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REGULAR ARTICLE

A study of soil methane sink regulation in two grasslands exposed to drought and N fertilization

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Abstract Oxidation by soil bacteria is the only biological sink for atmospheric methane (CH_4). There are substantial uncertainties regarding the global size of this sink, in part because the ecological controls of the involved processes are not well understood to date. We have investigated effects of severe summer drought and of nitrogen inputs (ammonium nitrate or cattle urine) on soil CH_4 fluxes in a field experiment. Soil moisture was the most important factor regulating the temporal dynamics of CH_4 fluxes. Simulated drought episodes altered the soil's water balance throughout the year, increasing CH_4 oxidation by 50% on an annual basis. N fertilizers exerted only small and transient effects at the ecosystem level. Laboratory incubations suggested that effects differed between soil layers, with larger effects of drought and N application in the top soil than in deeper layers. With soil moisture being the primary controlling factor of methanotrophy, a detailed understanding of the ecosystem's water balance is required to predict CH_4 budgets under future climatic conditions.

Keywords Ammonium nitrate · Cattle urine · Drought · Enzymatic inhibition · Grazing

Introduction

Methane (CH_4) is the second-most important anthropogenic greenhouse gas after carbon dioxide (CO_2), despite its atmospheric mixing ratio of only 1.8 $\mu L\ L^{-1}$ (IPCC 2007). While many CH_4 sources exist, both natural and anthropogenic, there is just one relevant biological sink for CH_4 – the oxidation of CH_4 in soils by methanotrophic bacteria (Dunfield 2007). Methanotrophs in wetland soils mainly thrive on CH_4 produced by methanogenesis in deeper soil layers where redox potential is low (Le Mer and Roger 2001). In contrast, well-aerated upland soils generally act as a net sink for atmospheric CH_4 (Conrad 2009). Global soil CH_4 sink estimates average around 30 Tg yr^{-1} (IPCC 2007), but this number is associated with large uncertainties (Smith et al. 2000), in part because the environmental

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regulation of the CH_4 uptake in upland soils is only poorly understood to date (Dunfield 2007; Bodelier and Laanbroek 2004).

Soil CH_4 uptake is generally limited by diffusion of CH_4 and dioxygen (O_2) through the soil profile to the sites occupied by CH_4 oxidizing bacteria (Del Grosso et al. 2000). The main factors determining soils gas diffusivity are soil porosity and water content. Soil moisture limits CH_4 and O_2 diffusion by filling small pore networks and, at high soil moisture, also macro-pores. Diffusion of CH_4 in water is slow and solubility low, so that soil moisture very effectively blocks CH_4 transport in soil. On the other hand, extreme water deficiency can reduce soil CH_4 uptake due to physiological stress (Nesbit and Breitenbeck 1992).

A number of studies have shown that fertilizers containing ammonium (NH_4^+) can reduce soil CH_4 oxidation in a variety of ecosystems (Bronson and Mosier 1994; Hütsch et al. 1994; Powlson et al. 1997). However, the mechanisms underlying this effect remain unclear. While NH_3 can inhibit the enzyme system responsible for the oxidation of CH_4 in methanotrophs (Dunfield and Knowles 1995), reports are inconsistent. Many experiments have shown patterns consistent with the hypothesis of competitive inhibition at the enzyme level (Mosier et al. 1991; Bronson and Mosier 1994; Willison et al. 1995). However, inhibitory effects occurred only with a delay in other studies (Hütsch et al. 1993; Gulledge et al. 1997). Still other experiments showed no effects of fertilizer application (Lessard et al. 1997), or even a positive effect of N application (Bodelier et al. 2000), indicating that the effect of N fertilization on CH_4 uptake of soils is more complex (Bodelier and Laanbroek 2004). These findings probably reflect the fact that NH_4^+, while inhibiting CH_4 assimilation, also is an essential nutrient for methanotrophs. It has also been proposed that reductions in CH_4 oxidation rates may result from general osmotic effects of the applied fertilizer (e.g. Gulledge and Schimel 1998).

Grazing animals redistribute ingested plant N in patchy form, resulting in local N deposition rates of several hundred or thousand kg N ha^{-1} under dung and urine patches (Haynes and Williams 1993). Many pasture soils oxidize atmospheric CH_4 (Smith et al. 2000), despite these very high patch-level N deposition rates. This contrasts the strong and persistant reductions in soil CH_4 uptake often found under agricultural fertilizer application (e.g. Hütsch et al. 1993). Is methanotrophy under excreta patches inhibited only temporarily, or are methanotrophs eliminated for longer time periods? Were methanotroph species in grazed ecosystems selected to tolerate these extreme conditions?

In the present study, we investigated soil CH_4 uptake in two multi-year field experiments in temperate grassland. N fertilizer was applied at rates equivalent to local N deposition by cattle, and severe summer drought was simulated with rain exclusion roofs. The objectives of this study were to analyze the mechanisms by which these experimental treatments affect soil CH_4 uptake, and to test how these interact.

Materials and methods

Field sites and experimental design

A field experiment simulating drought and N deposition by cattle was set up in September 2006 on two research farms representing typical Swiss grassland farming systems. The first site, *Früebüel* (Fig. 5 of online resource 1), is located on a montane plateau in central Switzerland and managed at intermediate intensity (8.5415° E, 47.1135° N, 1,000 ma.s.l.). Prior to this study, field plots were grazed by non-dairy cattle or mown for hay three to four times per year. The only fertilizer inputs were cattle excreta and manure from cattle kept in stables. The growing period lasts from April to October. The soil is a silt loam (37% sand, 56% silt and 7% clay, pH of ~4.7). The second site, *Alp Weissenstein* (Fig. 6 of online resource 1), is an extensively managed subalpine grassland in the eastern Swiss Alps (46.5833° E, 9.7859° N, 1,975 ma.s.l.). The growing period is short (mid May to mid September) and the site only grazed two to three times by non-dairy cattle and horses. No fertilizer is applied. The soil is a silt loam (35% sand, 59% silt and 6% clay) with a pH of ~5.0. Mean annual temperature and precipitation for the 1961–1990 period are approximately 6–7°C and 950 mm for Früebüel and 2°C and 1,350 mm for Alp Weissenstein (data provided by Swiss Federal Office of Meteorology and Climatology, values interpolated from nearby weather stations correcting for altitude).

The field experiment was organized as randomized complete split-plot design with five blocks per site.

Each block consisted of two 3.5 m×3 m plots, one of which was subject to simulated drought while the other served as control plot. Drought was simulated by excluding precipitation with rain exclusion roofs (Gilgen and Buchmann 2009) covered with a 200 µm thin plastic foil (Gewächshausfolie UV 5, folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). The central 2 m×2.2 m of each plot were subdivided into four subplots using polyvinyl chloride sheets reaching 15 cm depth. These subplots were either treated with ammonium nitrate (NH_4NO_3), non-dairy cattle urine, or served as unfertilized control (NIL). The forth subplot was not used in this study. This setup resulted in five replicates per site and treatment combination.

Ten-minute averages of soil temperature and moisture were recorded in two blocks per site (CR1000 data logger, Campbell Scientific Inc., Logan, UT, USA) at two depths (8 and 25 cm at *Früebüel* and 8 and 20 cm in the shallower soils at *Alp Weissenstein).* Temperature probes (AD592, Analog Devices, Norwood, MA, USA) were installed in all subplots, whereas soil moisture probes (EC-6, Decagon Devices Inc., Pullman, WA, USA) were installed in the unfertilized subplots only. Concomitantly with the regular CH_4 flux measurements, soil moisture was recoded manually in all plots (0–5 cm depth, ThetaProbe ML2x, Delta-T Devices Ltd., Burwell, Cambridge, UK).

Management

Grazing animals were excluded from October 2006 until the experiment was terminated in summer 2009. The sites were clipped at 4 cm height when the surrounding pastures were grazed by livestock or mown by the local farmer.

In 2007, the rain-exclusion roofs were installed from August 3 to September 27 and from July 31 to September 25 at *Früebüel* and *Alp Weissenstein*, respectively. In 2008, the roofs were installed from June 26 to August 13 and from July 14 to September 26, respectively.

The fertilizer treatment consisted of a small and a larger application in 2007 and a single large application in 2008. All applications took place when the rain exclusion roofs were installed. We split the fertilizer application in the first year into two portions because we had no a priori knowledge of the sensitivity of the soil CH_4 sink and did not want to completely inhibit it over prolonged periods. Urine was collected from non-dairy cattle. Both NH_4NO_3 and urine were applied as aqueous formulation (4.9 Lm^{-2}) and the same amount of water was applied to unfertilized control subplots (NIL treatment). The vast majority of urine N is in the form of urea, which quickly hydrolyses to NH_4^+ in soils (Haynes and Williams 1992). Since NH_3 is the chemical species generally believed to inhibit CH_4 oxidation, equivalent amounts of NH_4^+ and urea N were applied to subplots (5 g and 15 gN m^{-2} for the small and large fertilizer applications, respectively), which resulted in double the amount of N applied in the NH_4NO_3 fertilizer relative to the urine treatment.

CH_4 flux measurements

Static chambers (32 cm diameter×30 cm height) with a detachable lid were installed in the center of all subplots by carefully pre-trenching the soil with a spade fitting the curvature of the chamber and lowering these 19 cm into the ground (resulting in 11 cm chamber height and 8.85 L headspace). Soil CH_4 uptake rates were measured approx. every 2 weeks during the growing period, and more frequently in the first week after fertilizer applications. To measure CH_4 exchange rates, lids were attached to the chamber collars and headspace samples collected 5, 20 and 35 min after chamber closure. Gas samples were injected into pre-evacuated exetainers and analyzed for CH_4 in the laboratory (Agilent 6890 gas chromatograph equipped with a flame ionization detector, Agilent Technologies Inc., Santa Clara, CA, USA). The small concentration changes (generally around ~0.2 $\mu L\ L^{-1}$) did not allow to distinguish between zeroth or first order kinetics, and we therefore estimated CH_4 flux rates by linear regression. Correlation coefficients were generally very high ($r^2>0.97$) unless CH_4 exchange rates were close to zero.

CH_4 concentrations were measured in soil air collected from 50 cm long polypropylene tubes (Accurel PP V8/2 HF, Membrana GmbH, Wuppertal, Germany) installed horizontally at the same depths as the soil moisture and temperature probes. These tubes are permeable for gases including CH_4 but not for water. They were closed at the ends to equilibrate with the soil atmosphere. On sampling dates, equilibrated air was collected with a syringe and CH_4 concentrations analyzed as described above.

Soil analysis

When the experiment was terminated in 2009, soil blocks with 20 cm×20 cm surface area were excavated and divided into 0–5, 5–10 and 10–15 cm depth layers. CH_4 oxidation under standardized conditions was determined in fresh sieved soil (4 mm mesh size) corresponding to 100 g dry weight. This soil was adjusted to a water content of 0.3 g H_2O (g soil)$^{-1}$ and placed into 0.9 L gas-tight jars fitted with a septum. The soils were equilibrated at 20°C overnight, the jars opened for 30 min to aerate the samples, closed again, and CH_4 oxidation rates determined by measuring headspace CH_4 concentrations after 10, 160 and 310 min. Incubations were conducted under atmospheric concentrations, i.e. no extra CH_4 was injected into the headspace.

A separate incubation experiment tested effects of chemical species on soil CH_4 oxidation. The underlying rationale was that inhibitory effects could be due to osmotic effects rather than to effects of the N contained in the fertilizers. Sieved top soil (0–10 cm) samples equivalent to 100 g dry weight were amended with 8 mL aqueous solution containing 0.0, 0.088, 0.175, 0.35, 0.70 or 1.40 mmol of NH_4NO_3, ammonium chloride (NH_4Cl), potassium nitrate (KNO_3) or potassium chloride (KCl). Soils were incubated overnight in 0.9 L jars from which septa had been removed and blocked with paper tissue to allow some air exchange without drying of soils. Then, jars were aerated for 30 min and CH_4 oxidation rates measured as described above. The measurements of CH_4 consumption were repeated daily to test for a temporal component of effects. However, because there was none, these measurements were discontinued after 4 days and repeated measures averaged.

Data analysis

Data were analyzed using mixed-effects models fitted by maximum likelihood (*lme* function from the *nlme*-package of R 2.8.1, R Development Core Team 2010). The models included the nested random effects site, block, plot, and subplot while drought, fertilization, and, where appropriate, also soil layer, were the fixed effects tested. Effects were considered significant when $P<0.05$. All error estimates in text and figures are standard errors of treatment means.

The response of CH_4 oxidation to salt additions was described as sigmoidal function with intercept: $f_{CH4} = x_1 \cdot (1 - 1/(1 + \exp(-x_2 \cdot (\mathrm{conc} - x_3)))) + x_4$, where f_{CH4} is the measured flux rate, conc is the concentration of the added compounds, and $x_{1\ldots4}$ are shape parameters estimated by non-linear least squares fitting using the *optim* function of R.

Results

Soil temperature and moisture

Soil temperature did not depend on simulated drought or N fertilization (Fig. 1a). Soil moisture exhibited large intra-annual variation with pronounced drying cycles (Fig. 1b). The rain exclusion roofs reduced precipitation in drought-treated plots in *Früebüel* by 450 mm and 410 mm in 2007 and 2008, respectively. The corresponding reductions at *Alp Weissenstein* were 210 mm and 315 mm. These effects are equivalent to a reduction in annual precipitation of 25–30%, or of 30–40% on a vegetation period basis (Fig. 1c). Soil moisture in drought-treated plots was reduced by 40–60% when rain exclusion roofs were installed (Fig. 1b; $P<0.001$). The reduction in soil moisture persisted for weeks or even months after the rain exclusion roofs had been removed. Most remarkably, significantly reduced soil water contents were repeatedly found up to 1 year after the roofs had been removed (i.e. in spring and summer of 2008 and 2009).

Soil CH_4 exchange

CH_4 uptake rates were lower at *Früebüel* than at *Alp Weissenstein* (Fig. 2). Soil CH_4 uptake was strongly correlated with soil moisture (Fig. 2, $r^2=0.67$ for *Früebüel* and $r^2=0.80$ for *Alp Weissenstein*, respectively; $P<0.001$ for both sites), while soil temperature did not explain any additional variation when fitted after soil moisture in a multiple regression model. Rain exclusion roofs increased the soil CH_4 sink by over 100% at *Früebüel* and by over 200% at *Alp Weissenstein* when installed (Fig. 3a, $P<0.001$). After removal of the rain exclusion roofs, soil CH_4 uptake rates remained enhanced for several weeks while soil moisture was still reduced. Most interestingly, increased CH_4 sink rates were also found before the rain exclusion roofs were set up again in 2008 and 2009, i.e. up to 1 year after the rain exclusion roofs had been removed from the plots the year before.

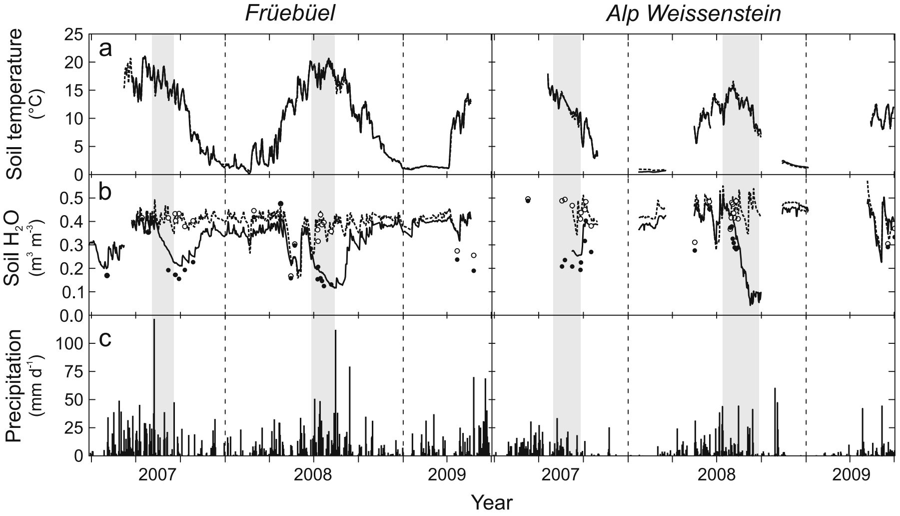
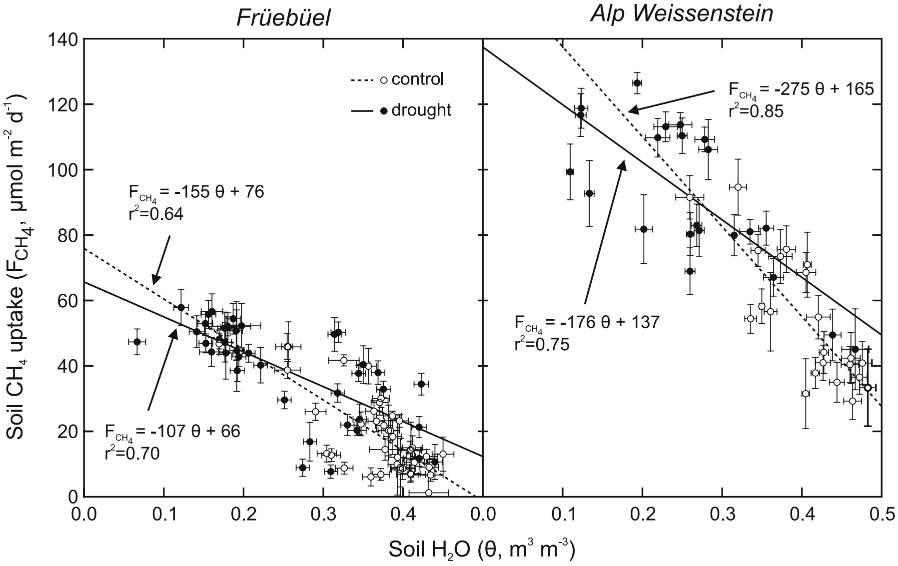


Fig. 1 **a** Soil temperature at 8 cm soil depth for control (*dashed line*) and drought-treated plots (*solid line*); differences are not visible most of the time since there were no significant differences. **b** Soil moisture at 8 cm depth for control (*dashed line*) and drought treated plots (*solid line*). Additional manual soil moisture measurements in 0–5 cm soil depth are indicated by dots (*white circle* control plots; *black circle* drought-treated plots). **c** Precipitation (data by Zeeman et al. 2010). *Grey* areas indicate periods with installed rain exclusion roofs

Both N fertilizers inhibited soil CH_4 uptake at both sites, but effects were limited to a period of a few weeks after fertilizer application and then vanished. Averaged over a 30-day period after N application, soil CH_4 uptake was reduced by both fertilizers ($P < 0.05$, average reduction of 13% over both sites). These inhibitory effects were relatively larger at *Früebüel* than at *Alp Weissenstein* ($P<0.05$ for site × N). At *Früebüel*, soil CH_4 uptake was repeatedly found to be more strongly inhibited by N application in the drought treatment than in the control plots ($P < 0.05$ for N × drought). However, these effects were transient and occurred only on the first day after fertilizer applications or shortly thereafter.

Fig. 2 Linear regression between soil CH_4 uptake and soil moisture for both experimental sites. Measurements were conducted between spring 2007 and summer 2009. *Error bars* indicate standard errors



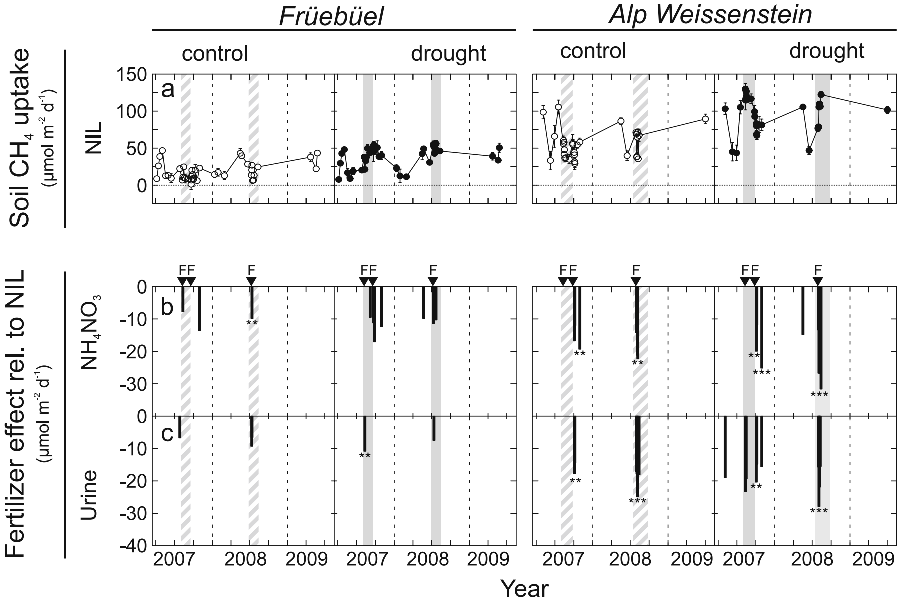


Fig. 3 Soil CH_4 uptake at the two experimental sites Früebüel and Alp Weissenstein. **a** shows CH_4 exchange rates in the unfertilised subplots (NIL treatment). **b** and **c** show effects of the added fertilizers relative to the NIL treatment; the presence of a *bar* indicates a significant effect at $P<0.05$; higher significances are indicated as ** $P<0.01$ and *** $P<0.001$; for clusters of bars, only the highest significance is indicated. *Grey* areas indicate periods when rain exclusion roofs were installed; F indicate fertilizer applications; *error bars* indicate the standard errors of treatment means

Soil CH_4 concentrations

Soil CH_4 concentrations always were below atmospheric concentration and decreased with depth (Table 1, $P<0.001$), indicating that both sites were a net sink for atmospheric CH_4. Rain exclusion roofs increased soil CH_4 concentrations when installed (+20% at *Früebüel*, $P<0.05$, and +70% at *Alp Weissenstein*, $P<0.001$, average over 2007 and 2008). This increase persisted for several weeks after removal of the rain exclusion roofs and closely followed the observed effects on soil moisture (data in online resource 2). As for soil moisture, a drought effect was detected in spring and summer of the next year (before rain exclusion roofs where re-installed) with increased soil CH_4 concentrations occurring mainly at 8 cm soil depth (data in online resource 2).

The application of NH_4NO_3 and urine had no significant effect on soil CH_4 concentrations, although soils at *Alp Weissenstein* exhibited a non-significant tendency towards increased soil CH_4 concentrations immediately after fertilizer application.

CH_4 oxidation of sieved soil

CH_4 oxidation rates of sieved soils exposed to standardized soil moisture and temperature depended on site and soil depth (Table 2, $P<0.001$ for site and

Table 1 Average soil CH_4 concentrations ($\mu L\ L^{-1}$) measured in two soil depths during the periods in which rain exclusion roofs were installed in 2007 and 2008

Treatment	Früebüel		Alp Weissenstein	
	8 cm	25 cm	8 cm	20 cm
Control	1.32±0.01	0.80±0.02	0.74±0.01	0.26±0.00
Drought	1.50±0.00	1.02±0.02	1.19±0.01	0.56±0.02

Table 2 CH_4 oxidation (nmol CH_4 (g dry soil)$^{-1}$ d^{-1}) of sieved soil exposed to ambient CH_4 concentrations, 20°C and with soil moisture adjusted to 0.3 m^3 H_2O m^{-3}

Treatment		Früebüel			Alp Weissenstein			average by treatment
		0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	
Control	NIL	0.43±0.04	0.45±0.04	0.39±0.06	2.18±0.21	1.93±0.16	1.24±0.12	1.10±0.14
	NH_4NO_3	0.33±0.09	0.44±0.07	0.33±0.06	1.83±0.25	2.06±0.26	1.44±0.31	1.07±0.15
	Urine	0.38±0.04	0.40±0.03	0.37±0.04	1.83±0.21	1.54±0.16	1.11±0.08	0.94±0.11
Drought	NIL	0.38±0.01	0.37±0.04	0.36±0.04	2.03±0.13	2.53±0.10	1.53±0.11	1.20±0.16
	NH_4NO_3	0.26±0.03	0.42±0.03	0.40±0.04	1.98±0.25	2.18±0.35	1.53±0.20	1.13±0.16
	Urine	0.35±0.04	0.44±0.05	0.42±0.05	1.98±0.25	1.89±0.21	1.05±0.11	1.02±0.13
Average by depth		0.36±0.01	0.42±0.01	0.37±0.01	1.89±0.08	1.93±0.08	1.21±0.07	1.03±0.05

$P<0.001$ for site×depth). The oxidation rates of sieved soil were approximately four times lower at *Früebüel* than at *Alp Weissenstein*.

The drought treatment had only little effect on CH_4 oxidation when soils were sieved and adjusted to identical water contents. At *Früebüel*, CH_4 oxidation in 0–5 cm soil depth slightly decreased under drought. At both sites, there was a tendency towards increased CH_4 oxidation in 10–15 cm soil depth.

N fertilization inhibited CH_4 oxidation of sieved soils, but effects were small and only present in the top soil layers. At *Früebüel*, fertilization reduced CH_4 oxidation in 0–5 cm soil depth with NH_4NO_3 causing a stronger reduction (−25%) than urine (−10%). At *Alp Weissenstein*, NH_4NO_3 reduced CH_4 oxidation in 0–5 cm soil depth, whereas urine caused a reduction in all soil depths.

The addition of salts containing NH_4^+ inhibited CH_4 oxidation in sieved soil (Fig. 4). The addition of NH_4^+-free salts also inhibited CH_4 oxidation, but only when added at much higher concentrations ($P<0.001$). NO_3^-, K^+ and Cl^- did not obviously differ in their effect.

Discussion

Our study indicates that soil water content is the dominant factor regulating the temporal dynamics of soil CH_4 uptake, at least in well-aerated loamy-textured soils. At the whole-ecosystem level, low soil moisture resulted in a larger soil CH_4 sink, even under extreme drought. The experimental treatments applied had contrasting effects: The simulated summer drought of a few weeks had effects that lasted over the entire year and substantially altered the ecosystem's CH_4 balance; in contrast, even high N fertilizer application rates exerted only a transient inhibition of the ecosystem's CH_4 sink, suggesting that N inputs by grazing animals have only a rather small influence on soil CH_4 oxidation, at least in the pastures investigated.

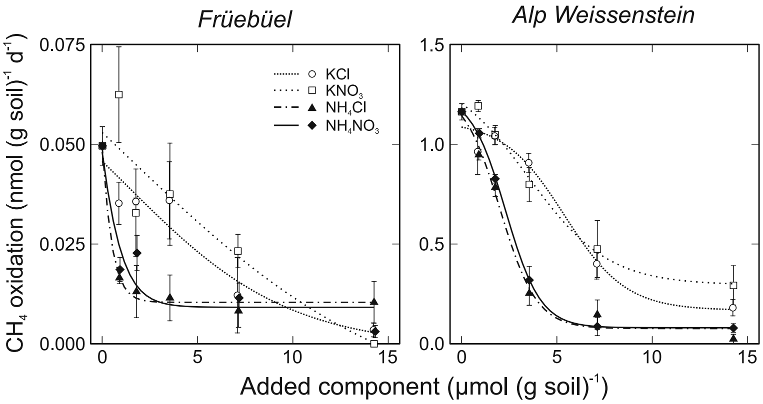


Fig. 4 CH_4 oxidation of sieved soil amended with different amounts of KCl, KNO_3, NH_4Cl or NH_4NO_3. The measured rates are expressed per soil dry weight. Lines are sigmoidal functions with intercept fitted for each compound (see Materials and Methods). *Error bars* indicate the standard errors of treatment means

Effects of soil moisture

The grasslands investigated continuously acted as net sinks for atmospheric CH_4. We never detected CH_4 emissions from soils, even when soils were waterlogged after heavy rain or snow melt. Together with the fact that soil CH_4 concentrations always decreased with depth, this suggests the absence of substantial methanogenesis in deeper soil layers; however, we cannot exclude the possibility of methanogenesis in anaerobic micro-sites.

Soil moisture was the single most important factor controlling soil CH_4 uptake, explaining 65–85% of the temporal within-site variation in CH_4 fluxes. The reductions in soil moisture also fully explained the increased ecosystem-level CH_4 sink under simulated drought. Strong negative correlations of soil moisture and soil CH_4 uptake have also been reported from other studies (e.g. Castro et al. 1994; Price et al. 2004; Smith et al. 2000). This effect can easily be understood given the much slower diffusion of CH_4 and O_2 in water than in air (Kruse et al. 1996) and the general substrate limitation of soil CH_4 oxidation (Degelmann et al. 2009). If soil internal methanogenesis occurred at all, it likely was more important in moister soils and thus contributed to the correlation we observed. Both investigated sites were of loamy soil texture. It may well be that soil moisture control of CH_4 uptake is most pronounced in this texture class. Pore water retention may be too low in sandy soils and too high in clayey soils to effectively modulate diffusive constraints over time. Across texture classes, however, soil texture is a good predictor of soil CH_4 uptake (Dörr et al. 1993).

Many laboratory studies have shown an optimum soil water content beyond which diffusion limitation reduced CH_4 oxidation and below which physiological stress limited the activity of methanotrophs (Gulledge and Schimel 1998; Del Grosso et al. 2000) and such effects were also reported from field studies (Borken et al. 2006; Davidson et al. 2008; Fiedler et al. 2008; Dobbie and Smith 1996). In our study, no indications for reductions in ecosystem-level CH_4 uptake under water deficiency were found, not even during sever summer droughts. One possible explanation could be that drought stress can physiologically limit CH_4 oxidation by methanotrophs in the top soil but that this does not necessarily reduce the soil CH_4 uptake rates because increased CH_4 oxidations in deeper, less dry soil layers can compensate for this effect.

Interestingly, the few weeks of simulated summer drought affected the soil's water balance throughout the entire year. Once the rain exclusion roofs had been removed, the recovery of soil moisture to ambient levels took several weeks to months and required multiple rain events. While the build-up of a soil moisture difference under the rain exclusion roofs can easily be explained by a reduction in precipitation, the mechanisms underlying the resilience after drought are less clear. Precipitation probably resulted in more runoff and deep seepage to ground water when the control plots were close to field capacity. However, there are also mechanisms which would tend to increase the soil moisture difference between the drought-treated and control plots and thus prolong the effect of drought. Drought induces a shrinking of soil aggregates, especially in soils with high clay content, and this often results in increased crack formation (Bronswijk 1988). This can lead to increased drainage after precipitation und a more efficient drying of soils due to a better coupling to the atmosphere (Ritchie and Adams 1974). In hydrophobic soils, water repellency often increases under drought (Doerr and Thomas 2000), which can substantially extend the time and amount of precipitation needed until a soil is re-saturated. Plants also affect evapotranspiration, but their role is less clear in the present study.

An intriguing effect found in our study was that effects of simulated summer drought on top soil moisture vanished in the winter following the treatment, but were found again in the next Spring and caused increased soil CH_4 uptake. This phenomenon was unexpected, since soils were covered by substantial amounts of snow during winter and the top soils of both control and drought-treated plots were water-saturated during snow-melt. We first suspected that the soils that had experienced simulated drought had developed hydrophobic properties; however, corresponding tests did not support this hypothesis (water droplet penetration time was less than 1 s in all samples; Dekker and Ritsema 1994). While vegetation can affect the soil's water balance, such an effect is rather unlikely since plant biomass did not differ at this time of the year (Hartmann et al., unpublished). We believe that the most likely explanation is that the top soil was re-saturated over winter but that deeper soil layers still exhibited water deficits relative to control plots. Drainage and capillary forces may then have led to an accelerated drying of the top soil of formerly drought-exposed plots. This appears possible given the large amount of precipitation excluded, exceeding winter precipitation at both sites. However, no moisture data is available for deeper soil layers, so that no complete soil water balance can be set up and this mechanism therefore remains speculation. Irrespective of the mechanisms involved, this experiment demonstrated that relatively short episodes of severe drought can affect the soil CH_4 balance for periods of up to 1 year.

We did not continuously measure CH_4 fluxes at our field sites. We calculated a rough estimate of the cumulative effect of simulated drought on soil CH_4 uptake by modeling CH_4 fluxes in dependence of soil moisture. At *Früebüel*, simulated drought caused a 50% increase in the amount of CH_4 oxidized in the course of the vegetation period (~4.5 and ~6.8 mmol CH_4 m^{-2} from April to October of 2007 and 2008 for control and drought-treatment, respectively; this corresponds to average soil uptake rates of ~21 and 32 μmol CH_4 m^{-2} d^{-1}, respectively). We were not able to run the same calculation for *Alp Weissenstein* due to incomplete soil moisture records; however, the available data suggests that the relative increase was in the same range.

Effects of N fertilization

The application of NH_4NO_3 and urine reduced soil CH_4 uptake in our study, but inhibitory effects were rather small and recovery of the soil CH_4 sink occurred within weeks, despite repeated applications of large amounts of N. These results are in line with other experiments testing effects of urine or NH_4NO_3 application to pasture soils which reported strong, but transient inhibitory effects that vanished within days to weeks (cattle urine: Li and Kelliher 2007; Liebig et al. 2008; NH_4NO_3: Steinkamp et al. 2001). In contrast to these findings, several other field and laboratory studies have reported larger and long-lasting inhibitory effects of N application. However, these studies were conducted in ecosystems that did not regularly experience high mineral N applications, either from grazing animals or from synthetic fertilizers (e.g. Hütsch et al. 1993; Bronson and Mosier 1994).

How can this discrepancy be resolved? One possibility is that decades of grazing already have resulted in reduced soil CH_4 uptake by eliminating methanotrophs from soil micro-sites that are strongly affected by high NH_4^+ concentrations, or by selecting for species that can tolerate high NH_4^+ concentrations. In this case, the application of extra N would have had only little effect because the system was already constrained. Also, the effect of NH_4^+ application strongly interacts with soil acitity (Hütsch et al. 1993, 1994), in part possibly because nitrification is reduced in acidic soils and more NH_4^+ can therefore accumulate. Cattle urine generally is alkaline (Haynes and Williams 1992), thus locally increasing soil pH in acidic soils and thus possibly protecting methanotrophic micro-organisms from adverse effects of NH_4^+.

At the end of our study, 1 year after the last fertilizer application, N-effects on CH_4 fluxes were detectable in the laboratory incubations of sieved soils but not in the soil CH_4 uptake rates measured in situ. The effects we found in sieved soil decreased with depth, possibly indicating a shift of the active methanotrophic zone towards deeper soil layers. One possible explanation for this discrepancy may be that a fertilizer-induced reduction in top-soil CH_4 oxidation was compensated by increased CH_4 uptake in deeper layers.

Several studies have suggested that soil CH_4 oxidation was not just inhibited by NH_4^+ but also by a general "salt effect" of the fertilizer additions (Nesbit and Breitenbeck 1992; Gulledge and Schimel 1998; Price et al. 2004). Our laboratory investigations however do not support this view. While inhibitory effects of NH_4^+-free salts were found, these were much weaker than when NH_4^+ was present. King and Schnell (1998) suggested that the inhibitory effect of NH_4^+-free salts may in fact be due to the desorption of NH_4^+ from mineral surfaces by ion exchange processes, i.e. be NH_4^+-effects in disguise. However, Gulledge and Schimel (1998) have argued that the observed inhibitory effects of non-ammoniacal salts cannot be attributed to desorbed NH_4^+ since the amounts of exchangeable NH_4^+ were too low. In our study, the amount of NH_4^+ potentially available for desorption was between 1.5 and 2.0 μmol (g soil)$^{-1}$ (soil extractions with 0.5 M KCl; Hartmann et al., unpublished). While the concentrations of ions added in these laboratory trials were small, we cannot exclude the possibility that desorbed NH_4^+ may have contributed to the effects observed.

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