## Anhang 1: N$_2$O-Flüsse im Vorversuch

(KAMMANN *et al.* 1998)

# Seasonal variability and mitigation options for $N_2O$ 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 $N_2O$ occurred mainly shortly after fertilization and during freeze–thaw periods. Additional field incubations (in jars) provided evidence that during frost periods $N_2O$ emissions as high as 22.000 ng $N_2O$–N $kg^{-1}$ soil $h^{-1}$ originated directly from the frozen topsoil. For the highest fertilized plots the freeze–thaw period accounted for 43 and 52% of the total annual $N_2O$ 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 $N_2O$ emissions. The results suggest that the ability of plant roots to take up $NO_3^-$ increases with increased cutting frequencies throughout the vegetation period, therefore reducing the amount of $NO_3^-$ available for soil denitrifying microorganisms.

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

## Introduction

About 70% of the global anthropogenic $N_2O$ 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 Bøckmann, 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 $N_2O$ 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

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(C) content can often be found in areas which are too wet for arable cropping, thus providing suitable conditions for biological denitrification and $N_2O$ emission (Firestone and Davidson, 1989; Granli and Bøckmann, 1994). Many investigations of $N_2O$ 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 $N_2O$ emissions from a long-term extensive managed, non-grazed grassland on mineral soil under various management regimes. The results presented here suggest management practices which could keep the $N_2O$ 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 $N_2O$ emissions during frost as well as freeze–thaw periods. It revealed the importance of this period for an annual $N_2O$ emission balance.

## Materials and methods

### Site description

The experimental site (Environmental Monitoring and Climate Impact Research Station Linden, ca. 4500 m$^2$) 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* subcommunity, is dominated by 12 grass species, 2 legumes and 15 non-leguminous herbs. The soil is classified as stagnofluvic 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 (3×3 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 $N_2O$ 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

Table 1

Soil characteristics of the Linden grassland site (Ap horizon, $n = 8$, ± SD in brackets)

| Ground water table depth[a] | >120 cm (plot no. 2) | <70 cm (all other plots) |
|---|---|---|
| pH (H$_2$O) | 5.9 | 6.2 |
| Organic carbon [%] | 3.0 (± 0.02)[b] | 6.6 (± 0.4)[b] |
| Organic nitrogen [%] | 0.3 (± 0.06) | 0.5 (±0.04) |
| Bulk density [g cm$^{-3}$] | 1.04 (± 0.06) | 0.88 (± 0.12) |
| Particle density [g cm$^{-3}$] | 2.61 (± 0.011)[b] | 2.51 (± 0.016)[b] |
| Soil porosity [%] | 60.2 (± 2.4) | 64.6 (± 4.4) |
| Sand (2000–63 µm) [%] | 43.2[c] | 10.0[c] |
| Silt (63–2 µm) [%] | 39.0[c] | 58.1[c] |
| Clay (<2 µm) [%] | 17.8[c] | 31.9[c] |

[a]Mean depth during summertime.
[b]$n = 3$.
[c]$n = 8$ mixed.

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 ($t_c = 60$ min) at times $t_0$, $t_{c/2}$ and $t_c$ with 60 ml syringes (Becton/Dickinson® Plastipak) fitted with 3-way stopcocks. At measurement days

Table 2

Fertilizer and cutting treatments (in brackets: number of application doses for total N amount) and hydrological parameters

| Plot no. | 1[a] | 2[a] | 3[a] | 4[b] | 5[a] | 6[a] | 7[a] | 8[a] | 9[c] |
|---|---|---|---|---|---|---|---|---|---|
| Fertilizer N (kg ha$^{-1}$ a$^{-1}$) | 0 | 40 (1) | 40 (1) | 80 (2) | 80 (2) | 120 (2) | 240 (3) | 400 (3) | 80 (2) |
| Number of cuts per year | 2 | 2 | 2 | 2 | 3 | 3 | 4 | 4 | 6 |
| Groundwater table[d] | <70 | >120 | <70 | <70 | <70 | <70 | 75–100 | <70 | <70 |
| Avg. soil mois. (vol.%)[e] | 34.8 | 28.1 | 53.9 | 43.8 | 44.6 | 43.4 | 32.1 | 41.1 | 44.6 |

Start of continuous measurements: [a]1.8.1996; [b]1.10.1996; [c]7.5.1997; [d]during summertime; [e]arithmetic means calculated from the period 10.2.1997 to 31.10.1997
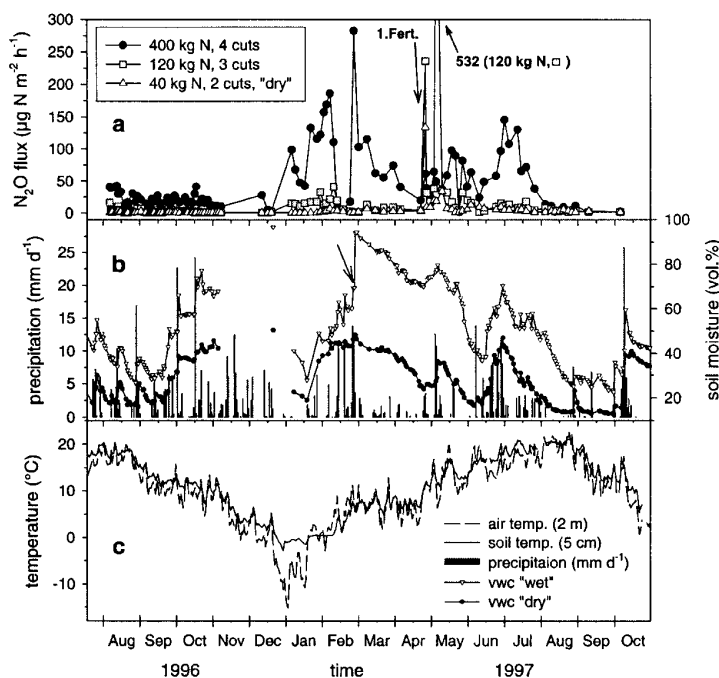
Fig. 1. Average $N_2O$ fluxes from three selected plots during the investigation period, August 1996–October 1997 (a) and related climatic parameters (b, c); see legend for both in (c). 'Wet' and 'dry' indicates data from the wettest and driest area of the site. vwc = volumetric water content (vol.%); 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 $N_2O$ free synthetic air. The $N_2O$ 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 h after sample collection. The gas chromatograph (HP 6890) was equipped with a $^{63}$Ni-electron 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. $N_2O$ 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 $N_2O$–N lost per fertilizer N applied was calculated as [annual $N_2O$–N loss$_{(fertilized)}$ – annual $N_2O$ 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, Fa. 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 ($\mu$g 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

| Fertilizer treatment (kg N $ha^{-1}$ $a^{-1}$)<br>cuts per year | | 0<br>2 | 40<br>2 (d) | 40<br>2 (w) | 80<br>2 | 80<br>3 | 120<br>3 | 240<br>4 | 400<br>4 |
|---|---|---|---|---|---|---|---|---|---|
| 30.4.1997 (6 days after<br>fertilization) | 0–5 cm | 0.19<br>*2.71* | 122.5<br>*94.4* | 2.67<br>*2.30* | 7.61<br>*3.23* | 113.6<br>*49.9* | 2.79<br>*3.72* | 6.48<br>3.26 | 221.7<br>97.8 |
| | 5–10 cm | 0.07<br>*2.39* | 2.83<br>*2.03* | 0.22<br>*1.12* | 0.38<br>*0.75* | 1.03<br>*1.18* | 0.32<br>*0.87* | 0.71<br>2.69 | 3.91<br>2.69 |
| | 10–15 cm | 0.03<br>*1.85* | 0.67<br>*1.21* | 0.27<br>*0.85* | 0.40<br>*0.53* | 1.37<br>*0.68* | 0.22<br>*0.43* | 0.15<br>0.76 | 1.19<br>0.76 |
| 22.5.1997[a] (one month<br>after fertilization | 0–5 cm | 0.12<br>*0.55* | 0.23<br>*1.30* | 0.37<br>*2.00* | 0.51<br>*2.22* | 0.81<br>*5.00* | 0.92<br>*5.97* | 359.7<br>*181.5* | 499.9<br>*321.3* |
| | 5–10 cm | 0.07<br>*0.18* | 0.16<br>*0.23* | 0.21<br>*0.47* | 0.34<br>*<0.1* | 0.41<br>*0.36* | 0.41<br>*0.38* | 36.2<br>*2.29* | 56.8<br>*1.97* |
| | 10–15 cm | 0.08<br>*0.17* | 0.18<br>*0.12* | 0.33<br>*0.49* | 0.29<br>*<0.1* | 0.45<br>*<0.1* | 0.27<br>*0.17* | 11.6<br>*1.05* | 7.43<br>*0.34* |

[a] 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 $NH_4$-N or $NO_3$-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 (max. $r = 0.6$).

Applied inorganic nitrogen ($NH_4^+$ and $NO_3^-$) 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 $\leq 1$ $\mu$g N $g^{-1}$ soil throughout the investigation period. Only on the highest fertilized plots, higher concentrations (among 6 and 80 $\mu$g $NO_3^-$-N $g^{-1}$ soil, at 0–5 cm) have been detected later on in the season and down to greater depth (max. 10 $\mu$g $NO_3^-$-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 ± SD 12.0 ± 29.5 and 4.2 ± 13.3 $\mu$g $N_2O$-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
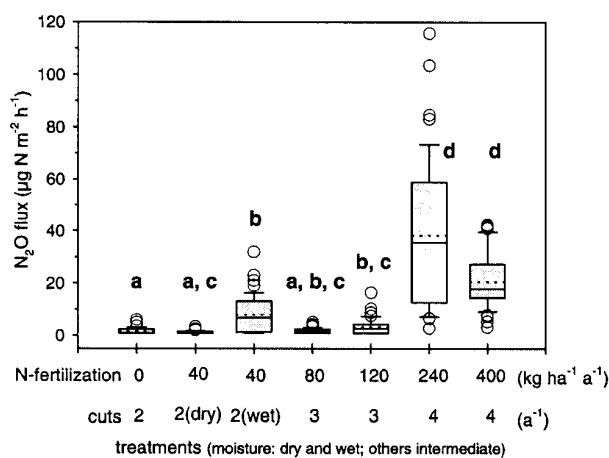
Fig. 2. Box- and whisker-plots of the $N_2O$ emission rates ($n = 54$) under different fertilizer and cutting regimes. Time period: 7 August 1996–20 December 1996. Solid lines in the boxes: medians; dotted lines: means; different indices show significant differences between treatments (Kruskal–Wallis-Test; Dunn's Test, $p < 0.05$).
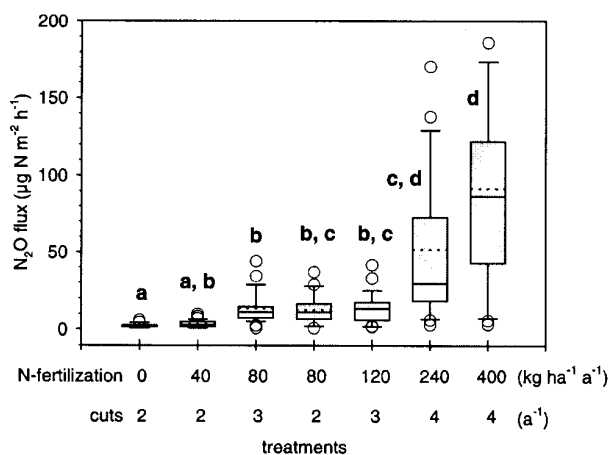


Fig. 3. Box- and whisker-plots of the $N_2O$ flux rates ($n = 22$; 40 kg N: $n = 44$) during the freeze–thaw period. 40 kg N treatment data were plotted together because there was no significant difference between them. Solid lines in the boxes: medians; dotted lines: means; different indices show significant differences between treatments (Kruskal–Wallis-Test; Dunn's Test, $p < 0.05$).

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

The percentage of $N_2O$–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).

## Influence of different cutting regimes

Preliminary results (Fig. 2) suggested that there may be an influence of cutting frequencies on $N_2O$ 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 $N_2O$ 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 $N_2O$ emissions significantly ($p = 0.018$; Fig. 4, right).

## $N_2O$ emissions from frozen soil

$N_2O$ emissions from frozen topsoils were studied during a severe frost period (December–January 1996/1997). Daily mean air temperatures as low as $-15°C$ (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). $N_2O$ emissions increased during the onset of freezing by a factor of 10–15 (e.g. to 100 µg $N_2O$–N m$^{-2}$ h$^{-1}$, highest fertilized plot, Fig. 1a). Jar incubations of frozen soil blocks taken from the top 5 cm indicated that $N_2O$ was emitted by the apparently frozen topsoil (Fig. 6). Furthermore, $N_2O$ emission rates were considerably higher from the soil that had previously been fertilized with $NH_4^+$ (5-fold, >2000 ng $N_2O$–N kg$^{-1}$ h$^{-1}$) or $NO_3^-$ (55-fold, >22,000 ng $N_2O$–N kg$^{-1}$ h$^{-1}$) compared to unfertilized soil.
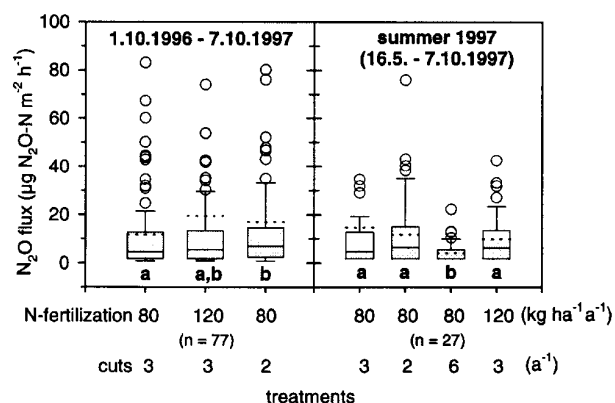


Fig. 4. Box- and whisker-plots of the $N_2O$ flux rates from the different cutting treatments receiving equal or comparable amounts of N fertilizer. Solid lines in the boxes: medians; dotted lines: means; different indices show significant differences between treatments (Kruskal–Wallis-test; Dunn's Test, $p < 0.05$). Three outlier values (peak rates after fertilization) are not shown in favour of a better ordinate scaling.
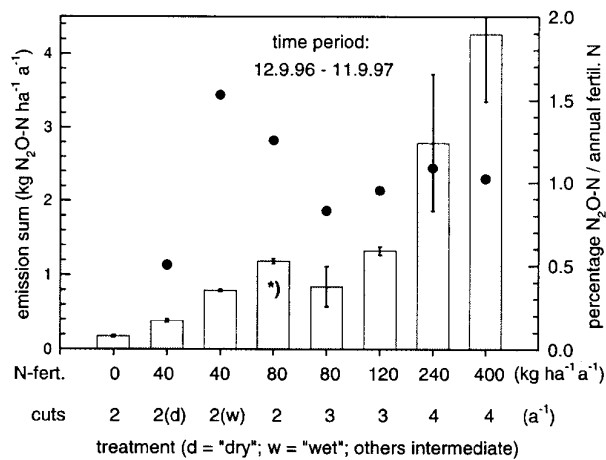
Fig. 5. Annual sums of $N_2O$ emissions (integrated on a day-to-day basis). Each bar: mean of the two maxichambers used per plot; up and down reaching caped lines indicate the upper and lower sums. • % $N_2O$–N emitted per annual fertilizer N applied; *Because of the later start, the period was two weeks shorter for this treatment.
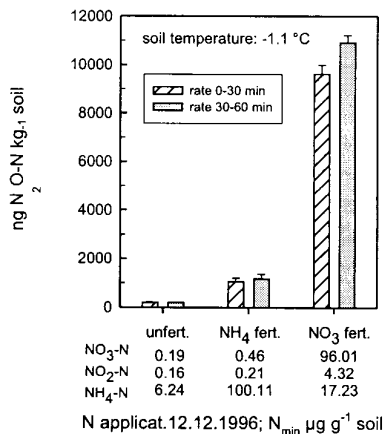


Fig. 6. $N_2O$ emissions from frozen soil blocks incubated in jars, 18 January 1997 (30-min rates, one hour incubation in total; n = 3 jars + SD). Incubation of unfertilized and previously fertilized soil (100 kg $NH_4$–N or $NO_3$–N applied on 12. December 1996). Below: amount of mineral N present in the frozen soil.

## Discussion

### Seasonal and annual $N_2O$ emission

Two main emission periods dominated the annual $N_2O$ losses: the period following the first fertilization and the off-season freeze–thaw period. Kaiser and Heinemeyer (1996) found similar $N_2O$ emission patterns from two arable soils in Germany. They reported $N_2O$ losses in the range of 1 kg N $ha^{-1}$ $a^{-1}$. This is almost equal to the results obtained in this study for the medium intensive fertilized plots (80–120 kg N $ha^{-1}$ $a^{-1}$). However, for the highest fertilized plots (240 and 400 kg N $ha^{-1}$ $a^{-1}$) the

emission period extended longer into the season with $N_2O$ 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 $N_2O$ emission 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 $ha^{-1}$ $a^{-1}$. Furthermore, the annual average $N_2O$ loss (3.8 kg N $ha^{-1}$ $a^{-1}$) as well as the $N_2O$ 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 $ha^{-1}$ $a^{-1}$ 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 $ha^{-1}$ $a^{-1}$ 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 $N_2O$ emission rates.

A higher ground water table significantly increased soil moisture and hence $N_2O$ emissions from the plots fertilized with 40 kg N $ha^{-1}$ $a^{-1}$ 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 $N_2O$ 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 Bøckmann, 1994).

In the IPCC 1995 report (Watson et al., 1996) an annual $N_2O$–N emission sum of 1 kg $ha^{-1}$ is used as a 'background level' to assess $N_2O$ emission from agricultural soils. Nitrous oxide emissions reported here from unfertilized and extensively fertilized plots (40 kg N $ha^{-1}$ $a^{-1}$) are with 0.18–0.79 kg $N_2O$–N $ha^{-1}$ $a^{-1}$ 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 $N_2O$ emissions (28.8% when fertilized with 80 kg N $ha^{-1}$ $a^{-1}$). Moreover, an increase to 6 cuts per year (80 kg N $ha^{-1}$ $a^{-1}$) decreased the $N_2O$ 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 $N_2O$ 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. Küppers 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 $N_2O$ 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 $N_2O$ 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.

### $N_2O$ emissions from frozen soil and during thawing

In this study we observed a considerable rise of $N_2O$ 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 $N_2O$ emissions from a frozen soil surface and interpreted it as a result of diurnal freeze–thaw cycles causing $N_2O$ 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 $N_2O$ 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 $NO_3^-$ 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 $N_2O$ emission rates from frozen soil

blocks were observed (>22,000 ng $N_2O$–N kg$^{-1}$ h$^{-1}$). In the $NH_4$-treated soil the nitrate concentration was similar to the unfertilized control. Therefore the 5-fold enhanced $N_2O$ 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 $N_2O$ flux rates as high as 411 $\mu$g $N_2O$–N m$^{-2}$ h$^{-1}$ (400 kg N ha$^{-1}$ a$^{-1}$ treatment) were detected while nitrate concentrations were quite low (<7 $\mu$g N g$^{-1}$). $N_2O$ flux rates during thawing were comparable to the results of Cates and Keeney (1987) who reported maximum $N_2O$ emission rates of 208 $\mu$g $N_2O$–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 $N_2O$ 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 $N_2O$ 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 $N_2O$ losses, especially when soils receive greater amounts of N fertilizer during the plant growing season.

### Conclusions

The study presented here deals with $N_2O$ emissions from long-term extensively managed, non-grazed grassland and therefore closes an existing data gap. The annual $N_2O$ losses from the unfertilized and extensively fertilized grassland were quite below the assumed agricultural background value of 1 kg $N_2O$–N ha$^{-1}$ a$^{-1}$ (IPCC 1995 report, Watson et al., 1996), demonstrating the potential for low $N_2O$ emissions from a nitrate limited extensively managed grassland ecosystem. An increase in the cutting frequency per year from 2 to 3 reduced the annual $N_2O$ losses by 29%, suggesting a practicable mitigation option. By application of the 80 kg N − 3 cuts treatment, an annual $N_2O$ 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 $N_2O$ producing denitrifiers, but with no reduction in the biomass yield. However, high fertilizer applications resulted in large $N_2O$ 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 $N_2O$ losses (up to 50%), thus stressing the importance of off-season $N_2O$ losses for the annual balance.

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# Anhang 2: Die Bodenluftsonden-Methode

(KAMMANN *et al.* 2001a: Druckfahne des im Juni 2001 erscheinenden Artikels)

# A new sampling technique to monitor concentrations of $CH_4$, $N_2O$ 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 $N_2O$) 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 $N_2O$, $CO_2$ 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 equalled 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 ($N_2O$ fluxes reviewed by, e.g., Aulakh *et al.*, 1992; Granli & Bøckmann, 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 $N_2O$ or $CH_4$.

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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-nm pore size that were used to measure various gases (Dowdell *et al.*, 1972; Dowdell & Smith, 1974; Egginton & Smith, 1986). Goodroad & 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 depths. 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 of 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 $O_2$, $CH_4$ and $CO_2$ 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 $^{15}NO_3^-$-labelled solution at various depths in the soil. Jacinthe & Dick (1996) tested the diffusivity of silicone tubing for large $N_2O$ concentrations in preliminary tests and in a column experiment in the laboratory. They found that $N_2O$ 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 $N_2O$ (30–60 $\mu$l l$^{-1}$), and Holter (1990), who measured $CH_4$ and $CO_2$ at large concentrations (9.6% $CH_4$, 7.2% $CO_2$), we tested for small near-ambient concentrations of $CO_2$, $N_2O$ and $CH_4$. When $CH_4$ is taken up by methane oxidizers in the soil, concentrations as small as 300 $\mu$l l$^{-1}$ $CH_4$ may result (Bender & Conrad, 1992; Mosier *et al.*, 1997). In addition, long periods of very small emissions of $N_2O$ 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 $N_2O$ can be expected at least in the topsoil, as found by Sotomayor & 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 $N_2O$, $CH_4$ and $CO_2$ 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 (Kalensee, 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 borer 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 $N_2O$, as tested by Jacinthe & Dick (1996), we measured the diffusivity of silicone for small concentration gradients of $CH_4$, $N_2O$ and
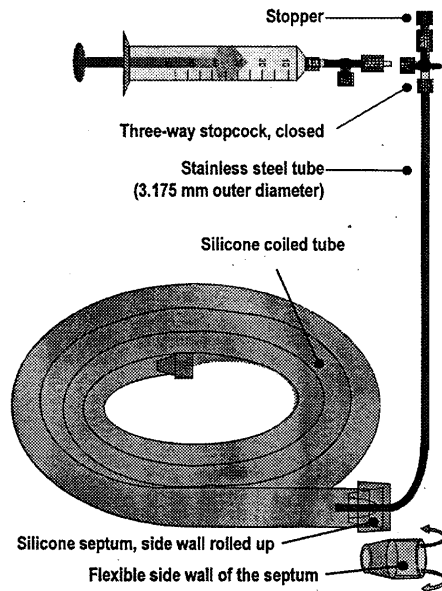
**Figure 1** Silicone soil air probe fitted with one stainless steel tube connection. The flat silicone coil is fixed with wire mesh (not shown) to maintain the flat 'snail' form. The silicone septum used for the steel tube connection has flexible side walls that could be rolled up and pulled over the silicone tubing to ensure a better fixing.
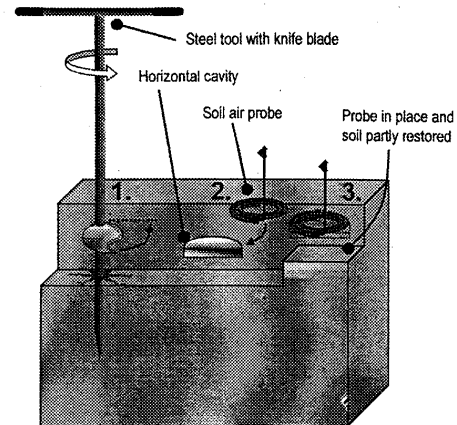


**Figure 2** Insertion of silicone probes into the soil: 1. A steel tool with a right-angled knife blade is driven into the bottom of the pit previously dug into the soil. When the tool is turned, the knife cuts a horizontal slit into the vertical side wall. By cutting a second slit 2–3 cm deeper, a 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.

$CO_2$. 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 $\mu$l $l^{-1}$ $CO_2$, 2.49 $\mu$l $l^{-1}$ $N_2O$ and 19.76 $\mu$l $l^{-1}$ $CH_4$ (in synthetic air; Deuste Steininger GmbH, Mühlhausen, Germany) and (ii, iii) synthetic air with 79.5% $N_2$ and 20.5% $O_2$ 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

$$D_t = D_0 + a\exp(-bt), \tag{1}$$

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:
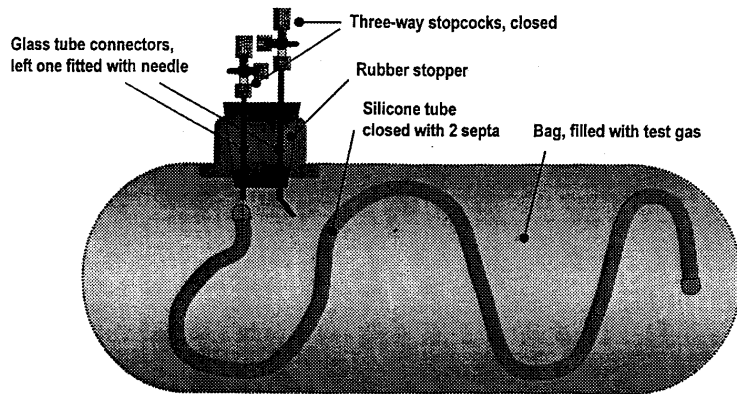
Figure 3 Bag with rubber stopper with a silicone tube enclosed, where glass connectors fitted with three-way stopcocks enable sampling of the atmosphere inside the bag as well as sampling of that inside the silicone tube by 60-ml syringes.

$$t = -\frac{1}{b}\ln\left(\frac{D_t - D_0}{a}\right). \qquad (2)$$

*Analytical technique*

The gas samples taken by syringes were analysed for $CO_2$, $N_2O$ and $CH_4$ automatically within 1–24 h following their collection, by a gas chromatograph (HP 6890) and the automatic sample system described by Loftfield *et al.* (1997). The chromatograph was equipped with an ECD ([63]Ni-electron capture detector, at 290°C) and an FID (flame ionization detector, at 230°C). The standard deviations of 10 measurements at ambient concentration were 4 $\mu$l $l^{-1}$ for $CO_2$, 4 $\mu$l $l^{-1}$ for $N_2O$ and 8 $\mu$l $l^{-1}$ for $CH_4$ 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 $N_2O$ was much smaller than used by Jacinthe & Dick (1996). As those authors presumed, $CO_2$ and $CH_4$ 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% equilibrium was reached on average after 2.9 h for $CO_2$, 1.5 h for $N_2O$ and 6.6 h for $CH_4$. The times Jacinthe & Dick (1996) found for $N_2O$ 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 $CO_2$ and $CH_4$ concentrations he used. With silicone tubing of 1 mm wall thickness, he found times of 1.45 h for $CH_4$ and 0.7 h for $CO_2$ until 95% equilibrium 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 $N_2O$ 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 $CH_4$ (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.
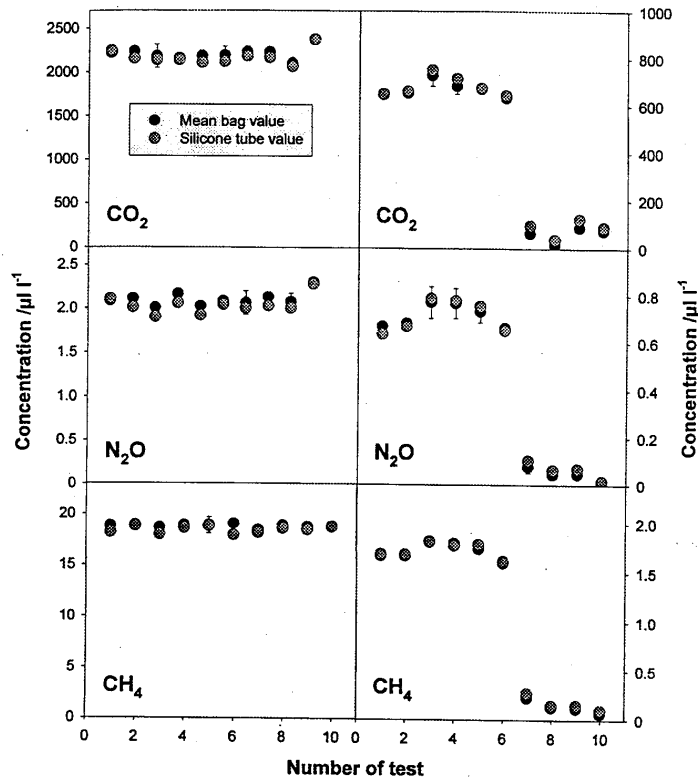
We did not test for diffusion of $O_2$, but Holter (1990) did, and he reported that $O_2$ took a little longer than $CH_4$ and $CO_2$ to reach 95% equilibrium. We observed smaller $O_2$ peaks in the FID chromatograms with rising water table in autumn 1998 and no $O_2$ peaks at all when large $CH_4$ concentrations ($> 100$ $\mu$l $l^{-1}$) occurred in the grassland soil (results not reported here). Therefore we suggest that the silicone probes might be valuable for measuring $O_2$ 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 $\pm$ 5.9 $\mu$l $l^{-1}$ for $CO_2$, 382 $\pm$ 2.9 $\mu$l $l^{-1}$ for $N_2O$ and 1900 $\pm$ 5.5 $\mu$l $l^{-1}$ for $CH_4$, respectively.

Figure 6 displays the concentrations of gas measured from August 1998 to January 2000 with three probes inserted at 5 cm

**Figure 4** Equilibration of the air inside the silicone tube with the surrounding atmosphere in the bag after 12–24 h at 20°C. The left-hand column shows the test series where the bag was filled with a standard gas, whereas in the right-hand column are graphs for the bag filled with synthetic air. The atmosphere in the silicone tubing contained either atmospheric concentrations at the beginning (left column; four small values in right column) or the standard gas (right column, larger values). Right column: the difference between the concentration in the atmosphere of the bag (which was set at 100%) and that in the tube was, averaged over the displayed measurements, 2.5% for $CO_2$, 3.2% for $N_2O$ and 2.2% for $CH_4$. Left column with smaller concentrations: the absolute difference between the atmosphere of the bag and that of the tube was, averaged over the displayed measurements, $18.7\,\mu l\ l^{-1}$ for $CO_2$, $18.8\,nl\ l^{-1}$ for $N_2O$ and $26.3\,nl\ l^{-1}$ for $CH_4$.

depth in $100\,m^2$ 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 $4290\,\mu l\ l^{-1}$ for $CO_2$, 1.58, 1.56 and $1.58\,\mu l\ l^{-1}$ for $CH_4$, and 0.37, 0.37 and $0.39\,\mu l\ l^{-1}$ for $N_2O$. On a wetter part of the experimental site, where the ground water 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 $13\,460\,\mu l\ l^{-1}$ for $CO_2$, $3.59\,\mu l\ l^{-1}$ for $CH_4$, and $0.61\,\mu l\ l^{-1}$ for $N_2O$ 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 $N_2O$ 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-
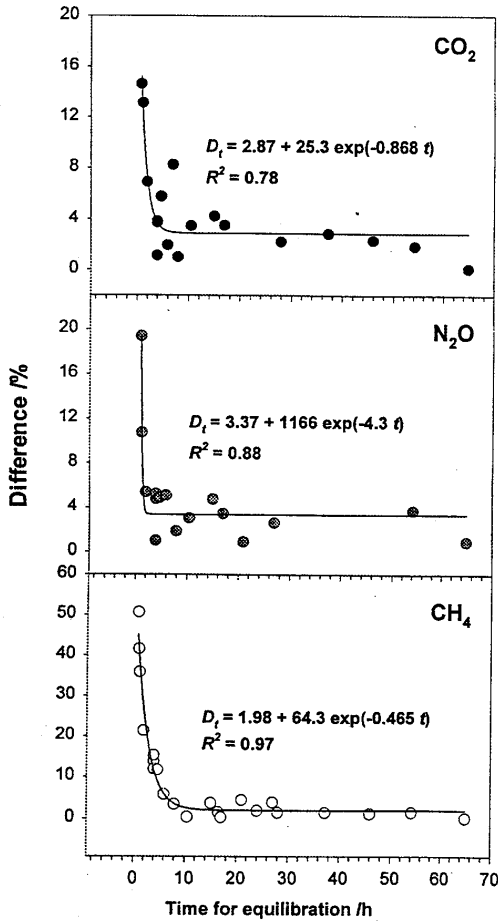
**Figure 5** Equilibration of the inner silicone tube atmosphere with the surrounding bag atmosphere with time. The 'per cent difference' is the difference between the mean of the atmosphere in the bag and the value measured in the silicone tube, expressed in percentage of the bag mean (= 100%). (For the exponential decline function fitted to the data see text.)

Plot CO₂: $D_t = 2.87 + 25.3 \exp(-0.868\ t)$, $R^2 = 0.78$

Plot N₂O: $D_t = 3.37 + 1166 \exp(-4.3\ t)$, $R^2 = 0.88$

Plot CH₄: $D_t = 1.98 + 64.3 \exp(-0.465\ t)$, $R^2 = 0.97$



**Figure 6** Mean concentrations and standard deviation of $CO_2$, $N_2O$ and $CH_4$ 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).

tured like this and inserted in July 1999 to measure a vertical soil air profile down to 50 cm depth in an experiment where ^{15}N fertilizer was applied on the soil surface (C. Müller, unpublished). None of the 'improved' probes has developed any leakage within 1 year of usage so far.

Another weak point could be the ageing of the polyethylene stopcocks, which should be covered with dark caps against the ultraviolet radiation. However, we detected no fatigue in the material during 18 months in any of the buried probes on our site (with uncovered stopcocks).
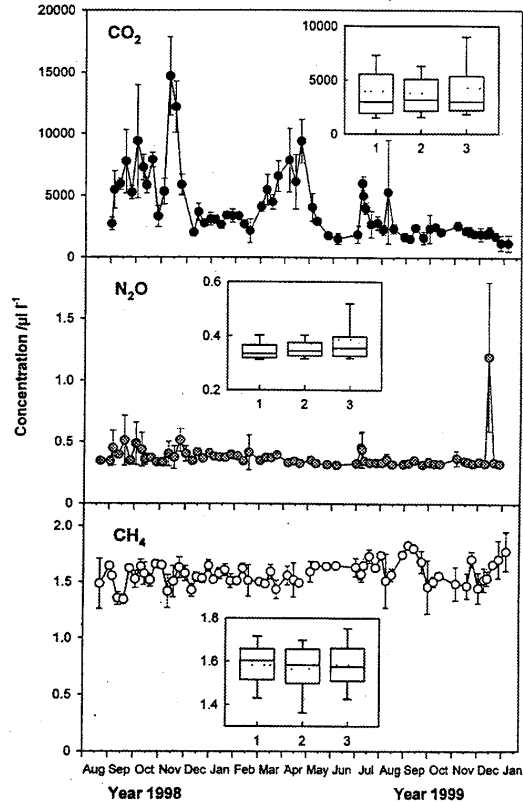
We suggest that a second steel tube with stopcock be inserted into the second silicone septum in a probe. Thus modified, the probe in the soil can be flushed with a tracer gas (e.g. ^{15}N-labelled $N_2O$ or other gases) so as to measure gas diffusion in the soil.

*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 $H_2O$ and lets gases such as $CO_2$, $N_2O$ and, especially interesting in wet soils, $CH_4$ 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 $N_2$, 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.

## Acknowledgements

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## Anhang 3: CH$_4$-Flüsse im Vorversuch

(KAMMANN *et al.* 2001b: Manuskript zur Veröffentlichung akzeptiert)

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# Methane fluxes from differentially managed grassland study plots: the important role of CH₄ oxidation in grassland with a high potential for CH₄ 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 ($NH_4NO_3$) fertilizer applied and in the height of the in ground water table. No inhibition of $CH_4$ oxidation occurred, regardless of the amount of annual N fertilizer applied. Instead, the mean $CH_4$ consumption rates were correlated with the mean soil moisture of the plots. However, the correlation between daily soil water content and corresponding $CH_4$ oxidation rate was always weak. During drought period (late summer) water stress was observed to restrict $CH_4$ oxidation rates.

The findings led to the question whether methane production with soil depth might modify the $CH_4$ 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 $CH_4$ sink capacity of a specific site was related to the vertical length of its $CH_4$ oxidizing column, i.e. the depth of the $CH_4$ producing horizon. Anaerobically incubated soil cores produced large amounts of $CH_4$ comparable to tropical rice paddy soil. Under field conditions, heavy autumnal rain in 1998 led to a dramatic increase of soil $CH_4$ concentrations up to 51 $\mu l \ l^{-1}$ at a depth of 5 cm. Nevertheless, no $CH_4$ was released when soil surface $CH_4$ fluxes were measured simultaneously. The results thus demonstrate the high $CH_4$ 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 $CO_2$ (Schimel and Gulledge, 1998), can be oxidized by aerobic methanotrophic bacteria, contributing about 5.8% to the atmospheric $CH_4$ 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 in 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 $N_2O$ 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 ($NH_4NO_3$) 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 $N_2O$ emissions, (for $N_2O$ 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 1$^{st}$ September 1998 to 6$^{th}$ 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 $CH_4$, $N_2O$ and $CO_2$ between the inner and outer atmosphere was only by diffusion through the walls of the silicone tube, whereas $H_2O$ 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-cm3 volume, excluding the first cm with thick grass roots) were obtained within a 0.25 m² 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 $N_2$ (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 CH$_4$ flux rates*

Methane oxidation dominated the direction of $CH_4$ fluxes in all treatment plots (August to November 1996 and April to November 1997, see Figure 1). However, single small events of $CH_4$ emissions were observed during autumn 1996 following rain events. The time courses of the $CH_4$ oxidation rates were similar on all treatment plots, but the average rates differed in magnitude (Figure 1; Figure 3). The $CH_4$ 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 $CH_4$ concentrations at 5 and 15 cm depth measured from September 1998 to January 2000 in the dry soil ($CH_4$ oxidation) were also poorly and negatively correlated to the corresponding soil moisture values, while the $CH_4$ 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 $CH_4$ 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 ($r^2 = 0.72$, $p < 0.001$, 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 m² 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$^{-1}$ CH$_4$ at 30-cm depth on the Dec. 1$^{st}$ 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. 17$^{th}$ the CH$_4$ concentration at 5-cm depth reached a maximum of 51-µl l$^{-1}$ CH$_4$ (Figure 7a). Nevertheless, when simultaneous CH$_4$ 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 CH$_4$ efflux could be detected, and no net CH$_4$ oxidation from the atmosphere took place.

The probe measurements also revealed that the length of the CH$_4$ oxidizing vertical soil column was not identical in all treatment plots. The lowest CH$_4$ 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 CH$_4$ concentrations reached values several-fold higher than the ambient atmospheric concentration (Table 3).

## 4. Discussion

### 4.1. Influence of different N fertilizer levels on CH$_4$ oxidation

The mean CH$_4$ oxidation rates observed during the experimental period ranged from -10.1 µg CH$_4$-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 CH$_4$-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 CH$_4$-C m$^{-2}$ h$^{-1}$ from native and annual prairie that was burned. Mosier et al. (1991) monitored rates between 15 and 26 µg CH$_4$-C m$^{-2}$ h$^{-1}$ in highland and lowland prairie and unfertilized meadow. Boeckx et al. (1997) found rates of 25 µg CH$_4$-C m$^{-2}$ h$^{-1}$ from clay grassland soil (incubation of soil cores).

In many other investigations, the application of NH$_4^+$ 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 $O_2$) 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 $CH_4$ 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 $CH_4$ oxidation rates. A wetter treatment plot oxidized on an average less $CH_4$ than a drier one (Figure 1).

However, on a day-to-day basis, there was only a very weak correlation between decreasing $CH_4$ oxidation rates and increasing soil moisture values (Table 2). In contrast, the soil air $CH_4$ concentrations exhibited a (also weak) negative correlation, i.e. decreasing $CH_4$ concentrations when soil moisture increased. Such a finding could only be due to a higher $CH_4$ oxidation rate with increasing moisture in the surrounding soil. Moreover, correlation of the $CH_4$ 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 $CH_4$ concentrations (r = 0.09).

Taken together, the results do not support the common perception of a growing soil water film restricting $CH_4$ (and $O_2$) fluxes from the atmosphere to the $CH_4$ oxidizing soil microorganisms. The soil air probe measurements demonstrated that the length of the $CH_4$ oxidizing vertical soil column, in combination with the duration of the $CH_4$ production at greater depths contribute to the magnitude of the $CH_4$ fluxes *into* the soil. Whalen et al. (1996) and Mosier et al. (1997) described increasing $CH_4$ production as a cause for declining $CH_4$ oxidation rates in ecosystems with frequent $CH_4$ production. In the present study, the grassland soil has a large $CH_4$ 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 $CH_4$ production rates (max. 19.6 µg $CH_4 \, g^{-1 \, dwt} \, day^{-1}$) were comparable to the rates reported by Wassmann et al. (1998) (max. 13.6 µg $CH_4 \, g^{-1 \, dwt} \, day^{-1}$) 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 $O_2$ conditions unfavorable to $CH_4$ production (Conrad, 1989; Knowles, 1993). Peters and Conrad (1995) were even able to induce $CH_4$ production in desert soil slurries by anaerobic incubation.

Our data indicate that $CH_4$ 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 $CH_4$ production in near-surface soil horizons occurred, which means shorter vertical $CH_4$ oxidizing soil columns above the $CH_4$ 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$^{-1}$ $CH_4$ for the measurement period.

Therefore it can be concluded that in the grassland ecosystem investigated here, the depth of the $CH_4$ producing horizon (the length of the $CH_4$ oxidizing vertical soil column), in combination with the duration of subsurface $CH_4$ production contribute to the overall methane oxidation rate at the soil surface. $CH_4$ 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 $CO_2$ (Hungate et al., 1997; Dacey et al., 1994; Megonigal and Schlesinger, 1997) may increase the magnitude and frequency of $CH_4$ production periods and hence, diminish the $CH_4$ soil sink capacity of an ecosystem even if it does not usually emit methane.

In contrast to the weak correlation between $CH_4$ flux rates/$CH_4$ concentrations and the soil moisture, a significant restriction of $CH_4$ oxidation with declining soil moisture (desiccation) was observed in this study. Methane oxidation rates decreased with declining soil moisture from more than $25 \, \mu g \, C \, m^{-2} \, h^{-1}$ to $6 \, \mu g \, C \, m^{-2} \, h^{-1}$ (i.e. 76% decline). Restriction of $CH_4$ 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 $CH_4$ 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 $CH_4$ 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 $CH_4$ 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) $CH_4$ 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 $CH_4$ 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. CH$_4$ oxidation as a 'biofilter' function*

The silicone probe measurements revealed that situations with above-ambient CH$_4$ concentrations in the topsoil might frequently occur in semi-wet, C-rich ecosystems. Nevertheless, no net 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 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 CH$_4$ oxidation prevented the flux of 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 CH$_4$ flux balance throughout the year. We therefore concur with Whalen et al. (1996) and Hütsch (1998b) that the process of biological CH$_4$ oxidation should not only be regarded as an important global CH$_4$ sink, but also as a very effective 'biofilter'. Such a filter modifies 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**
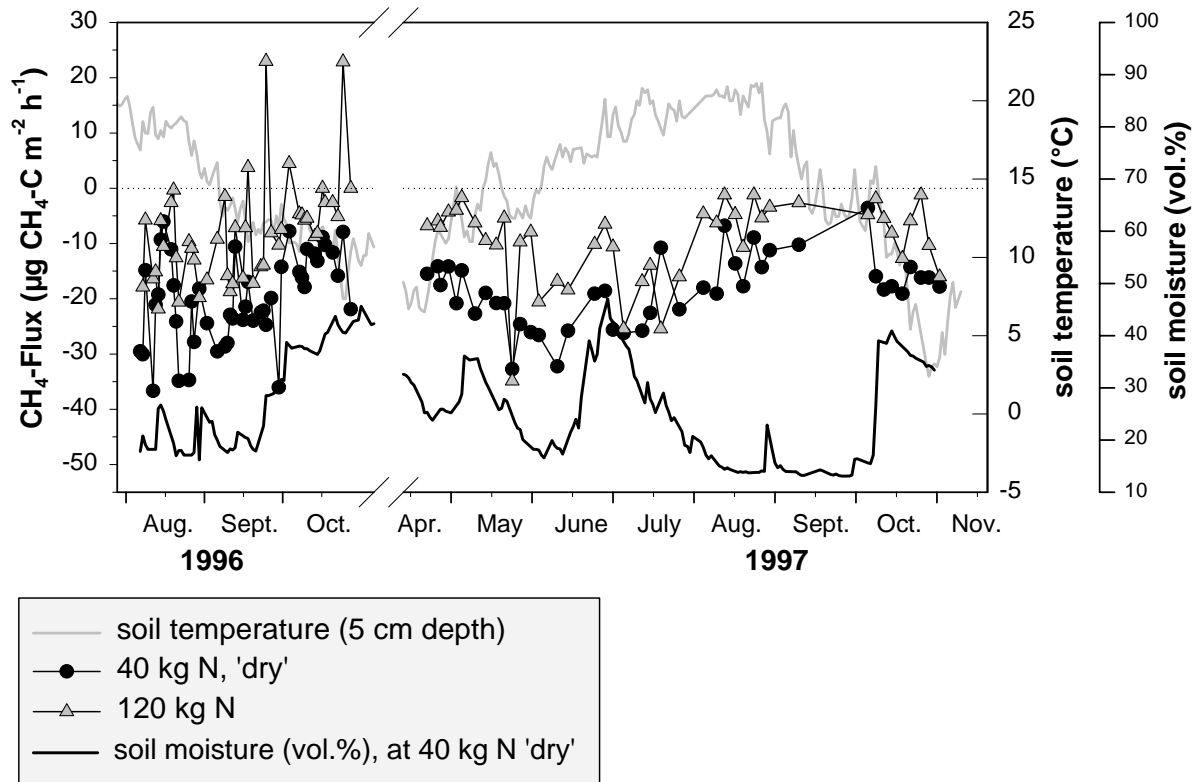*(C. Kammann, L. Grünhage, H.-J. Jäger, G. Wachinger)*



**Figure 1:** Time series of the average $CH_4$ flux rates between two treatment plots and the atmosphere and corresponding soil moisture and temperature.
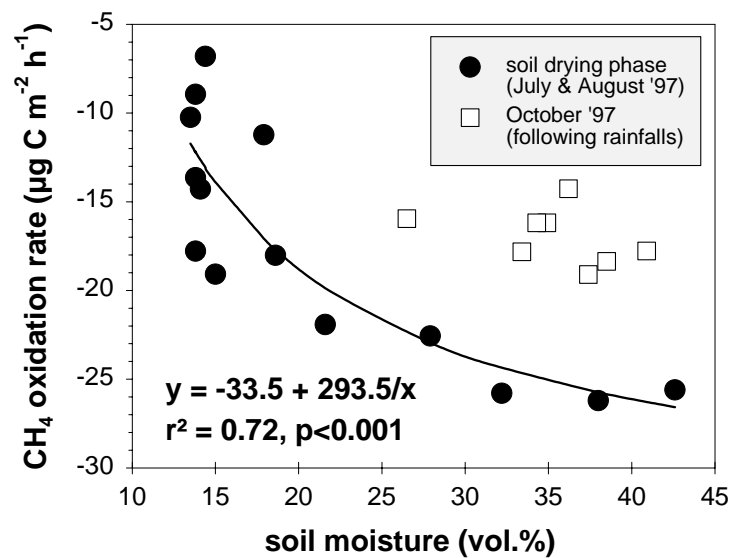


**Figure 2:** Declining $CH_4$ oxidation rates in the 40 kg N, 'dry' treatment with soil desiccation during a drought (July to September 1997) and recovery following the first rain in October 1997.
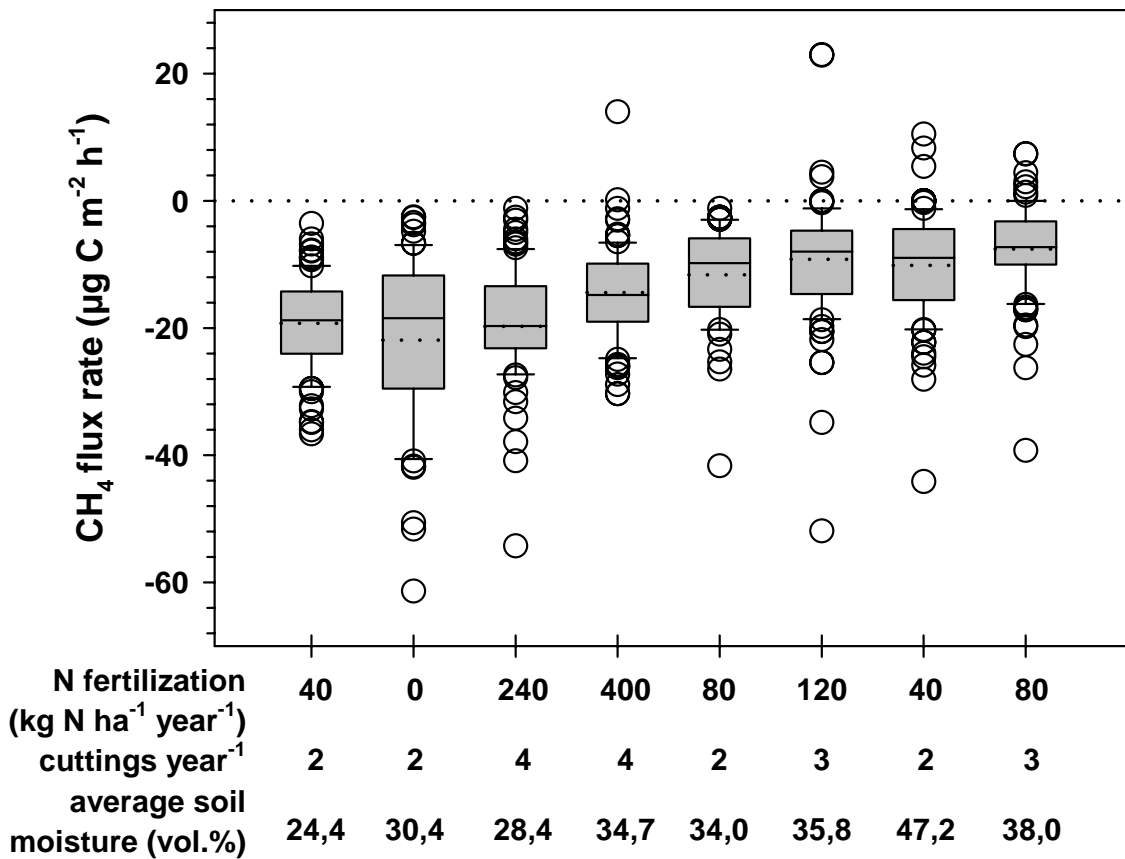
**Figure 3:** Box-Plots of the $CH_4$ flux rates of all treatments during the time period shown in Figure 1. Below, the fertilization level and the average soil moisture during the measurement period is given.



**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.

**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 hights (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: Atmosheric $CH_4$ 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 $N_2O$-Flüsse im Untersuchungszeitraum 1996/1997 wurde für die einzelnen Dünge- und Schnittbehandlungen in und KAMMANN *et al.* (2000b) ausführlich dargestellt und wird daher an dieser Stelle nicht erneut gezeigt.



**Abbildung A.4-A**: Bodentemperaturverlauf in 5 cm Tiefe bei den mit 40 kg N ha$^{-1}$ a$^{-1}$ gedüngten Parzellen. Schwarze Linie: Differenz der trockenen und der feuchten Parzelle (Temp.(2.5) – Temp.(2.4)). Der Vergleich zeigt, daß sich die trockenere Parzelle rascher abkühlte bzw. erwärmte als die feuchte Parzelle.

Wichtig für die $N_2O$-Flußmessungen im FACE-Experiment:

- $N_2O$-Emissionen der beiden mit 40 kg N ha$^{-1}$ a$^{-1}$ gedüngten Parzellen ganzjährig auf relativ niedrigem Niveau (zwischen 10 und 30 µg N m$^{-2}$ h$^{-1}$), mit Ausnahme des 3 – 4-wöchigen Zeitraums Düngung (April 1997)

- Die feuchtere der beiden 40-kg-Parzellen (2.4) zeigte fast immer die höheren $N_2O$-Flußraten. Jahresbilanz: $N_2O$-N-Verluste der feuchten 40-kg-N-Parzelle fast doppelt so hoch wie die der trockenen 40-kg-N-Parzelle (0,38 *versus* 0,75 kg N ha$^{-1}$ a$^{-1}$).

- 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.

- Je stärker die N-Düngung während der Vegetationsperiode 1996, desto höher das N$_2$O-Emissionsniveau während der Frost-Tau-Periode 1996/1997

- Bei den beiden höchsten Düngungsstufen: maximale N$_2$O-Flußraten nicht nach der Düngung, sondern während der Frost-Tau-Periode 1996/1997.

- Korrelationen der N$_2$O-Emissionen mit den Faktoren Bodenfeuchte und -temperatur war stets sehr schlecht (maximal: r = 0,55; mit Bodentemperatur), zurückzuführen auf die für Grünland typische N-Limitierung von Nitrifikation und v.a. Denitrifikation (JARVIS 1996; GRANLI & BØCKMANN 1994).

- 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 N$_2$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 N$_2$O-Emissionen (in µg N m$^2$ h$^{-1}$): Mittlere N$_2$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).

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

Jahres-Zeitraum: n = 77; n = 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 N$_2$O-Flüsse auf; es konnte durch Zusatzversuche gezeigt werden, daß im scheinbar hartge-

froren Boden biologische Denitrifikationsaktivität zu hohen N$_2$O-Emissionen führte (vgl. MÜLLER *et al*. 1997; KAMMANN *et al*. 1998, KAMMANN *et al*. 2000b, und Zitate hierin). Argumente und Belege:

- o 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)

- o Inkubation von mit Stichsäge und Beitel aus dem Grünlandboden geschnittenen gefrorenen Bodensäulen bei unter 0°C: hohe N$_2$O-Flüsse in Abhängigkeit von der verfügbaren N-Form (v.a. Denitrifikation > 90 %): N$_2$O nicht aus ungefrorenen Bodentiefen

- o Rechenexempel (KAMMANN *et al*. 2000b): Maximal 1/20 der Freiland-N$_2$O-Emissionen bei Frost, bzw. 1/200 der N$_2$O-Emissionen aus den inkubierten NO$_3^-$-Bodensäulen hätte durch Austreiben von im Bodenwasser gelöstem N$_2$O (Ausfrieren) stammen können.



**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).
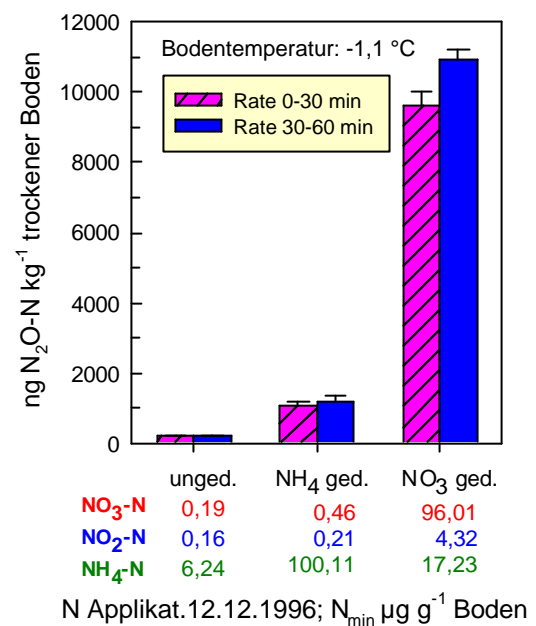
**Abb. A.4-C**: N$_2$O-Emissionen aus gefrorenen Bodensäulen, 6 Bodensäulen pro Glas, n=3 Gläser (+ Standardabweichung). Der Boden war vor der Frostperiode unterschiedlich gedüngt worden. Unter den Säulenlabels: Gehalte des gefrorenen Bodens an Nitrat, Nitrit und Ammonium (KCl-Extraktion von gefrorenen Bodensäulen)

- • Hohe N$_2$O-Emissionen während des Auftauens des Grünlandbodens: Die *off-season* Frost-Tauperiode verursachte bei der höchsten Düngungsstufe mehr als die Hälfte der N$_2$O-Jahresemissionen (vgl. KAMMANN *et al*. 1998 und Zitate hierin). Bei geringerer Düngung: "nur" 15 bis 30 % der N$_2$O-Jahresbilanz.

- • Limitierender Faktor während des Tauens: Nitratverfügbarkeit; im Winter (fehlende Wurzelkonkurrenz) reichten jedoch geringe Nitratmengen, um hohe Auftau-N$_2$O-Peaks zu erzielen (vgl. Verlauf auf den hochgedüngten 240- und 400-kg-N-Parzellen, KAMMANN *et al.* 2000b). Zur Diskussion der Hintergründe der winterlichen N$_2$O-Emissionen siehe KAMMANN *et al.* 1998 in Anhang 1.

Die Bodenluft-Sondenmethode (Anhang 2) wurde entwickelt, um u.a. die Frage nach einer möglichen $N_2O$-Akkumulation unterhalb einer gefrorenen Bodenschicht zu klären, da andere Autoren dies bei hohen während des Winters gemessenen $N_2O$-Emissionen bei Frost vermuteten (Bsp. BURTON & BEAUCHAMP 1994; GOODROAD & KEENEY 1984).
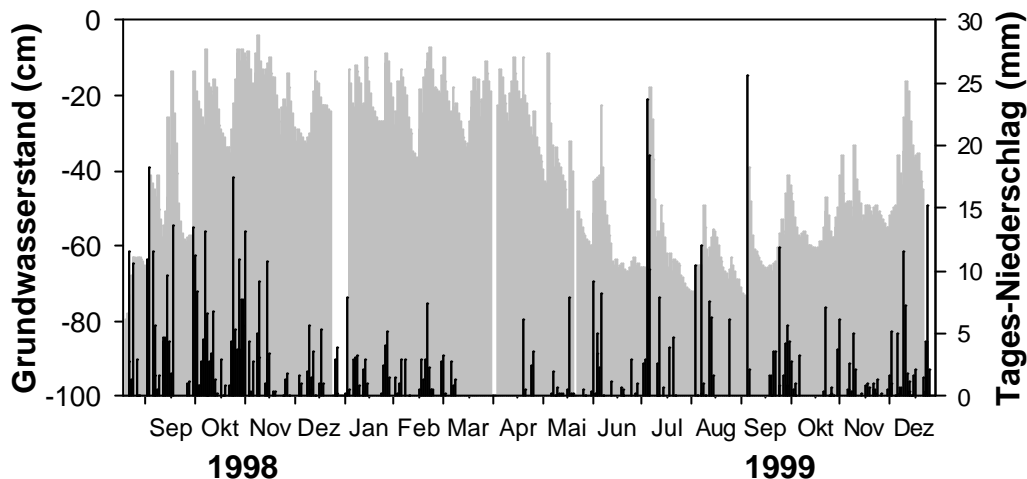
*A.4.2 $N_2O$-Konzentrationen in der Bodenluft*



**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.

Wie Abb. A.4-A sowie Abb. 3.1-C (Lufttemperaturen) zeigen, trat nach dem strengen Winter 1996/1997 keine vergleichbarer Frost mehr auf, so daß die Beantwortung der Frage nach der Akkumulation von $N_2O$ unter einer Eisbarriere noch aussteht. Im September und Oktober 1998 ließen hohe Niederschläge den Grundwasserstand stark ansteigen (Abb. A.4-D). Während die $N_2O$-Bodenluft-Konzentrationen das ganze Jahr hindurch selbst bei hoher Düngung nicht sehr groß waren, schossen sie mit dem Grundwasseranstieg steil in die Höhe, v.a. in 15 und 30 cm Tiefe (Abb. A.4-E). Sie gingen aber in 30 cm Tiefe fast ebenso rasch wieder auf einen geringeren Level zurück (Abb. A.4-E). Zumindest in dieser Tiefe waren die Sonden zeitweilig überflutet bzw. lagen an der Grenze des Grundwasserstands (Abb. A.4-D), so daß die fortdauernde Anaerobiose eine Nitratnachlieferung durch Nitrifikation erschwerte bzw. verhinderte. Wechselten Anaerobiose und $O_2$-Versorgung durch den schwankenden Wasserstand einander ab (vgl. Abb. A.4-E, 15 cm Tiefe), waren erneute Peaks die Folge, die jedoch an Höhe und Dauer abnahmen. Die erneuten Peaks lassen sich über Nitratnachlieferung durch Nitrifikation während der Sauerstoffverfügbarkeit, im Wechsel mit guten Denitrifikationsbedingungen durch den hohen Wasserstand erklären (GRANLI & BØCKMANN 1994). Die wechselnden Bedingungen sorgen nach der Übersicht der o.g. Autoren in der Summe oft für höhere Emissionen als beständige Anaerobiose allein.
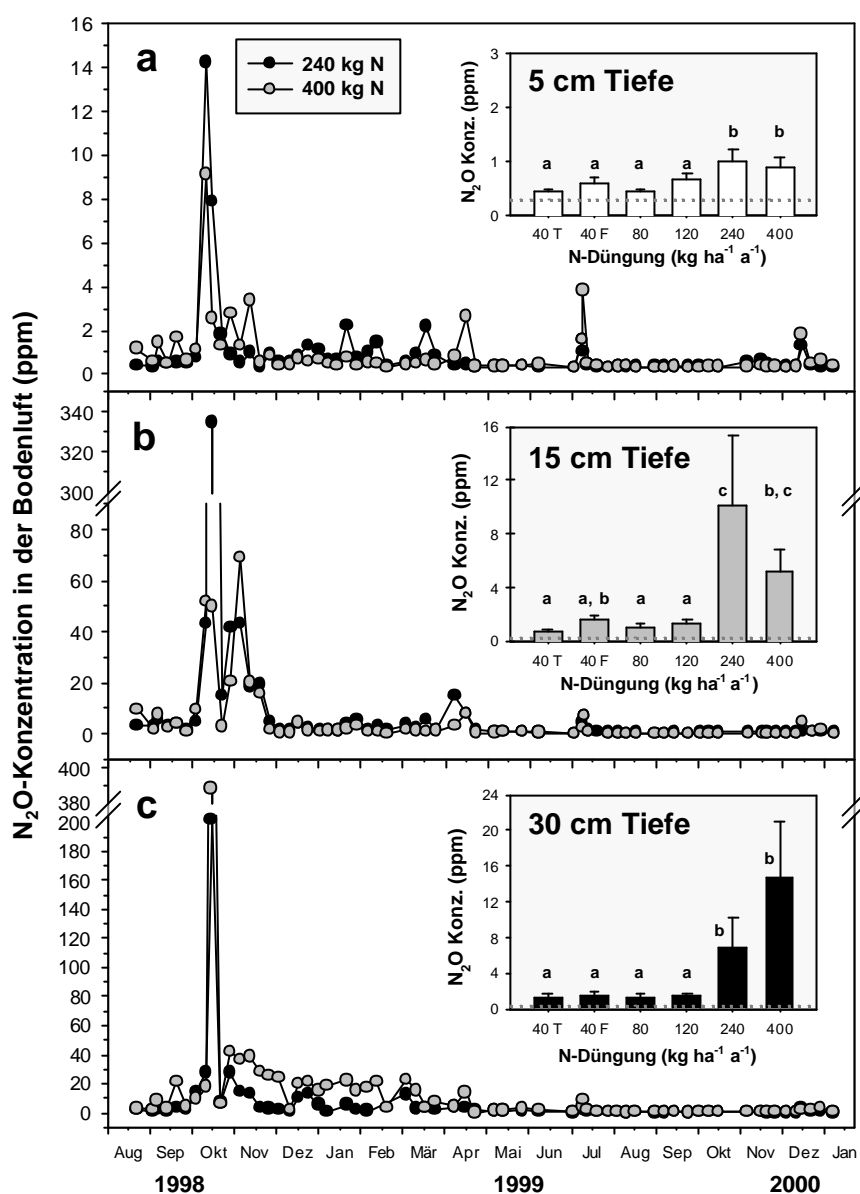
4

**Abbildung A.4-E**: Verlauf der N$_2$O-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 N$_2$O-Konzentrationen + Standardfehler im Meßzeitraum für die Bodenluftsonden-Parzellen. 40 T = trockene Parzelle 2.5, 40 F = feuchte Parzelle 2.4. Graue gepunktete Linie: Atmosphärische N$_2$O-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 N$_2$O-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 betrugen[1] (30 cm Tiefe), lagen die Maximalwerte der geringer gedüngten Flächen (15 und 30 cm Tiefe) alle zwischen 12 und 18 ppm N$_2$O, d.h. etwa um den Faktor 20 niedriger.

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[1] Da die gemessenen N$_2$O-Konzentrationen das Detektionslimit überschritten, wurde eine konservative Abschätzung der Konzentration vorgenommen: Die Eichgerade wurde linear extrapoliert, obwohl sie in Wirklichkeit bei hohen Konzentrationen nach oben abknickt. Daher kann davon ausgegangen werden, daß die wirklichen Konzentrationen noch über den angegebenen Werten lagen.
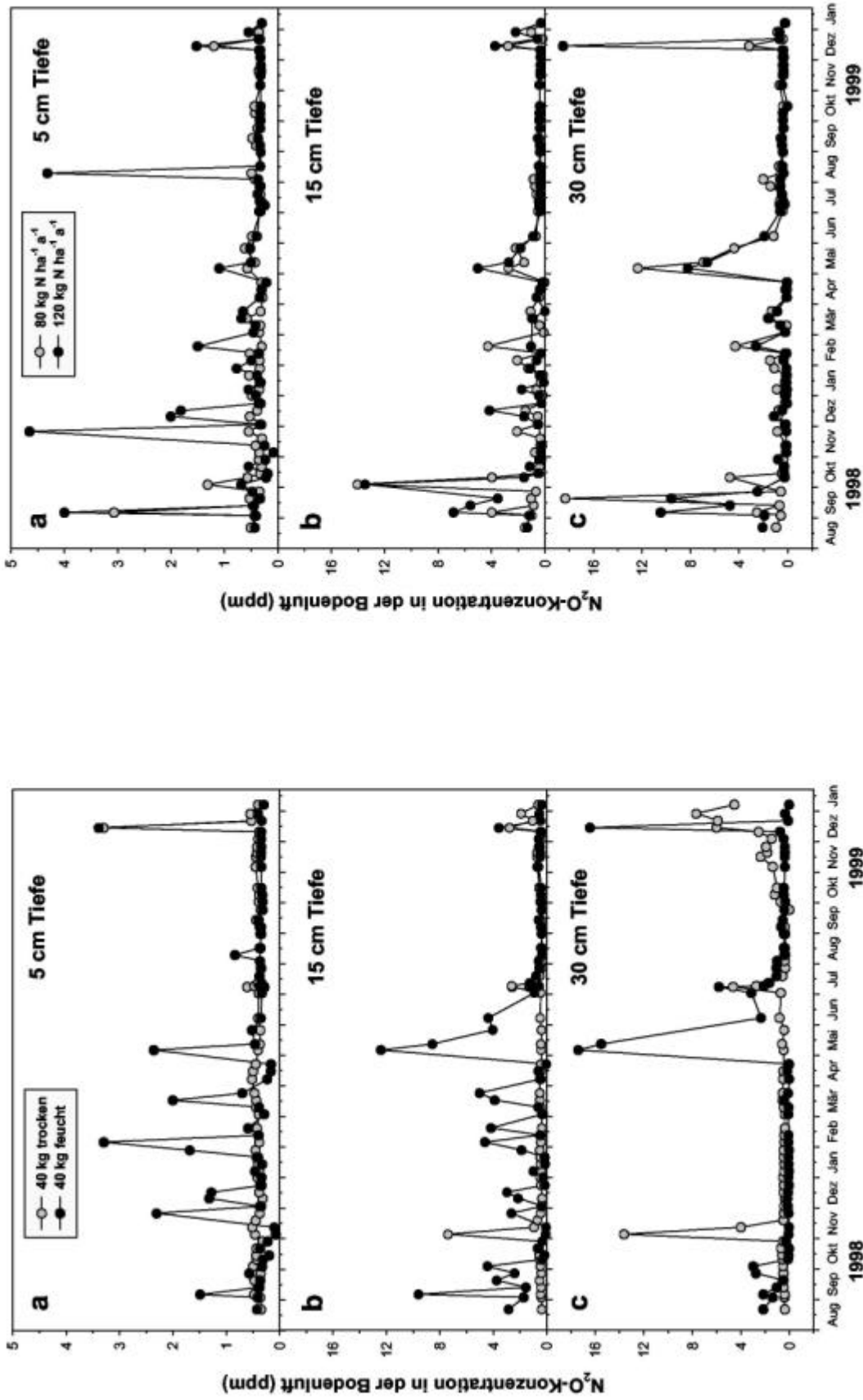
**Abbildung A.4-F:** N₂O-Konzentrationen in der Bodenluft der mit 40 kg N ha⁻¹ a⁻¹ gedüngten Parzellen (links) und der mit 80 und 120 kg N ha⁻¹ a⁻¹ gedüngten Parzellen (rechts) in 5, 15 und 30 cm Tiefe im Meßzeitraum vom 20.08.1998 bis zum 06.01.2000.

**Tabelle 3.3-A**: N$_2$O-Konzentrationen (ppm) in der Bodenluft unterschiedlich gedüngter Parzellen in verschiedenen Tiefen in der Zeit vom 20.08.1998 bis 06.01.2000, ± Standardabweichung.

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

Die N$_2$O-Konzentrationen in der Bodenluft bestätigen somit den zuvor bereits abgeleiteten Sachverhalt: Nur bei ausreichender Nitratverfügbarkeit (hier: auf den hoch-gedüngten Parzellen) ist eine sehr hohe N$_2$O-Produktion (und in Folge hohe N$_2$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 N$_2$O (Abb. A.4-F).

Die aus der Meßperiode resultierenden mittleren Bodenluft-N$_2$O-Konzentrationen (Tab. A.4-D) stimmen mit den Ergebnissen anderer Autoren überein: In 5 cm Tiefe lagen sie zwischen 460 bis 670 ppb N$_2$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 Prärie-boden und kultiviertem Ackerboden für einen vergleichbaren Zeitraum Mittelwerte von 403 und 1090 ppb N$_2$O angeben. In weniger effektiven Nitrat-absorbierenden Ackerböden (Maisanbau, 150 kg N ha$^{-1}$ a$^{-1}$ lagen die N$_2$O-Mittelwerte in 5 cm Tiefe hingegen höher, bei 1320 und 1250 ppb N$_2$O (zwei verschiedene Beprobungsperioden, BURTON & BEAUCHAMP 1994).

Der hier gemessene Jahresverlauf der N$_2$O-Konzentrationen unterschiedet sich mit seinen Herbst- und Frühjahrsmaxima bei jeweils guten Denitrifikationsbedingungen jedoch deutlich von Jahresgängen, 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 N$_2$O-Produktionsprozeß zurückführen. Obwohl der Anteil des Nitrifikations-N$_2$O an den Gesamt-N$_2$O-Emissionen im Sommer durchaus 60 % betragen kann (vgl. 5.8), sind die N$_2$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 Nitratverfüg-barkeit, 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.

- Statt dessen korrelierte die $CH_4$-Oxidation der einzelnen Parzellen mit der mittleren Bodenfeuchte bzw. mit der Länge der oxidierenden vertikalen Bodensäule über dem methanproduzierenden Horizont, vgl. Methanproduktion in der Tiefe, A.4.4.

- Dies spiegelte sich *nicht* in einer guten Korrelation zwischen dem Verlauf der Bodenfeuchte und der Methanoxidationsraten innerhalb einer Parzelle wieder ($R^2$ maximal 0,32), wie sie häufig von anderen Autoren beschrieben wird.

- Ebenfalls schlecht: Die Korrelation der Bodenluft-$CH_4$-Werte in 5 cm Tiefe oder tiefer mit der täglich gemessenen Bodenfeuchte. Ausnahme: Bodenfeuchtewerte unter 50 Vol.-% ($R^2 = 0,6$); hier war die Korrelation zwischen Bodenluft-$CH_4$ und Bodenfeuchte *negativ* ($CH_4$-Konzentrationen sinkend mit steigender Bodenfeuchte).

- Rückgang der $CH_4$-Oxidationsraten im Spätsommer 1997 durch Wasserstreß

*A.4.4   Methankonzentrationen in der Bodenluft*

Wie Abb. A.4-G zeigt, schnellte die $CH_4$-Konzentration in der Bodenluft mit dem Anstieg des Grundwasserspiegels nach starken Herbstniederschlägen 1998 rasch in die Höhe. Dabei wurden Maximalwerte von 236 ppm $CH_4$ in 30 cm Tiefe (am 01.12.1998) und 51 ppm $CH_4$ in 5 cm Tiefe (am 17.11.1998) erreicht. Die Methanwerte der 50-cm-Sonde waren nur deshalb meist niedriger als die in 30 cm Tiefe, weil die Sonde die längste Zeit während des Winters 1998/1999 unterhalb des Grundwasserspiegels lag und die Werte somit die im Wasser gelöste $CH_4$-Konzentration wiedergeben. Zeitgleich hohe $CH_4$- und hohe $N_2O$-Konzentrationen schlossen sich nahezu aus (Abb. A.4-H).

Herrschten reduzierende Verhältnisse für die $CH_4$-Produktion, so lagen die $N_2O$-Konzentrationen stets um Null. Zugleich war in solchen Proben im FID-Chromatogramm der $O_2$-Peak verschwunden. Bei denjenigen Punkten in Abb. A.4-H, bei denen sowohl die $N_2O$- als auch die $CH_4$-Werte erhöht waren, lagen die Sonden, in denen dies gemessen wurde, nie unter dem Wasserspiegel. Daher ist anzunehmen, daß aufsteigendes $CH_4$ in $N_2O$-produzierende Horizonte mit höherem Redoxpotential gelangte.

Während aller Phasen mit oberflächennahen (5 cm Tiefe), über-atmosphärischen $CH_4$-Konzentrationen wurden wiederholt mit beiden Gasauffanghauben auf Parzelle 2.4 und auch auf der Nachbarparzellen 7.4 und 8.4 $CH_4$-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).
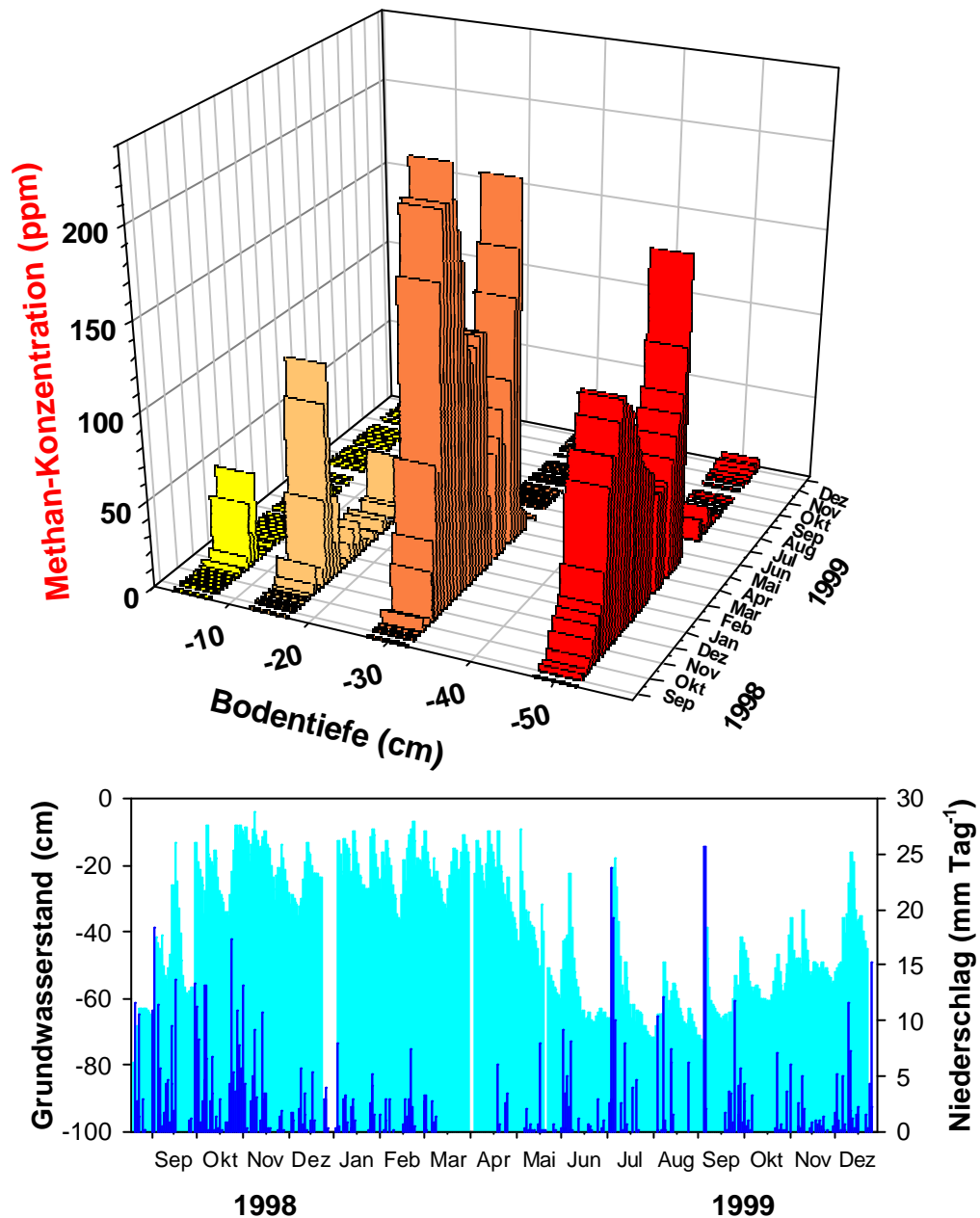


**Abbildung 3.2-A**: Methankonzentration in der Bodenluft der feuchtesten Parzelle 2.4 (40 kg N ha$^{-1}$ a$^{-1}$) in 5, 15, 30 und 50 cm Tiefe (obere Graphik) und Grundwasserstand sowie Tagesniederschläge dieses Zeitraums (untere Graphik). Man beachte, daß die Sonde in 50 cm Tiefe lange Zeit unter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

Diese Befunde belegen eindrucksvoll die CH$_4$-Oxidationskapazität des untersuchten Grünland-Ökosystems. Von Feuchtgebieten wie Sümpfen, Mooren oder Reisfeldern ist bekannt, daß bis zu 80 % des produzierten Methans in der aeroben Rhizosphäre, dem Aerenchym der Pflanzen oder in den aeroben obersten Boden- oder Wasser-Millimetern biologisch oxidiert wird, bevor es emittiert werden kann (CONRAD & ROTHFUSS 1991; BUTTERBACH-BAHL *et al.* 1997; WATANABE *et al.* 1997). WHALEN *et al.* (1990) und WHALEN *et al.* (1996) konnten zeigen, daß die Methan-Oxidation die CH$_4$-Emissionen

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 $CH_4$-Flüsse in die Atmosphäre modifiziert bzw. vermindert.
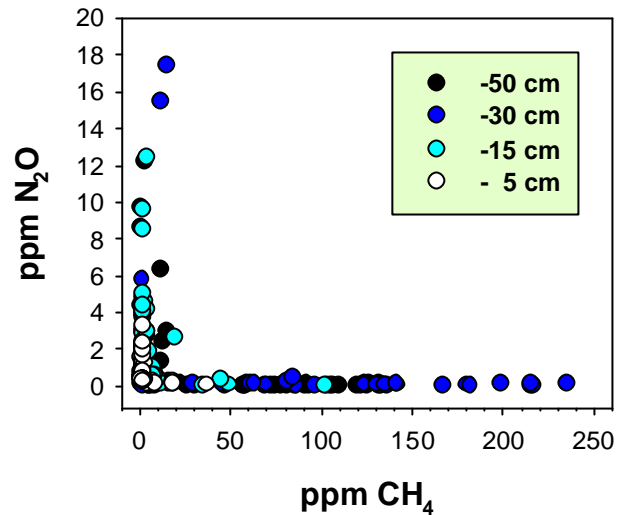


**Abbildung A.4-H**: Beziehung zwischen den zeitgleich gemessenen $CH_4$- und $N_2O$-Konzentrationen in der Bodenluft der Parzelle 2.4 (40 kg N, feucht) in verschiedenen Tiefen.

Jede Verminderung der $CH_4$-Oxidationskapazität der Böden, sei es durch Land-nutzungseinflüsse wie Rodung, Düngung oder Pflügen, oder auch möglicherweise durch erhöhtes $CO_2$, könnte daher im globalen Maßstab einen größeren Einfluß auf die Jahresbilanzen der $CH_4$-Flüsse von methanproduzierenden Ökosystemen haben als bislang angenommen.

### A.4.5 Die closed-chamber-Methode und Grenz-$CH_4$-Flüsse: Methodenkritik

Während des Höchststandes der je gemessenen Bodenluft-$CH_4$-Konzentrationen am 17. November 1998 (Abb. A.4-I) wurden am gleichen Abend noch $CH_4$-Flußmessungen unmittelbar neben den Bodenluft-Sonden durchgeführt (Abb. A.4-J) und dabei eine starke Schwankung der $CH_4$-Konzentrationen unter der Haube festgestellt. Diese betrug ein Vielfaches der Meßgenauigkeit des GC, und analytische Fehler können als Ursache für diese Erscheinung ausgeschlossen werden. Schon am nächsten Tag (18. November 1998, Abb. A.4-J) war diese Erscheinung verschwunden, und die $CH_4$-Konzentration unter den Hauben änderte sich während der Bedeckung im Prinzip nicht mehr (d.h. es erfolgte auch keine Netto-Aufnahme von $CH_4$ aus der Atmosphärenluft).
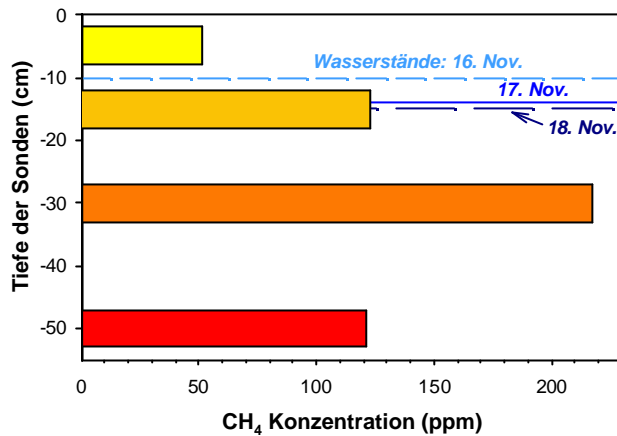
**Abb. A.4-I**: CH$_4$-Konzentrationen in der Bodenluft zum Zeitpunkt des höchsten oberflächennahen CH4-Stands (gemessen am 17.11.1998 um etwa 18:00 Uhr).

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

Während der Winterperiode bei hoher Bodenfeuchte und z.T. auch hohen Grundwasser-ständen konnten häufiger CH$_4$-Konzentrationsschwankungen in den Kammern während der Bedeckungszeit beobachtet werden, die das drei- bis vierfache der GC-Genauigkeit (2 x Standardabweichung) betrugen. Bei solchen Schwankungen konnte keine Flußrate bestimmt werden, da der Korrelationskoeffizient des Anstiegs bzw. Abfalls der Methan-konzentrationen während der Bedeckungszeit zu schlecht war ("Grenz-CH$_4$-Flüsse").
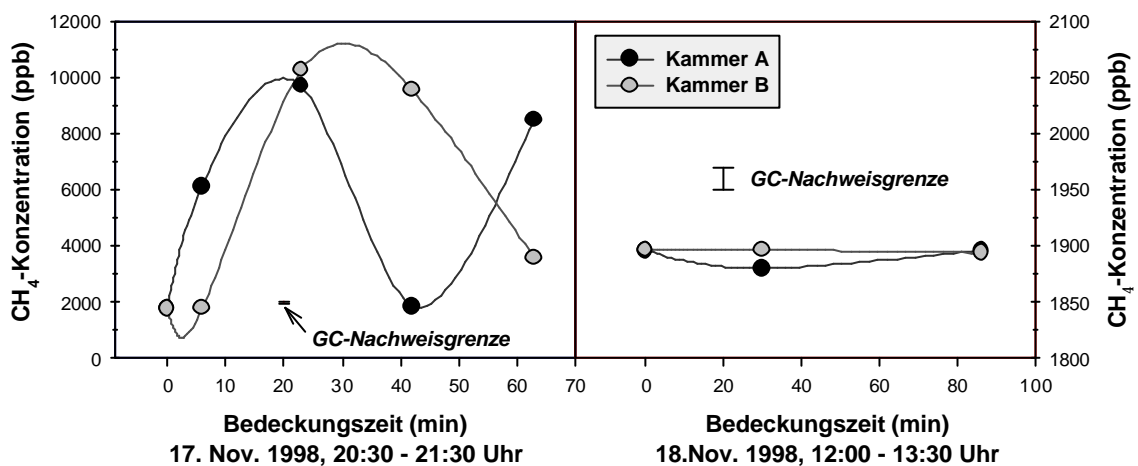


**Abbildung A.4-J**: Verlauf der CH$_4$-Konzentrationen in den Hauben auf Parzelle 2.4 (40 kg N ha$^{-1}$ a$^{-1}$, feucht) am 17. November abends (links), und einen Tag später mittags (rechts). GC-Nachweisgrenze = 2x Standardabweichung von 6 Messungen eines Standards. Man beachte die unterschiedliche Ordinaten-skalierung links und rechts.

Der Einblick in die Bodenluft-Methankonzentrationen am 17. November 1998 lieferte einen Erklärungsansatz für solche CH$_4$-Flußmessungen mit den geschlossenen Hauben: Die hohen Methankonzentrationen in den obersten cm des Bodens führten möglicher-weise zu aufsteigenden CH$_4$-Blasen wie von FECHNER-LEVY & HEMOND 1996) für das

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 Grundwasserstands 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 anaerober Inkubation waren einige $CH_4$-Werte bei einmaligem 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 $CH_4$ 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 $CH_4$-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 $CO_2$ (Typ 3; Abb. A.4-K). Die höchsten gemessenen $CH_4$-Produktionsraten lagen bei 55 µg $CH_4$-C 100 $cm^{-3}$ $h^{-1}$ (entsprechend 19,6 µg $CH_4$ $g^{-1}$ $Tag^{-1}$ (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 $CH_4$ $g^{-1}$ $Tag^{-1}$, WAßMANN *et al.* 1998).
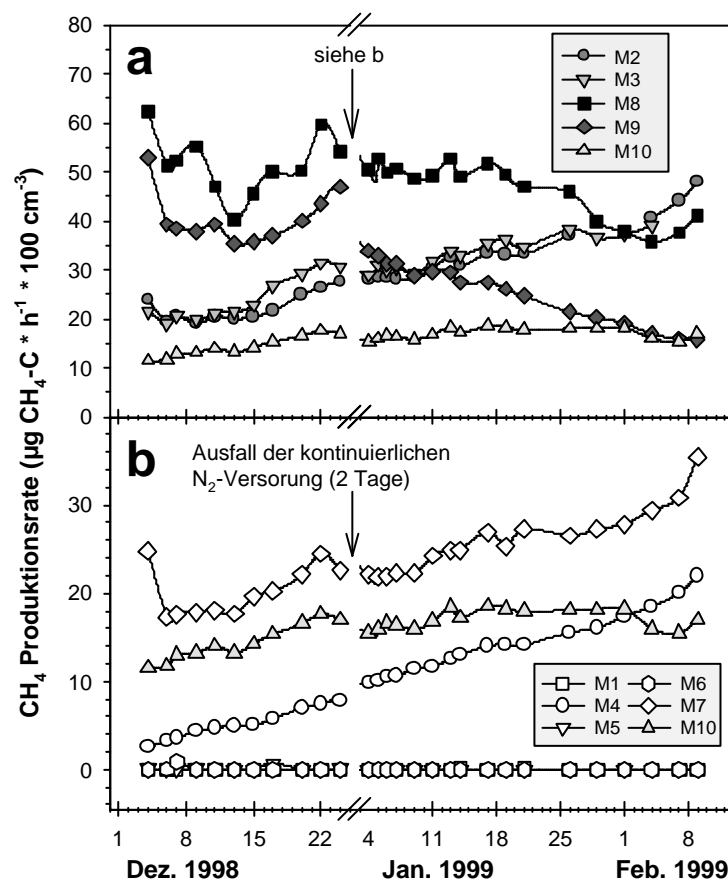


**Abbildung A.4-K**: Methanproduktion der anaerob inkubierten Bodenkerne (Durchströmungstechnik). Wassergehalt = Feldkapazität. **a**) anfangs hochproduktive Kerne; **b**) gering oder nicht $CH_4$ produzierende Kerne. (M10 ist zur besseren Vergleichbarkeit in **a** *und* **b** dargestellt).

Interessant ist die Korrelation zwischen den $CH_4$-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.

In Mikrokosmos-Untersuchungen konnten BORKEN *et al.* (2000) zeigen, daß *Lumbricus terrestris*, zusammen mit Birkenblätter-Substrat sowie seinen Ausscheidungen inkubiert, kurzfristig $CH_4$ produzierte (unter aeroben Verhältnissen). Die $CH_4$-Oxidationskapazität des getesteten Waldbodens wurde in dieser Studie durch das Vorhandensein der Regenwürmer signifikant reduziert. Dabei blieb unklar, ob das $CH_4$ von den Würmern selbst produziert wurde, oder ob es auf Methanogene im Gastrointestinaltrakt der Würmer zurückging (BORKEN *et al.* 2000). In einer Untersuchung von KARSTEN & DRAKE (1997) wurden im Gastrointestinaltrakt zweier anderer Regenwurmarten keine kultivierbaren Methanogenen gefunden. Zudem konnten PETERS & CONRAD (1995) und PETERS & CONRAD (1996) sogar in anaerob inkubierten Wüstenböden (Bodensuspensionen) Methanogenese induzieren, d.h. Methanogene sind anscheinend wiederstandsfähiger gegen $O_2$ als bislang angenommen. Die hier gezeigten Ergebnisse lassen aber zumindest denkbar erscheinen, daß Regenwürmer Träger methanogener Bakterienpopulationen waren, die möglicherweise auch im Kot der Tiere vorhanden waren und beim Einsetzen von Anaerobiose rasch für hohe Methanproduktionsraten sorgten.

Es wäre interessant, die Hypothese zu testen, ob die Induzierbarkeit von Methanogenese im Lindener Grünland grundsätzlich an lebende Regenwürmer und/oder ihren Kot gebunden ist, da ja einige Kerne keinerlei $CH_4$ produzierten. ZALLER & ARNONE (1999) konnten zeigen, daß die Menge der Ausscheidungen (*surface cast production*) von Regenwürmern unter erhöhtem $CO_2$ in Kalkgrünland um 35 % zunahm. Unter diesen Voraussetzungen könnten Regenwürmer möglicherweise einen Einfluß auf die potentielle Methanproduktion bzw. die Netto-$CH_4$-Oxidation des Bodens unter erhöhtem $CO_2$ haben.

Generell ist eine hohe $CH_4$-Produktionsrate (bei Anaerobiose) aber vor allem an das Vorhandensein von frischem organischem Material geknüpft (vgl. WACHINGER *et al.* 2000). Einer der inkubierten 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 hindeuten könnten (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.
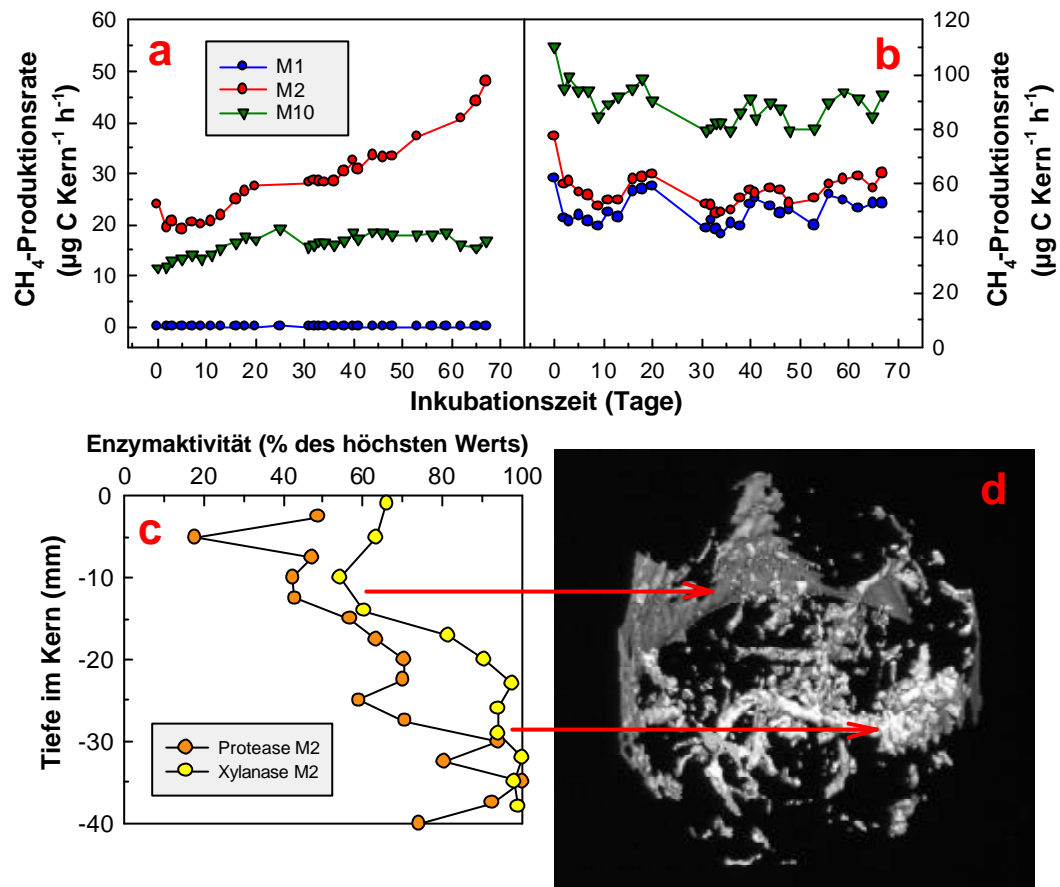
**Abbildung A.4-L**: **a**) Methanproduktion und **b**) CO$_2$-Produktion von drei inkubierten Kernen (drei verschiedenen "Methanproduktions-Typen"). **c**) Aktivitäten zweier extrazellulärer Zersetzer-Enzyme im Kern M2 (Mirkotomschnitte, G. Wachinger), und d) Computertomographisches Dichtebild des Kerns M2 mit Wurzelstruktur (Prof. Alzen u. Mitarbeiter, bearbeitet von G. Wachinger). Hell = geringe Dichte. Die roten Pfeile zwischen **c** und **d** kennzeichnen ungefähr die Schicht der entsprechenden Enzymaktivität.



**Abbildung A.4-M**: Temperaturabhängigkeit der Methanproduktion nach Beendigung der isothermen Inkubation (20 °C). Die Temperaturmessung erfolgte mit einem in Kern M1 inserierten Thermometer. Dieser Kern wurde, ebenso wie die anderen 6 Kerne, kontinuierlich von N$_2$-Gas durchströmt.

Am 11. Februar wurde die isotherme Inkubation der Bodenkerne beendet, und sechs $CH_4$-produzierende Kerne wurden im Zwischenraum natürlichen Temperatur-schwankungen ausgesetzt (vgl. 2.13; Abb. A.4-M). Zunächst reduzierten sich bei einer Absenkung der Temperatur von 20 auf 5,0 °C die $CH_4$-Produktionsraten drastisch um im Schnitt 94,4 %. Berechnet man aus diesem Temperaturschritt den $Q_{10}$-Wert (s.u.), so resultiert ein untypisch hoher Wert von 6,9 (Mittel der 6 Kerne).

Nach der Gleichgewichtseinstellung bei geringeren Temperaturen hingegen konnte aus den in Abb. A.4-M gezeigten Daten ein $Q_{10}$-Wert von im Mittel 3,5 berechnet werden (6 Kerne; Einzelwerte siehe Tab. A.4-K). Die Berechnung des $Q_{10}$ erfolgte nach THORNLEY & JOHNSON 1990) und MÜLLER (1999) nach folgender Gleichung:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{(10/(T_2 - T_1))} \qquad \underline{\text{mit:}} \qquad k_1, k_2: \text{Raten bei Temperaturen } T_1, T_2$$

d.h. für die Berechnung des $Q_{10}$ in Tab. A.4-C resultierte: $\qquad Q_{10} = \dfrac{k_2}{k_1}$

**Tabelle A.4-C**: Berechnung des $Q_0$ der $CH_4$-Produktion (etabliert bei 20 °C): Lineare Regression der $CH_4$-Produktionsraten vom 11./12. Feb. 1999 in Abhängigkeit von der Temperatur (Daten Abb. A.4-M); Rate (µg C 100 cm$^{-1}$ 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 $Q_{10}$ siehe Text.

| Bodenkern | Koeff. b[1] | Koeff. b[0] | R² | $k_1$ Rate (2 °C) | $k_2$ Rate (12 °C) | Q10 (2 → 12 °C) |
|---|---|---|---|---|---|---|
| M2 | 0,4233 | 1,0245 | 0,77 | 1,87 | 6,10 | 3,3 |
| M3 | 0,4423 | 1,2395 | 0,78 | 2,12 | 6,55 | 3,1 |
| M7 | 0,3571 | 0,5690 | 0,82 | 1,28 | 4,85 | 3,8 |
| M8 | 0,5824 | 0,8593 | 0,81 | 2,02 | 7,85 | 3,9 |
| M9 | 0,1940 | 0,2731 | 0,82 | 0,66 | 2,60 | 3,9 |
| M10 | 0,1710 | 0,3831 | 0,75 | 0,73 | 2,44 | 3,4 |

Der "Zusammenbruch" der $CH_4$-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 $CH_4$-Emissionen aus Moorböden fanden SAARNIO *et al.* (2000) bei Bodentemperaturen von 5 °C Effluxraten von etwa 0,6 bis 1,2 mg $CH_4$-C m$^{-2}$ h$^{-2}$. Errechnet man für die inkubierten Kerne mittels der in Tab. A.4-C angegebenen linearen Regressionsgleichung die mittlere Emissions-rate 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 $CH_4$-C m$^{-2}$ h$^{-1}$ – vergleichbar den Werten von SAARNIO *et al.* (2000). Der wesentliche Faktor für das "Zustandekommen" hoher $CH_4$-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 $CH_4$-Profile im Boden entstehen wie im Jahr 1998.

## A.4.7  Literatur

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## Anhang 5: Zusätzliche Hauptversuchsergebnisse
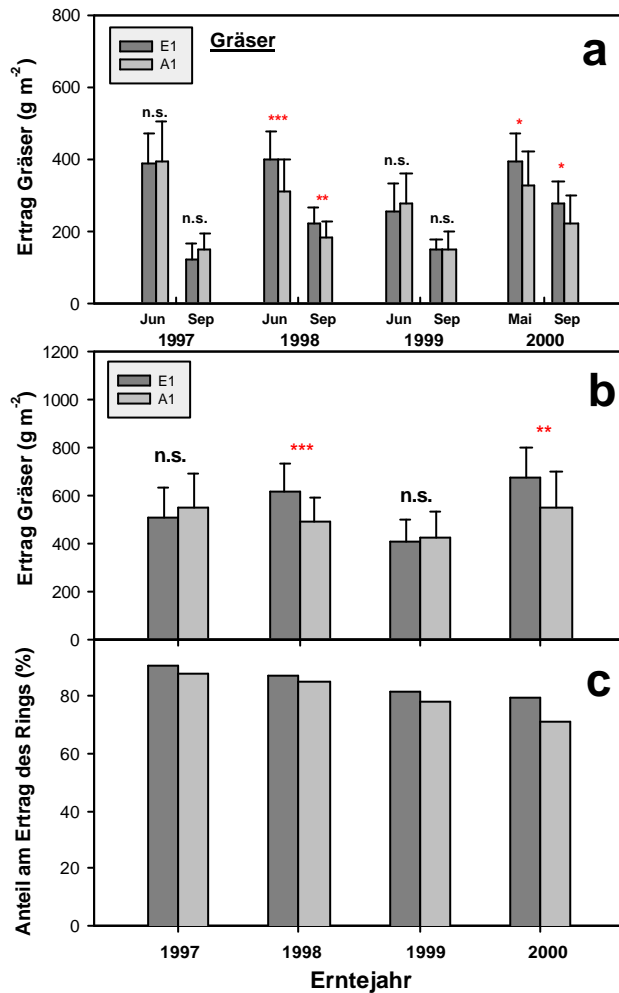
(Abbildungen zum Kapitel 3.3, Biomasse-Erträge)

**Abbildung A-3.3-J**: Gras-Ertrag im Ringpaar 1 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamtertrag des Rings.
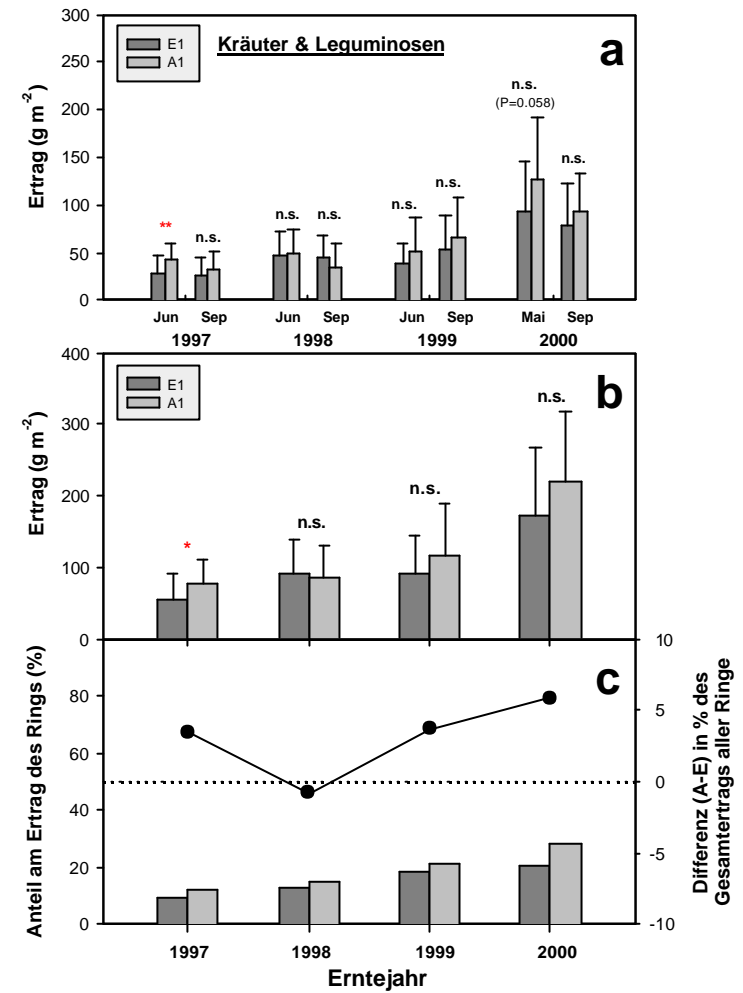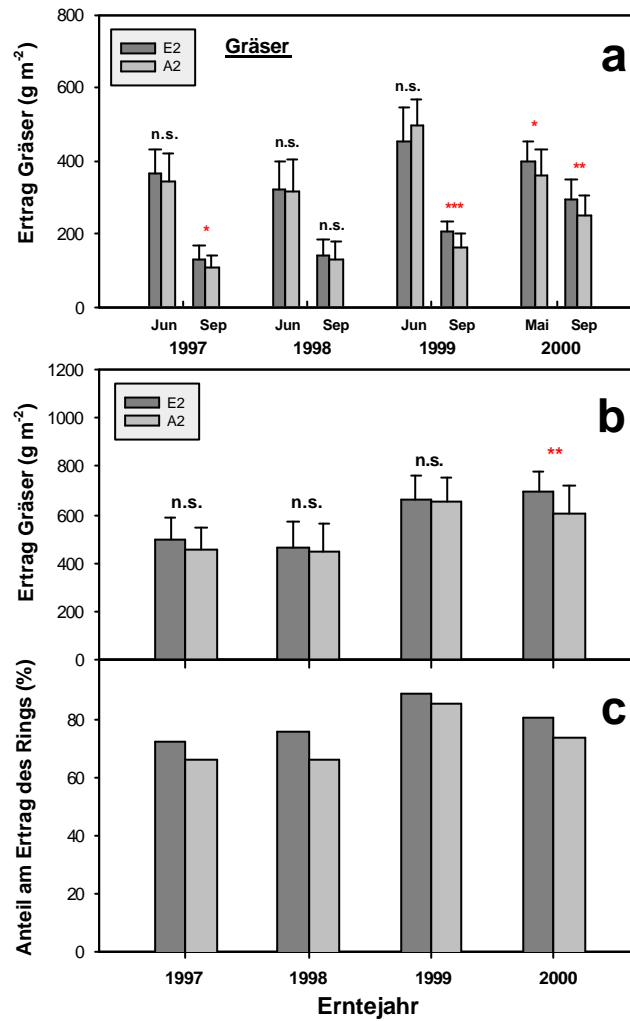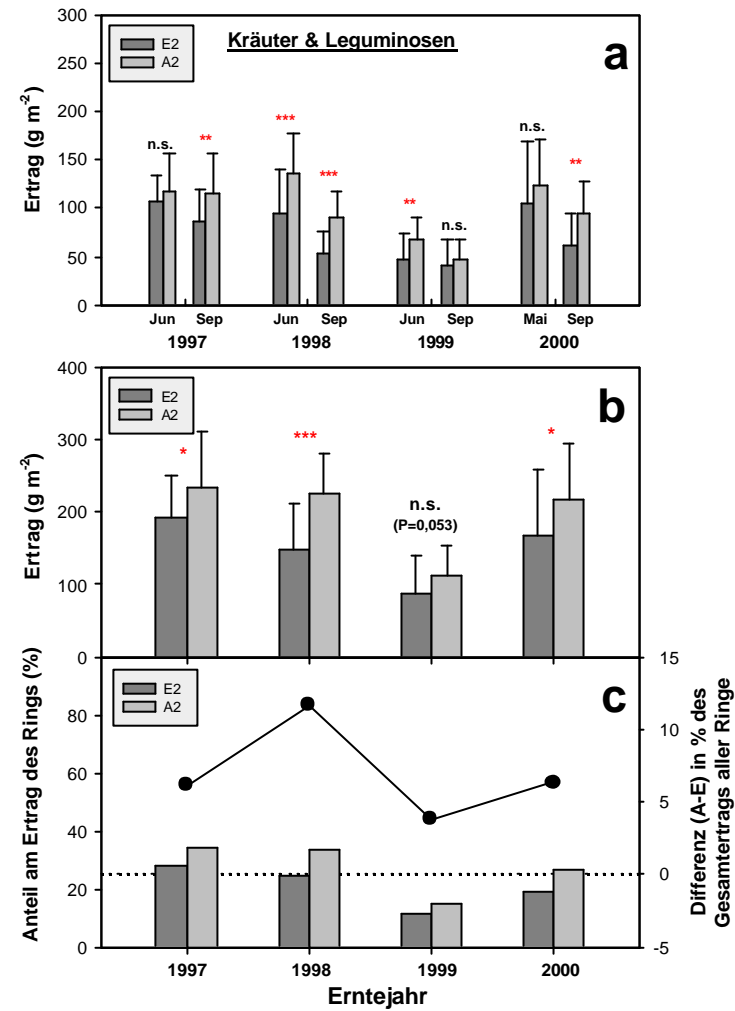
*Statistik: wie in Abb. 3.3-B*

**Abbildung A-3.3-K**: Ertrag "Krautige Pflanzen" im Ringpaar 1 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamt-Ertrag des Rings. Punkt-Linien-Darstellung: Differenz zwischen A1 und E1 in Prozent des Gesamtertrags aller Flächen (E1 bis A3).
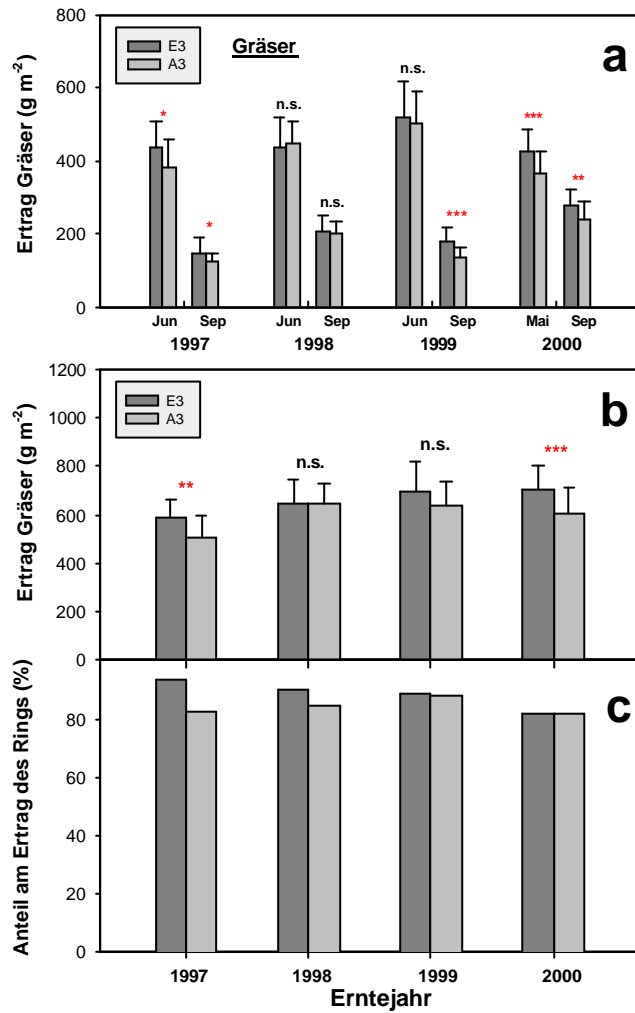
**Abbildung A-3.3-L**: Gras-Ertrag im Ringpaar 2 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamtertrag des Rings.

*Statistik: Wie in Abb. 3.3-B*

**Abbildung A-3.3-M**: Ertrag "Krautige Pflanzen" im Ringpaar 2 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamt-Ertrag des Rings. Punkt-Strich-Darstellung: Differenz zwischen A1 und E1 in Prozent des Gesamtertrags aller Flächen (E1 bis A3).

**Abbildung A-3.3-N**: Gras-Ertrag im Ringpaar 3 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge, und **c**) Prozentualer Anteil am Gesamtertrag des Rings.
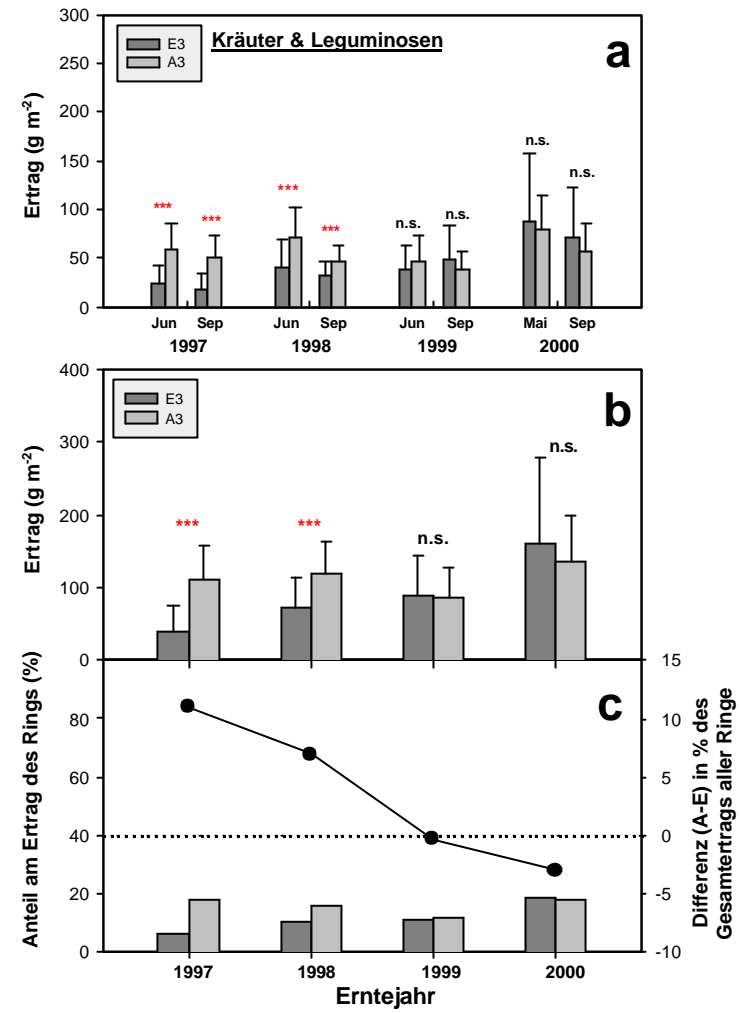
*Statistik: Wie in Abb. 3.3-B*

**Abbildung A-3.3-O**: Ertrag "Krautige Pflanzen" im Ringpaar 3 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge, und **c**) Prozentualer Anteil am Gesamt-Ertrag des Rings. Punkt-Strich-Darstellung: Differenz zwischen A1 und E1 in Prozent des Gesamtertrags aller Flächen (E1 bis A3).
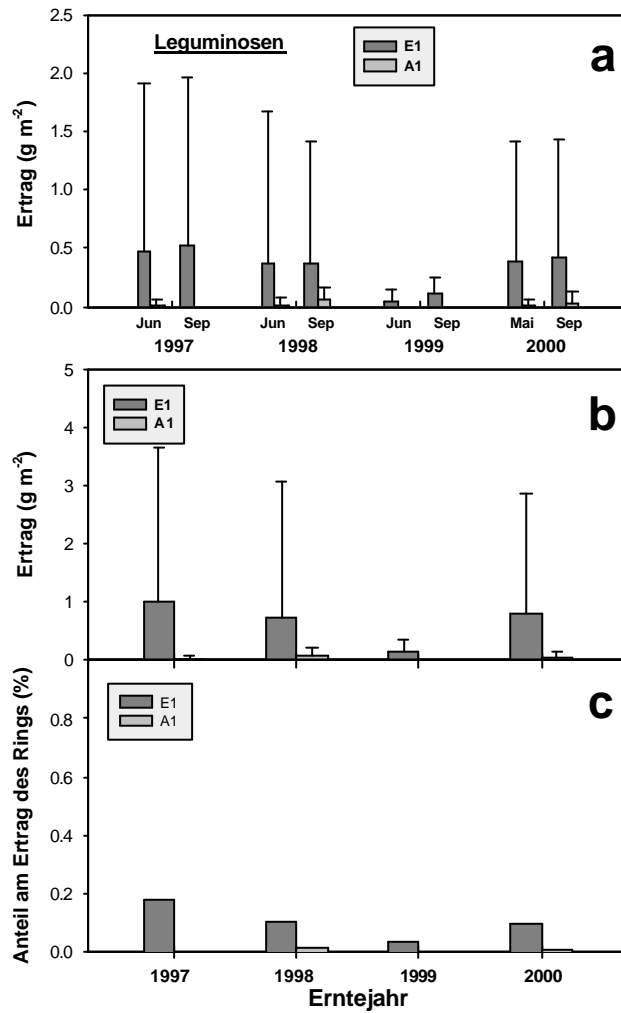
**Abbildung A-3.3-P**: Leguminosen-Ertrag im Ringpaar 1 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamtertrag des Rings. *Ordinatenskalierung den Erntedaten angepaßt!*
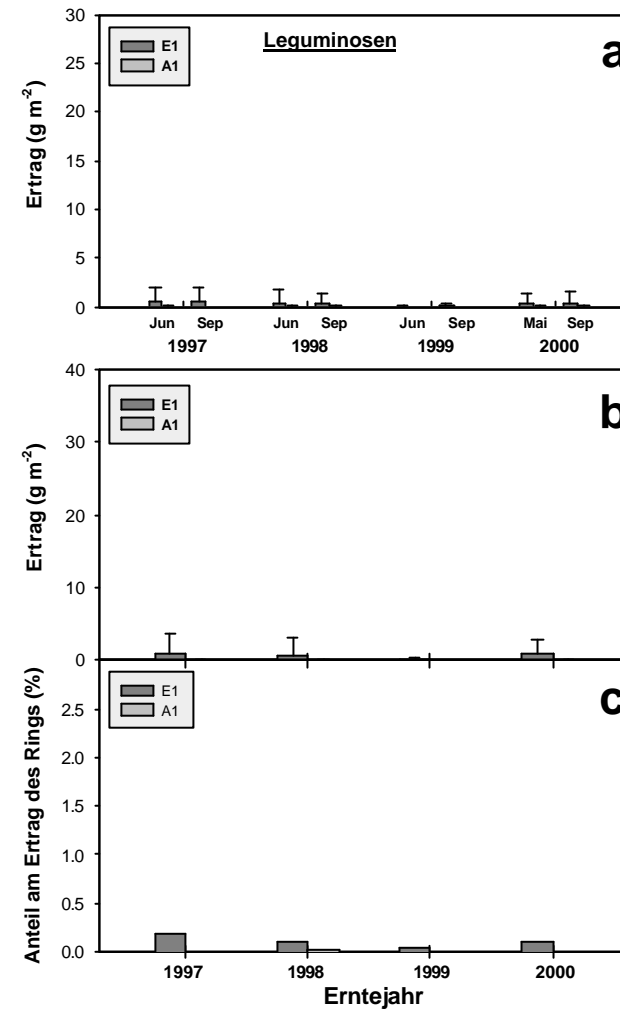
**Abbildung A-3.3-Q**: Leguminosen-Ertrag im Ringpaar 1 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge,und **c**) Prozentualer Anteil am Gesamtertrag des Rings. *Ordinatenskalierung wie bei Ringpaar 2 und 3!*

**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!*
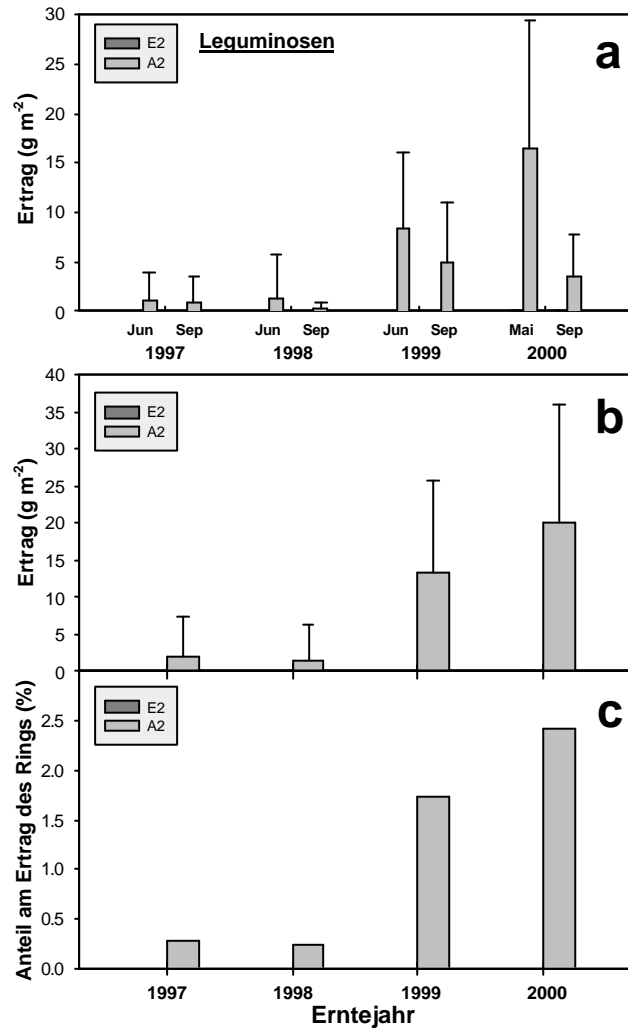
**Abbildung A-3.3-S**: Leguminosen-Ertrag im Ringpaar 3 von 1997 bis 2000 **a**) Einzel-Ernten, **b**) Jahres-Erträge, und **c**) Prozentualer Anteil am Gesamtertrag des Rings. *Ordinatenskalierung wie bei Ringpaar 1 und 2!*
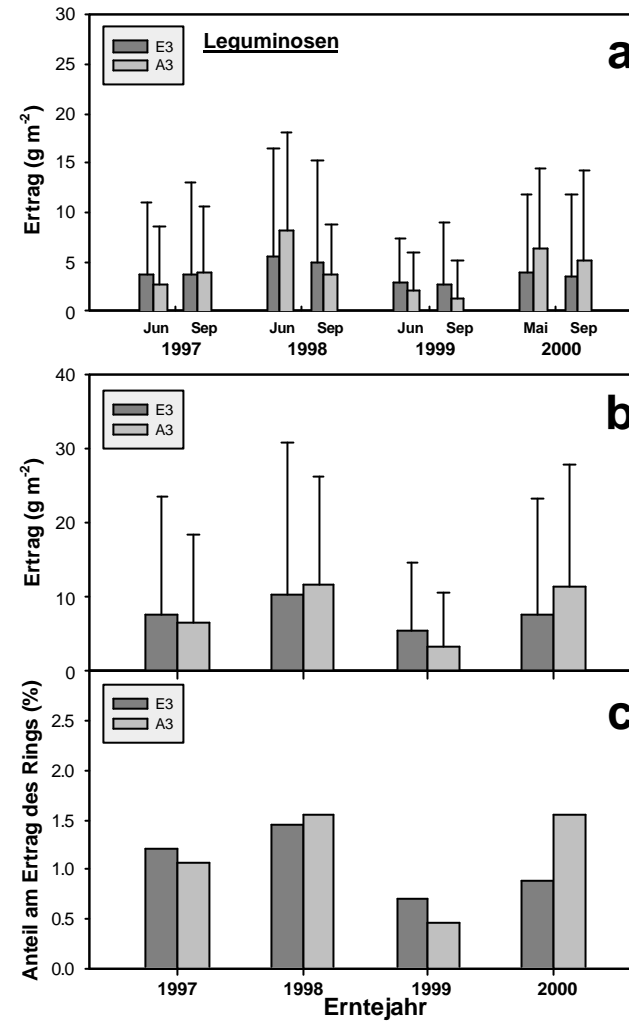
5

**Tabelle 3.3-D**: Zweifaktorielle Varianzanalyse der Erträge der **ersten Ernte**        **Tabelle 3.3-E**: Zweifaktorielle Varianzanalyse der Erträge der **zweiten Ernte**

<u>Für beide Tabellen gilt</u>: Erster Faktor: $CO_2$-Applikation; Zweiter Faktor: Lage der Ringpaare 1 bis 3 (variierende Bodenfeuchte). "Interaktion signifikant": $CO_2$-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)

**Tabelle 3.3-D (1. Ernte)**

| Jahr | CO₂-Applikation Gesamt-Ertrag | Gräser | Kräuter & Legu. | Lage 1–3 (Bodenfeuchte) Gesamt-Ertrag | Gräser | Kräuter & Legu. | Interaktion Gesamt-Ertrag | Gräser | Kräuter & Legu. |
|---|---|---|---|---|---|---|---|---|---|
| 1993 | n.s. 0,154 | | | * 0,039 2>1 | | | *** <0,001 A2>E2 E3>A3 | | |
| 1994 | n.s. 0,185 | | | *** <0,001 2>1 2>3 | | | n.s. 0,244 | | |
| 1995 | n.s. 0,982 | | | * 0,013 3>1 | | | n.s. 0,590 | | |
| 1996 | n.s. 0,228 | | | n.s. 0,487 | | | n.s. 0,338 | | |
| 1997 | n.s. 0,866 | n.s. 0,092 | *** <0,001 A>E | n.s. 0,055 | ** 0,003 3>2 | *** <0,001 2>1 2>3 | n.s. 0,335 | n.s. 0,154 | * 0,040 A1>E1 A3>E3 |
| 1998 +CO₂ | n.s. 0,764 | * 0,025 E>A | *** <0,001 A>E | *** <0,001 3>1 3>2 | *** <0,001 3>2 3>1 | *** <0,001 A>E | *** <0,001 E1>A1 | * 0,06 E1>A1 | * 0,015 A2>E2 A3>E3 |
| 1999 +CO₂ | * 0,029 A>E | n.s. 0,214 | ** 0,002 A>E | *** <0,001 2>1 3>1 | *** <0,001 2>1 3>1 | * 0,015 2>1 2>3 | n.s. 0,162 | n.s. 0,276 | n.s. 0,571 |
| 2000 +CO₂ | ** 0,004 E>A | *** <0,001 E>A | n.s. 0,135 | n.s. 0,485 | * 0,043 3>1 | * 0,015 2>3 | n.s. 0,326 | n.s. 0,636 | n.s. 0,162 |

**Tabelle 3.3-E (2. Ernte)**

| Jahr | CO₂-Applikation Gesamt-Ertrag | Gräser | Kräuter & Legu. | Lage 1–3 (Bodenfeuchte) Gesamt-Ertrag | Gräser | Kräuter & Legu. | Interaktion Gesamt-Ertrag | Gräser | Kräuter & Legu. |
|---|---|---|---|---|---|---|---|---|---|
| 1993 | n.s. 0,640 | | | ** 0,010 2>1 | | | ** 0,005 A1>E1 E3>A3 | | |
| 1994 | *** <0,001 A>E | | | *** <0,001 2>1 2>3 3>1 | | | n.s. 0,136 | | |
| 1995 | n.s. 0,625 | | | ** 0,008 2>1 | | | n.s. 0,524 | | |
| 1996 | n.s. 0,473 | | | n.s. 0,318 | | | n.s. 0,586 | | |
| 1997 | * 0,010 A>E | n.s. 0,323 | *** <0,001 A>E | *** <0,001 2>1 2>3 | * 0,047 [E3>E1 A1>A2 A1>A3]ʸ | *** <0,001 2>1 2>3 | n.s. 0,175 | *** <0,001 A1>E1 E2>A2 E3>A3 | * 0,024 A2>E2 A3>E3 |
| 1998 +CO₂ | n.s. 0,604 | * 0,015 E>A | *** <0,001 A>E | *** <0,001 1>2 3>2 | *** <0,001 1>2 3>2 | *** <0,001 2>1 2>3 | *** <0,001 E1>A1 A2>E2 | n.s. 0,146 | *** 0,024 A2>E2 A3>E3 |
| 1999 +CO₂ | *** <0,001 E>A | *** <0,001 E>A | n.s. 0,591 | ** 0,004 2>3 | *** <0,001 2>1 2>3 | ** 0,008 1>2 1>3 | *** <0,001 E2>A2 E3>A3 | ** 0,011 E2>A2 E3>A3 | n.s. 0,169 |
| 2000 +CO₂ | *** <0,001 E>A | *** <0,001 E>A | n.s. 0,085 | n.s. 0,082 | n.s. 0,135 | * 0,017 1>3 | n.s. 0,246 | n.s. 0,680 | * 0,011 A2>E2 |