Our data indicate that CH4 production can be induced quickly in grassland soils after the onset of favorable conditions, e.g. 3-4 weeks of anaerobic incubation at 20°C or in-situ, 3
weeks after the rise in the water table at about 10°C soil temperature. The soil air probes revealed that during certain time periods CH₄ production in near-surface soil horizons occurred, which means shorter vertical CH₄ oxidizing soil columns above the CH₄ producing soil layer. The duration of the periods with concentrations above ambient at 5 cm depth was long enough in the wettest plot to yield an average concentration of 3.9 µl l⁻¹ CH₄ for the measurement period.

Therefore it can be concluded that in the grassland ecosystem investigated here, the depth of the CH₄ producing horizon (the length of the CH₄ oxidizing vertical soil column), in combination with the duration of subsurface CH₄ production contribute to the overall methane oxidation rate at the soil surface. CH₄ production at greater depth might be more common in many other non-aquatic ecosystems than previously thought. If so, any changes in soil conditions, e.g. an increasing input of easily degradable organic carbon by plants, under elevated atmospheric CO₂ (Hungate et al., 1997; Dacey et al., 1994; Megonigal and Schlesinger, 1997) may increase the magnitude and frequency of CH₄ production periods and hence, diminish the CH₄ soil sink capacity of an ecosystem even if it does not usually emit methane.

In contrast to the weak correlation between CH₄ flux rates/CH₄ concentrations and the soil moisture, a significant restriction of CH₄ oxidation with declining soil moisture (desiccation) was observed in this study. Methane oxidation rates decreased with declining soil moisture from more than 25 µg C m⁻² h⁻¹ to 6 µg C m⁻² h⁻¹ (i.e. 76% decline). Restriction of CH₄ oxidation has been described by Striegl et al. (1992) for desert soils and also for more comparable ecosystems; Whalen and Reeburgh (1996) for boreal soils and Boeckx and van Cleemput (1996) for landfill cover soils. All these authors attribute the declining CH₄ oxidation rates to biological water stress at low soil moisture. Boeckx et al. (1997) suggest that in very wet soils slight desiccation may cause accelerated N mineralization, hence inhibiting CH₄ oxidation. This explanation seems unlikely in our case when the lack of in situ N inhibition is considered. However, a water content of 40-50% water-filled pore space (WFPS) where CH₄ oxidation rates started to decline, should not be normally considered as water stress. Thus it can be assumed that in the semi-wet grassland ecosystem, the majority of the (active) CH₄ oxidizer population is associated with larger soil particles such as sand grains (sand grains covered by microbial biofilms, Conrad, 1996; Hütsch 1998c) or is mainly located at the side walls of soil macropores. This hypothesis is in line with the findings of Born et al. (1990) and Boeckx et al. (1997) who demonstrated that higher CH₄ oxidation rates were always found in coarse textured sandy soils, whereas the lowest rates occurred in finer textured clay soils. Restriction of methane oxidation might hence occur quite frequently in temperate humid grasslands.
4.3. \( \text{CH}_4 \) oxidation as a 'biofilter' function

The silicone probe measurements revealed that situations with above-ambient \( \text{CH}_4 \) concentrations in the topsoil might frequently occur in semi-wet, C-rich ecosystems. Nevertheless, no net \( \text{CH}_4 \) fluxes to the atmosphere were measurable in such situations. It is known from semi-aquatic ecosystems such as rice paddies that up to 80% of the \( \text{CH}_4 \) produced is oxidized in the aerobic rhizosphere, the aerenchyma of the plants, in the aerobic surface water film or top few millimeters of the soil before it escapes to the atmosphere (Conrad and Rothfuss, 1991; Butterbach-Bahl et al., 1997; Watanabe et al., 1997). Whalen et al. (1990, 1996) showed that \( \text{CH}_4 \) oxidation prevented the flux of \( \text{CH}_4 \) to the atmosphere in a landfill cover soil and in a wet tundra soil.

Our findings demonstrate that this is also true for a 'sink' ecosystem, which normally has a negative \( \text{CH}_4 \) flux balance throughout the year. We therefore concur with Whalen et al. (1996) and Hütsch (1998b) that the process of biological \( \text{CH}_4 \) oxidation should not only be regarded as an important global \( \text{CH}_4 \) sink, but also as a very effective 'biofilter'. Such a filter modifies \( \text{CH}_4 \) fluxes which otherwise would reach the atmosphere, even in ecosystems that are not expected to produce methane such as the grassland investigated here.

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Figures 1-8

Figure 1: Time series of the average CH$_4$ flux rates between two treatment plots and the atmosphere and corresponding soil moisture and temperature.

Figure 2: Declining CH$_4$ oxidation rates in the 40 kg N, 'dry' treatment with soil desiccation during a drought (July to September 1997) and recovery following the first rain in October 1997.
**Figure 3**: Box-Plots of the CH$_4$ flux rates of all treatments during the time period shown in Figure 1. Below, the fertilization level and the average soil moisture during the measurement period is given.

**Figure 4**: a) Correlation between mean soil moisture, or b) annual N fertilizer application, and corresponding mean CH$_4$ flux rates for the measurement period.
<table>
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<th>Date</th>
<th>CH4 production rate (µg CH4-C * h⁻¹ * 100 cm⁻³)</th>
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**Figure 5:** Methane production from incubated grassland soil cores (top 1-5 cm), carried out at 20°C. The 10 cores with intact soil structure were taken from a grassland area of 0.25 m² that is very unlikely to have been exposed to flooding for the last decade. Only the period of flow through sampling is shown (see text).
Figure 6: a) CH$_4$ concentrations in 5 cm depth in the 40 kg 'dry' treatment during 1998 - 1999. In the grey area, the soil moisture values were above 50 vol.%, whereas in the white section they were below 50 vol.%. b) Correlation of all 5 cm CH$_4$ values with soil moisture (dashed line, $p = 0.005$) and of the below-50% soil moisture values with corresponding CH$_4$ concentrations (straight line, $p < 0.001$). The probes at 15 and 30 cm depth exhibited similar fluctuations compared to the 5 cm probe, but with lower CH$_4$ concentration levels.
**Figure 7:** a) Soil (air) methane concentrations in different depths (5, 15, 30 and 50 cm) in the 'wet' 40 kg N treatment. b) Daily precipitation sums (black bars) and water table heights (grey bars), precipitation data until Dec. 2nd 1999. The data gap in the March/April 1999 precipitation values was caused by instrumental failure; the missing water table values are associated with holidays.
Figure 8: a) Methane concentrations in various treatment plots in 5, 15 and 30 cm depth averaged over the measurement period (capped lines: standard errors). The concentrations varied especially during periods with changes between methane oxidation and production. Dotted line: Atmospheric CH₄ concentration b) For easier comparison, the ‘wet’ treatment is displayed together with the 80 kg N treatment also shown in (a).
Anhang 4: Unveröffentlichte Vorversuchsergebnisse

Stichpunktartig zusammengefasste hypothesenrelevante Vorversuchsergebnisse, und (hauptsächlich) unveröffentlichte Vorversuchsergebnisse
A.4 Unveröffentlichte und hypothesenrelevante Vorversuchsergebnisse

In diesem Anhang werden kurz unveröffentlichte Ergebnisse der Vorversuchsperiode vorgestellt, die für die Hypothesenbildung zur Kernfrage der vorliegenden Arbeit von Bedeutung waren. Wenn sie hypothesenrelevant sind, werden auch die bereits veröffentlichten Ergebnisse kurz und stichpunktartig vorgestellt, für Details und Abbildungen wird aber auf die jeweiligen Veröffentlichungen (d.h. Anhang 1 bis 3) verwiesen.

A.4.1 Lachgas-Flüsse im Lindener Grünland

Der Verlauf der N\textsubscript{2}O-Flüsse im Untersuchungszeitraum 1996/1997 wurde für die einzelnen Düng- und Schnittbehandlungen in und \textsc{Kammann et al.} (2000b) ausführlich dargestellt und wird daher an dieser Stelle nicht erneut gezeigt.

![Boden temperaturverlauf](image)

\textbf{Abbildung A.4-A:} Boden temperaturverlauf in 5 cm Tiefe bei den mit 40 kg N ha\textsuperscript{-1} gedüngten Parzellen. Schwarze Linie: Differenz der trockenen und der feuchten Parzelle (Temp.(2.5) – Temp.(2.4)). Der Vergleich zeigt, daß sich die trockenerere Parzelle rascher abkühlt bzw. erwärmt als die feuchte Parzelle.

\textbf{Wichtig für die N\textsubscript{2}O-Flußmessungen im FACE-Experiment:}

- N\textsubscript{2}O-Emissionen der beiden mit 40 kg N ha\textsuperscript{-1} gedüngten Parzellen ganzjährig auf relativ niedrigem Niveau (zwischen 10 und 30 µg N m\textsuperscript{2} h\textsuperscript{-1}), mit Ausnahme des 3-4-wöchigen Zeitraums Düngung (April 1997)

- Die feuchtere der beiden 40-kg-Parzellen (2.4) zeigte fast immer die höheren N\textsubscript{2}O-Flußraten. Jahresbilanz: N\textsubscript{2}O-N-Verluste der feuchten 40-kg-N-Parzelle fast doppelt so hoch wie die der trockenen 40-kg-N-Parzelle (0,38 \textit{versus} 0,75 kg N ha\textsuperscript{-1} a\textsuperscript{-1}).
• Bis zu einer N-Applikation von 120 kg ha\(^{-1}\) a\(^{-1}\): Wochen nach der ersten Düngung = Hauptemissionsperiode. Erst ab N-Applikation von 240 und 400 kg N ha\(^{-1}\) a\(^{-1}\): Emissionen ganzjährig auf höherem Niveau.


• KCl-Bodenextraktionen: sehr rasche N-Aufnahme nach Düngung; ganzjährig sehr niedrigen Nitrat- (unter 0,01 ppm) und Ammoniumkonzentrationen (unter 6 ppm) bis zu einer N-Düngungsstufe von 120 kg N ha\(^{-1}\) a\(^{-1}\) (vgl. KAMMANN et al. 1998)

• Die \(\text{N}_2\text{O}\)-Emissionen konnten durch eine Erhöhung der Schnittfrequenz z.T. signifikant verringert werden (Tab. A.4-A). Die Reduktion trat vor allem während der Vegetationsperiode auf. In der Jahresbilanz betrug die Reduktion fast 30\%. Das Emissionsmuster der Parzelle 80 kg N ha\(^{-1}\) a\(^{-1}\) + 2 Schnitte ähnelte stärker der mit 120 kg N ha\(^{-1}\) a\(^{-1}\) gedüngten Parzelle als der gleich gedüngten 3-Schnitt-Variante (Graphiken in KAMMANN et al. 2000b). In diesem Ergebnis drückte sich (vgl. Diskussion bei KAMMANN et al. 1998 und Zitate hierin) die Fähigkeit der Pflanzen aus, mit der mikrobiellen Biomasse um verfügbaren Stickstoff zu konkurrieren.

Tabelle A.4-A: Einfluß der Schnittfrequenz auf die \(\text{N}_2\text{O}\)-Emissionen (in \(\mu\)g N m\(^{-2}\) h\(^{-1}\)): Mittlere \(\text{N}_2\text{O}\)-Flußraten ± Standardabweichung (in Klammern: Median) der betreffenden Zeitperiode. Blau, in Klammern: Jahres-Emissions-summe der 3-Schnitt-Behandlung in Prozent des 2-Schnitt-Werts. Unterschiedliche Indizes innerhalb einer Zeitperiode zeigen eine signifikante Differenz zwischen den Behandlungen an (Man-Whitney- oder Kruskal-Wallis-Test mit anschließendem Student-Newman-Keuls-Test, alle Tests \(P < 0,05\)).

<table>
<thead>
<tr>
<th>Zeitperiode</th>
<th>80 kg N ha(^{-1}) a(^{-1}) 2 Schnitte a(^{-1})</th>
<th>80 kg N ha(^{-1}) a(^{-1}) 3 Schnitte a(^{-1})</th>
<th>80 kg N ha(^{-1}) a(^{-1}) 6 Schnitte a(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eine Jahresperiode (1.10.96 – 7.10.97)</td>
<td>16,9 ± 4,5 (6,56) (a)</td>
<td>11,6 ± 2,8 (6,22) (b)</td>
<td>Messbeginn: 07.05.1997</td>
</tr>
<tr>
<td>Winter 1996/1997 (20.12.96 - 01.04.97)</td>
<td>11,9 ± 2,1 (11,4) (a)</td>
<td>12,6 ± 2,3 (9,29) (a)</td>
<td>Messbeginn: 07.05.1997</td>
</tr>
<tr>
<td>Sommer 1997 (07.05. – 07.10.97)</td>
<td>11,7 ± 2,6 (5,53) (a)</td>
<td>14,6 ± 7,3 (4,94) (a)</td>
<td>4,07 ± 0,6 (3,14) (b)</td>
</tr>
<tr>
<td>Jährl. (\text{N}_2\text{O})-N Emissions-summe (kg N ha(^{-1}) a(^{-1})*)</td>
<td>1,186 (100%)</td>
<td>0,845 (71,3%)</td>
<td>(kein vollständ. Jahr)</td>
</tr>
</tbody>
</table>

Jahres-Zeitraum: \(n = 77\); \(n = \text{Mittelwert der beiden Maxikammern pro Parzelle und Meßtag.} \)*) die jährliche Integrationsperiode für die 2-Schnitt-Parzelle war zwei Wochen kürzer (vgl. 2.5).

• In der Winter-Periode 1996/1997 traten während strengen Frosts hohe \(\text{N}_2\text{O}\)-Flüsse auf; es konnte durch Zusatzversuche gezeigt werden, daß im scheinbar hartge-
frorenen Boden biologische Denitrifikationsaktivität zu hohen N₂O-Emissionen führte (vgl. MÜLLER et al. 1997; KAMMANN et al. 1998, KAMMANN et al. 2000b, und Zitate hierin). Argumente und Belege:

- Lindener Grünlandboden (relativ hoher organischer C- und Ton-Gehalt) bei Temperaturen unter 0°C nur scheinbar fest gefroren: TDR-Sensoren zeigten ungefrorenen Restwassergehalt an (Abb. A4-B, -C)
- Inkubation von mit Stichsäge und Beitel aus dem Grünlandboden geschnittenen gefrorenen Bodensäulen bei unter 0°C: hohe N₂O-Flüsse in Abhängigkeit von der verfügbaren N-Form (v.a. Denitrifikation > 90 %): N₂O nicht aus ungefrorenen Bodentiefen

Abb. A.4.-B: Situation im Grünlandboden ("trockene" Parzelle 2.5, 40 kg N ha⁻¹ a⁻¹) nach 3 bis 4 Wochen Frosteinwirkung: Die 0°C-Grenze war unter 20 cm Tiefe gesunken. Daher konnte davon ausgegangen werden, daß die TDR-Sensoren ausschließlich das ungefrorene Bodenwasser anzeigten (PATTERSON & SMITH 1981; PATTERSON & SMITH 1984).


A.4.2 N₂O-Konzentrationen in der Bodenluft

Abbildung A.4-D: Verlauf des Grundwasserstands sowie Tages-Niederschlagssummen im Block 4 (feuchte Parzellen, Pegel 6) während der Bodenluftsonden-Messungen. Fehlende Werte: Feiertage. Messungen stets werktags; bis zu 2 fehlende Tage (Sa; So) wurden extrapoliert.

Anhang 4

Abbildung A.4-E: Verlauf der N₂O-Konzentrationen in der Bodenluft der beiden hochgedüngten Parzellen (240 und 400 kg N ha⁻¹ a⁻¹) in 5 cm Tiefe (a), 15 cm Tiefe (b) und 30 cm Tiefe (c).

Kleine inserierte Graphiken: Mittlere N₂O-Konzentrationen + Standardfehler im Meßzeitraum für die Bodenluftsonden-Parzellen. 40 T = trockene Parzelle 2.5, 40 F = feuchte Parzelle 2.4. Graue gepunktete Linie: Atmosphärische N₂O-Konzentration (310 ppb). Unterschiedliche Indizes: Signifikante Unterschiede zwischen den Parzellen (Kruskal-Wallis-Anova, Dunn's Test, P < 0,05)

Im Prinzip gilt der gleiche Verlauf für die N₂O-Bodenluft-Konzentrationen der geringer gedüngten Parzellen (40 bis 120 kg N ha⁻¹ a⁻¹; Abb. A.4-F), nur waren die erreichten Maximalkonzentrationen sehr viel geringer: Während sie bei den 240 und 400-kg-N-Flächen im Herbst 1998 maximal 334 und 388 ppm betrugen¹ (30 cm Tiefe), lagen die Maximalwerte der geringer gedüngten Flächen (15 und 30 cm Tiefe) alle zwischen 12 und 18 ppm N₂O, d.h. etwa um den Faktor 20 niedriger.

Abbildung A.4-F: N₂O-Konzentrationen in der Bodenluft der mit 40 kg N ha⁻¹ a⁻¹ gedüngten Parzellen (links) und der mit 80 und 120 kg N ha⁻¹ a⁻¹
gedüngten Parzellen (rechts) in 5, 15 und 30 cm Tiefe im Meßzeitraum vom 20.08.1998 bis zum 06.01.2000.
Tabelle 3.3-A: \( \text{N}_2\text{O} \)-Konzentrationen (ppm) in der Bodenluft unterschiedlich gedüngter Parzellen in verschiedenen Tiefen in der Zeit vom 20.08.1998 bis 06.01.2000, ± Standardabweichung.

<table>
<thead>
<tr>
<th>Tiefe</th>
<th>40 kg N, T</th>
<th>40 kg N, F</th>
<th>80 kg N</th>
<th>120 kg N</th>
<th>240 kg N</th>
<th>400 kg N</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 cm</td>
<td>0,46 ± 0,37</td>
<td>0,61 ± 0,69</td>
<td>0,46 ± 0,38</td>
<td>0,67 ± 0,90</td>
<td>0,99 ± 1,98</td>
<td>0,91 ± 1,29</td>
</tr>
<tr>
<td>15 cm</td>
<td>0,72 ± 1,00</td>
<td>1,69 ± 2,39</td>
<td>1,06 ± 1,89</td>
<td>1,28 ± 2,15</td>
<td>10,09 ± 42,29</td>
<td>5,25 ± 12,57</td>
</tr>
<tr>
<td>30 cm</td>
<td>1,36 ± 2,21</td>
<td>1,48 ± 3,59</td>
<td>1,39 ± 2,91</td>
<td>1,44 ± 3,13</td>
<td>6,87 ± 25,97</td>
<td>14,7 ± 48,97</td>
</tr>
<tr>
<td>50 cm</td>
<td>—</td>
<td>1,75 ±4,51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Die \( \text{N}_2\text{O} \)-Konzentrationen in der Bodenluft bestätigen somit den zuvor bereits abgeleiteten Sachverhalt: Nur bei ausreichender Nitratverfügbarkeit (hier: auf den hochgedüngten Parzellen) ist eine sehr hohe \( \text{N}_2\text{O} \)-Produktion (und in Folge hohe \( \text{N}_2\text{O} \)-Emission) zu erwarten. Selbst bis zu einer Düngung von 120 kg N ha\(^{-1}\) a\(^{-1}\) jedoch blieben die Spitzenwerte immer unter 20 ppm \( \text{N}_2\text{O} \) (Abb. A.4-F).

Die aus der Meßperiode resultierenden mittleren Bodenluft-\( \text{N}_2\text{O} \)-Konzentrationen (Tab. A.4-D) stimmen mit den Ergebnissen anderer Autoren überein: In 5 cm Tiefe lagen sie zwischen 460 bis 670 ppb \( \text{N}_2\text{O} \) (bis 120 kg N ha\(^{-1}\) a\(^{-1}\)). Selbst bei den beiden höchsten Düngungsstufen betrugen die Mittelwerte in 5 cm Tiefe 990 und 910 ppb. Diese Werte entsprechen denen von SOTOMAYOR & RICE (1999), die in nicht-kultiviertem Prärieboden und kultiviertem Ackerboden für einen vergleichbaren Zeitraum Mittelwerte von 403 und 1090 ppb \( \text{N}_2\text{O} \) angeben. In weniger effektiven Nitrat-absorbierenden Ackerböden (Maisanbau, 150 kg N ha\(^{-1}\) a\(^{-1}\) lagen die \( \text{N}_2\text{O} \)-Mittelwerte in 5 cm Tiefe hingegen höher, bei 1320 und 1250 ppb \( \text{N}_2\text{O} \) (zwei verschiedene Beprobungsperioden, BURTON & BEAUCHAMP 1994).

Der hier gemessene Jahresverlauf der \( \text{N}_2\text{O} \)-Konzentrationen unterschied sich mit seinen Herbst- und Frühjahrsmaxima bei jeweils guten Denitrifikationsbedingungen jedoch deutlich von Jahressängen, die SOTOMAYOR & RICE (1999) oder CATES & KEENEY (1987) in den Prärieböden feststellten: Hier stiegen die Bodenluft-Konzentrationen im Frühjahr und Sommer mit steigenden Bodentemperaturen sehr deutlich an, was die Autoren v.a. auf Nitrifikation als den dominierenden \( \text{N}_2\text{O} \)-Produktionsprozeß zurückführen. Obwohl der Anteil des Nitrifikations-\( \text{N}_2\text{O} \) an den Gesamt-\( \text{N}_2\text{O} \)-Emissionen im Sommer durchaus 60 % betragen kann (vgl. 5.8), sind die \( \text{N}_2\text{O} \)-Emissionen in dieser Zeit jedoch verhältnismäßig gering.

Die Bodenluft-Sonden-Messungen bestärken daher die vorherigen Befunde dazu, was die Jahresbilanzen im Lindener Grünland gestaltet: Gute Denitrifikationsbedingungen (kurzfristige hohe Wasserstände bzw. Bodenfeuchte) bei möglichst hoher Nitratverfügbarkeit, z.B. nach der Düngung, ohne Pflanzenwurzelkonkurrenz (d.h. während der Vegetationsruhe) oder bei Frier-Tau-Zyklen.
A.4.3 Die CH4-Flüsse im Lindener Grünland

- Methanoxidation verringerte sich nach N-Düngergaben im Gegensatz zu den Befunden zahlreicher anderer Autoren nicht (zu den folgenden Punkten siehe KAMMANN et al. 2001b in Anhang 3 und Zitate hierin).
- Zwischen der Höhe der Methanoxidation einer Parzelle und der jährlich applizierten N-Menge konnte keine Beziehung gefunden werden.
- Dies spiegelte sich nicht in einer guten Korrelation zwischen dem Verlauf der Bodenfeuchte und der Methanoxidationsraten innerhalb einer Parzelle wieder (R² maximal 0,32), wie sie häufig von anderen Autoren beschrieben wird.
- Ebenfalls schlecht: Die Korrelation der Bodenluft-CH4-Werte in 5 cm Tiefe oder tiefer mit der tätig gemessenen Bodenfeuchte. Ausnahme: Bodenfeuchtwerte unter 50 Vol.-% (R² = 0,6); hier war die Korrelation zwischen Bodenluft-CH4 und Bodenfeuchte negativ (CH4-Konzentrationen sinkend mit steigender Bodenfeuchte).
- Rückgang der CH4-Oxidationsraten im Spätsommer 1997 durch Wasserstreich

A.4.4 Methankonzentrationen in der Bodenluft


Herrschten reduzierende Verhältnisse für die CH4-Produktion, so lagen die N2O-Konzentrationen stets um Null. Zugleich war in solchen Proben im FID-Chromatogramm der O2-Peak verschwunden. Bei denjenigen Punkten in Abb. A.4-H, bei denen sowohl die N2O- als auch die CH4-Werte erhöht waren, lagen die Sonden, in denen dies gemessen wurde, nie unter dem Wasserspiegel. Daher ist anzunehmen, daß aufsteigendes CH4 in N2O-produzierende Horizonte mit höherem Redoxpotential gelangte.

Während aller Phasen mit oberflächennahen (5 cm Tiefe), über-atmosphärischen CH4-Konzentrationen wurden wiederholt mit beiden Gasauffanghauben auf Parzelle 2.4 und auch auf der Nachbarparzellen 7.4 und 8.4 CH4-Flußmessungen durchgeführt. Der Abstand zwischen den eingebauten Bodenluftsonden, deren Werte in Abb. A.4-G dargestellt sind, und den Bodenrahmen der Parzelle 2.4 betrug nur 1 bis 1,5 m. Dennoch
konnte zu keinem Zeitpunkt eine Netto-CH$_4$-Abgabe in die Atmosphäre gemessen werden – nicht einmal bei 51 ppm CH$_4$ in 5 cm Tiefe (vgl. A.4.5).

Abbildung 3.2-A: Methan-Konzentration in der Bodenluft der feuchtesten Parzelle 2.4 (40 kg N ha$^{-1}$ a$^{-1}$) in 5, 15, 30 und 50 cm Tiefe (obere Graphik) und Grundwasserstand sowie Tagesniederschläge dieses Zeitraums (untere Graphik). Man beachte, daß die Sonde in 50 cm Tiefe lange Zeit unter Wasser lag.

aus Deponieböden bzw. aus feuchtem Tundra-Böden regelrecht "verhinderte". Die vorliegenden Ergebnisse zeigen, daß der als "Biofilter" wirkende Prozeß der Methanoxidation möglicherweise häufiger als bislang angenommen die CH₄-Flüsse in die Atmosphäre modifiziert bzw. vermindert.

![Abbildung A.4-H: Beziehung zwischen den zeitgleich gemessenen CH₄- und N₂O-Konzentrationen in der Bodenluft der Parzelle 2.4 (40 kg N, feucht) in verschiedenen Tiefen.](image)

Jede Verminderung der CH₄-Oxidationskapazität der Böden, sei es durch Landnutzungseinflüsse wie Rodung, Düngung oder Pflügen, oder auch möglicherweise durch erhöhtes CO₂, könnte daher im globalen Maßstab einen größeren Einfluß auf die Jahresbilanzen der CH₄-Flüsse von methanproduzierenden Ökosystemen haben als bislang angenommen.

### A.4.5 Die closed-chamber-Methode und Grenz-CH₄-Flüsse: Methodenkritik

Anhang 4

Der Wasserstand im Boden war während dieser Periode oberflächennah; da der Pegel 6, von dem die Werte stammen, in ca. 10 m Entfernung zur Parzelle 2.4 liegt, war der Wasserstand dort wahrscheinlich sogar höher, denn Parzelle 2.4 war die feuchteste der Reihe 4 (Beobachtung: der Untergrund setzte dort beim Laufen Wasser frei). Während der Meßtage sank der Grundwasserspiegel langsam und beständig (Abb. A.4-I).


Auftriebsverhalten von Schwingrasen in borealen Mooren beschrieben, oder auch vorstellbar wie in einem Gärballon. Da diese aber nicht frei wegdiffundieren konnten, sondern von den geschlossenen Hauben über den bedeckten Bodenflächen "festgehalten" wurden, konnten die CH$_4$-Oxidierer die hohen Konzentrationen wieder reduzieren (bis zur nächsten aufsteigenden Methanblase).

Schon eine geringfügig stärkere Aerobisierung des obersten Bodenhorizonts (absinkender Grundwasserstand, sich entleerende, zuvor wassergefüllte Bodenporen) bis zum Mittag des folgenden Tages reichte aus, um das höchstwahrscheinlich immer noch von unten aufsteigende Methan wieder vollständig zu oxidieren, bevor es die Atmosphäre (auf diese Weise) hätte erreichen können.

Daher wäre es denkbar, daß mit den geschlossenen Hauben CH$_4$-Emissionen im Grenzbereich zwischen CH$_4$-Produktion in der Tiefe und Oxidation im obersten Horizont permanent unterschätzt werden.

Die im vorherigen Punkt geschilderten Befunde implizierten bereits die Bedeutung des Grundwasserstandes bzw. der Lage des CH$_4$-produzierenden Horizonts für die Stärke der an der Bodenoberfläche gemessenen CH$_4$-Oxidationsraten. In der Tat spiegelten die über den Untersuchungszeitraum gemessenen mittleren Bodenluft-Profile die CH$_4$-Oxidationskapazität eines Standorts wider (KAMMANN et al. 2001b): Je näher an der Bodenoberfläche der (gemittelte) "Methanhorizont" mit überatmosphärischen CH$_4$-Werten lag, desto geringer waren im Mittel die CH$_4$-Oxidationsraten der betreffenden Parzelle. Im untersuchten Lindener Grünland muß dieser Effekt bei Betrachtung der CH$_4$-Oxidationsraten mit in Betracht gezogen werden.

A.4.6 Das Methanproduktions-Potential des Grünlandbodens

In einem Zusatzexperiment wurde durch Inkubation von Bodenkernen mit ungestörter Bodenstruktur (vgl. WACHINGER et al. 2000) das Methanproduktionspotential des Lindener Grünlandbodens näher untersucht. Die Initiation dieses Versuchs erfolgte etwa zeitgleich mit den hohen Niederschlägen im Herbst 1998 (Probennahme am 14. Oktober). Dabei wurden am Nordrand der Parzelle TF (bzw. VII; vgl. Abb. 2.4-A) auf einer ca. ¼ m² großen Fläche aus 1 bis 5 cm Tiefe 10 Bodenkerne mit Stechzylindern entnommen, auf Feldkapazität entwässert, und, wenn vorhanden und nicht entfernbar, mitsamt Regenwürmern anaerob (zunächst statisch) inkubiert. Nach etwa drei bis vier Wochen begann in den meisten Kernen die Methanproduktion. Diejenigen Kerne, die zu diesem Zeitpunkt keine CH$_4$-Produktion zeigten, wiesen auch bis zum Ende des CH$_4$-Experiments nach etwa 3 Monaten noch keine CH$_4$-Produktion auf (Abb. A.4-K). Sie zeigten aber eine (anaerobe) CO$_2$-Produktion, die in der gleichen Größenordnung wie die der hochproduktiven CH$_4$-Kerne lag (Abb. A.4-L). Nach etwa 6 Wochen anaeroben Inkubation waren einige CH$_4$-Werte bei einmalig Durchspülen eines Kerns mit 50 ml N$_2$ (= 50 ml Probe) so hoch, daß die GC-Nachweisbarkeit nach oben hin überschritten
wurde (mehrere hundert ppm CH₄ mit stärkster Signaldämpfung). Daher wurde der Versuchsansatz auf die Durchströmungstechnik umgestellt (Messungen ab Anfang Dezember; Ergebnisse Abb. A.4-K).

Drei Methanproduktions-Typen traten auf (Abb. A.4-K): Bei Typ 1 (Kerne M2, M3, M4 und M7) stieg die CH₄-Produktion von Anfang bis Ende des Experiments kontinuierlich an, was auf eine wachsende Methanogenen-Population bei reichlich vorhandenem organischem Substrat hindeutet. Bei Typ 2 sank die Methanproduktion im Laufe der Inkubation, v.a. während des zweiten Monats (Kerne M8 und M9). Hier verringerte sich möglicherweise die Verfügbarkeit des vorhandenen Substrats für die methanogene Lebensgemeinschaft relativ rasch. Kern M10 zeigte ein intermediäres Verhalten, einen leichten Anstieg bis zur Hälfte der Inkubationszeit und danach konstante Produktion. Der Rest der inkubierten Kerne produzierte niemals Methan, wohl aber CO₂ (Typ 3; Abb. A.4-K). Die höchsten gemessenen CH₄-Produktionsraten lagen bei 55 µg CH₄-C 100 cm⁻³ h⁻¹ (entsprechend 19,6 µg CH₄ g⁻¹ Tag⁻¹ (Bodentrockengewicht), wenn mit einer Lagerungsdichte von 0,9 g cm⁻³ umgerechnet wird). Diese Rate ist vergleichbar mit Werten, die bei 25 °C an anaerob inkubierten tropischen Reisböden gemessen wurden (z.B. 13,6 µg CH₄ g⁻¹ Tag⁻¹, WABMANN et al. 1998).

Abbildung A.4-K: Methanproduktion der anaerob inkubierten Bodenkerne (Durchströmungstechnik). Wassergehalt = Feldkapazität. a) anfangs hochproduktive Kerne; b) gering oder nicht CH₄ produzierende Kerne. (M10 ist zur besseren Vergleichbarkeit in a und b dargestellt).
Interessant ist die Korrelation zwischen den CH₄-Produktionsraten, die von Versuchsbeginn an hoch waren, mit dem Vorhandensein von Regenwürmern: In den Kernen M3, M4, M7 M8 und M9 wurden bei Beginn der anaeroben Inkubation Regenwürmer mit eingeschlossen (die sich nicht "retten" ließen). Es läßt sich aber nicht gänzlich ausschließen, daß auch andere Kerne kleine Regenwürmer enthielten, die evtl. bei Versuchsbeginn nicht sichtbar darin eingeschlossen wurden.


Generell ist eine hohe CH₄-Produktionsrate (bei Anaerobiose) aber vor allem an das Vorhandensein von frischem organischem Material geknüpft (vgl. WACHINGER et al. 2000). Einer der inkubierte Kerne, M2, wurde in Kooperation mit Herrn Prof. Dr. Alzen (Leiter der Kinderradiologie, Gießen) und Frau Dr. Gisela Wachinger (Universität Hohenheim) am Computertomographen sowie über Mikrotomschnitte und nachfolgende enzymatische Tests eingehender untersucht (Abb. A.4-L). In Kern M2 fanden sich keine großen Regenwurmgänge (Abb. A.4-L, d), die auf einen eingeschlossenen Wurm hindeuteten (das Vorhandensein von Kot ist natürlich nicht auszuschließen). Dagegen wurde eine größere Wurzelstruktur identifiziert (Pfeil in Abb. A.4-L, d) und diese korrelierte mit hoher enzymatischer Aktivität in der entsprechenden Schichttiefe des Kerns.

Am 11. Februar wurde die isotherme Inkubation der Bodenkerne beendet, und sechs CH₄-produzierende Kerne wurden im Zwischenraum natürlichen Temperatschwankungen ausgesetzt (vgl. 2.13; Abb. A.4-M). Zunächst reduzierten sich bei einer Absenkung der Temperatur von 20 auf 5,0 °C die CH₄-Produktionsraten drastisch um im Schnitt 94,4 %. Berechnet man aus diesem Temperaturschritt den Q₁₀-Wert (s.u.), so resultiert ein untypisch hoher Wert von 6,9 (Mittel der 6 Kerne).


\[ Q_{10} = \left( \frac{k_2}{k_1} \right)^{(10/(T_2-T_1))} \]

mit: \( k_1, k_2 \): Raten bei Temperaturen \( T_1, T_2 \)

d.h. für die Berechnung des Q₁₀ in Tab. A.4-C resultierte:

\[ Q_{10} = \frac{k_2}{k_1} \]

Tabelle A.4-C: Berechnung des Q₁₀ der CH₄-Produktion (etabliert bei 20 °C); Lineare Regression der CH₄-Produktionsraten vom 11./12. Feb. 1999 in Abhängigkeit von der Temperatur (Daten Abb. A.4-M); Rate (µg C 100 cm⁻³ h⁻¹) = b[1] * Temp. (°C) + b[0]; R² = Bestimmtheitsmaß der linearen Regression; \( k_1, k_2 \): Produktionsraten bei 2 und 12 °C, berechnet nach der o.g. Gleichung. Berechnung des Q₁₀ siehe Text.

<table>
<thead>
<tr>
<th>Bodenkern</th>
<th>Koeff. b[1]</th>
<th>Koeff. b[0]</th>
<th>R²</th>
<th>( k_2 ) Rate (2 °C)</th>
<th>( k_2 ) Rate (12 °C)</th>
<th>Q₁₀ (2 → 12 °C)</th>
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<tbody>
<tr>
<td>M2</td>
<td>0,4233</td>
<td>1,0245</td>
<td>0,77</td>
<td>1,87</td>
<td>6,10</td>
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<td>0,78</td>
<td>2,12</td>
<td>6,55</td>
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<td>M7</td>
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<td>0,5690</td>
<td>0,82</td>
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<tr>
<td>M8</td>
<td>0,5824</td>
<td>0,8593</td>
<td>0,81</td>
<td>2,02</td>
<td>7,85</td>
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</tr>
<tr>
<td>M9</td>
<td>0,1940</td>
<td>0,2731</td>
<td>0,82</td>
<td>0,66</td>
<td>2,60</td>
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<tr>
<td>M10</td>
<td>0,1710</td>
<td>0,3831</td>
<td>0,75</td>
<td>0,73</td>
<td>2,44</td>
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Der "Zusammenbruch" der CH₄-Produktion durch die Abkühlung verdeutlichte, wie sensitiv die Methanproduktion gegenüber sinkenden Temperaturen ist: Während bei Raumtemperatur Raten wie in tropischen Reisböden gemessen werden konnten, sanken die Produktionsraten bei starker Verringerung der Temperatur augenblicklich auf ein "boreales" Niveau ab. Bei Messungen von Jahresgängen der CH₄-Emissionen aus Moorböden fanden SAARNIO et al. (2000) bei Bodentemperaturen von 5 °C Effluxraten von etwa 0,6 bis 1,2 mg CH₄-C m⁻² h⁻². Errechnet man für die inkubierte Kerne mittels der in Tab. A.4-C angegebenen linearen Regressionsgleichung die mittlere Emissionsrate bei 5 °C (und rechnet diese auf die Fläche von 1 m² um), ergibt sich aus einer Rate von 2,5 µg C 100 cm⁻³ h⁻¹ ein Efflux von 1,0 mg CH₄-C m⁻² h⁻¹ – vergleichbar den Werten von SAARNIO et al. (2000). Der wesentliche Faktor für das "Zustandekommen" hoher CH₄-Emissionen im Lindener Grünland war somit nicht nur das Auftreten von
Überstauungsbedingungen, sondern auch die dabei herrschende Bodentemperatur. Diese war im regenreichen Herbst 1998 ungewöhnlich hoch; sie lag in der Phase, in der sich die hohen Methankonzentrationen im Boden ausbildeten, bei etwa 12 bis 14 °C. Im darauf folgenden Herbst 1999 war der Grundwasserstand im Mittel sehr viel tiefer, zudem lagen die Bodentemperaturen bei Einsetzen von Anaerobiose in 50 cm Tiefe (tiefste Sonde) bei etwa 5 °C oder niedriger (Temperaturfühler in 20 cm Tiefe). Unter diesen Bedingungen konnten keine vergleichbaren CH₄-Profile im Boden entstehen wie im Jahr 1998.

A.4.7 Literatur


Saarnio, S., Saarinen, T., Vasander, H. & Silvola, J. (2000): A moderate increase in the annual CH\textsubscript{4} efflux by raised CO\textsubscript{2} or NH\textsubscript{4}NO\textsubscript{3} supply in a boreal oligotrophic mire. *Global Change Biology* **6**, 137-144.


Anhang 5: Zusätzliche Hauptversuchsergebnisse

(Abbildungen zum Kapitel 3.3, Biomasse-Erträge)
Anhang 5
zu 3.3 Biomasse-Erträge


Statistik: wie in Abb. 3.3-B


Statistik: Wie in Abb. 3.3-B


Statistik: Wie in Abb. 3.3-B

Abbildung A-3.3-P: Leguminosen-Ertrag im Ringpaar 1 von 1997 bis 2000
a) Einzel-Ernten, b) Jahres-Erträge, und c) Prozentualer Anteil am Gesamtertrag des Rings. Ordinatenskalierung den Erntedaten angepaßt!

Abbildung A-3.3-Q: Leguminosen-Ertrag im Ringpaar 1 von 1997 bis 2000
a) Einzel-Ernten, b) Jahres-Erträge, und c) Prozentualer Anteil am Gesamtertrag des Rings. Ordinatenskalierung wie bei Ringpaar 2 und 3!
Abbildung A-3.3-R: Leguminosen-Ertrag im Ringpaar 2 von 1997 bis 2000
a) Einzel-Ernten, b) Jahres-Erträge, und c) Prozentualer Anteil am Gesamtertrag des Rings. Ordinatenskalierung wie bei Ringpaar 1 und 3!

Abbildung A-3.3-S: Leguminosen-Ertrag im Ringpaar 3 von 1997 bis 2000
a) Einzel-Ernten, b) Jahres-Erträge, und c) Prozentualer Anteil am Gesamtertrag des Rings. Ordinatenskalierung wie bei Ringpaar 1 und 2!
### Tabelle 3.3-D: Zweifaktorielle Varianzanalyse der Erträge der ersten Ernte

Für beide Tabellen gilt: Erster Faktor: CO₂-Applikation; Zweiter Faktor: Lage der Ringpaare 1 bis 3 (varierende Bodenfeuchte). "Interaktion signifikant": CO₂-Ergebnis abhängig vom Einfluß der Bodenfeuchte. Statistik: vgl. Tab. 5.3-C; Y: Innerhalb einer Lage (E+A) keine Signifikanzen, nur innerhalb der E- bzw. A-Gruppe (angegeben)

<table>
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<th>Jahr</th>
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### Tabelle 3.3-E: Zweifaktorielle Varianzanalyse der Erträge der zweiten Ernte

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### Anhang 5 zu 3.3 Biomasse-Erträge