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The inhibitory effect of difluoromethane on CH_4 oxidation in reconstructed peat columns and side-effects on CO_2 and N_2O emissions at two water levels

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ABSTRACT

Methane emissions from soils are the net result of two processes: methane (CH_4) production and CH_4 oxidation. In order to understand how both processes respond to environmental changes, it is necessary to distinguish between CH_4 production and oxidation. In bacterial cultures and small soil samples, difluoromethane (CH_2F_2) was found to inhibit CH_4 oxidation reversibly, without affecting CH_4 production. Hence, CH_2F_2 allows the study of CH_4 production directly and of CH_4 oxidation indirectly. To our knowledge, however, the inhibitory effect of CH_2F_2 within soil columns has not yet been evaluated. We therefore tested which CH_2F_2 concentration is needed for complete inhibition of CH_4 oxidation in reconstructed 28 cm high peat soil columns under different water levels (WL). We found that soil columns require considerably higher headspace CH_2F_2 concentrations for complete inhibition of CH_4 oxidation than small soil samples. Inhibition remained complete until ca. 24 h after CH_2F_2 exposure. Then, the inhibitory effect diminished. The time needed for the inhibitory effect to disappear depended on WL; at a low WL of −15 cm, the inhibitory effect declined slowly and oxidation rates recovered by 90% only after 12 days. At WL = −5 cm, CH_4 oxidation recovered much faster (90% recovery after ca. 3 days). Last, CH_2F_2 addition significantly decreased the N_2O emissions, whereas CO_2 emissions remained unaltered.

1. Introduction

Methane (CH_4), the second most important greenhouse gas (IPCC, 2001), is produced in soils when organic matter is degraded anaerobically by methanogenic bacteria (Oremland, 1988; Conrad, 1989). In aerobic soils or aerobic surface layers on top of anaerobic soils, methanotrophic bacteria can oxidize CH_4 to CO_2 (Cicerone and Oremland, 1988). The net CH_4 flux between soil and atmosphere thus results from two opposite processes. These processes often respond differently to environmental changes. Water level drawdown, for example, decreases the production of CH_4, whereas it can enhance CH_4 oxidation if CH_4 remains readily available (Jungkunst and Fiedler, 2007). In order to understand how climate and other environmental factors control methane emissions, and to accurately incorporate these responses into models, it is essential to study the separate responses of both production and oxidation of CH_4.

Methane production and oxidation can be differentiated by using selective inhibitors. Several inhibitors have been used to inhibit CH_4 oxidation, e.g., acetylene and methyl fluoride, but most of them resulted in an irreversible inhibition or affected not only CH_4 oxidation, but also CH_4 production (Frenzel and Bosse, 1996; Janssen and Frenzel, 1997; Matheson et al., 1997). Matheson et al. (1997) and Miller et al. (1998), however, discovered that difluoromethane (CH_2F_2) can inhibit CH_4 oxidation without affecting CH_4 production. Moreover, they reported that the inhibition is completely reversible and that CH_2F_2 can be quickly removed from the system as it is very soluble in water. Difluoromethane was used in several experiments on small soil samples (e.g., Teh et al., 2005, 2006), but to our knowledge, a thorough evaluation of the inhibitory effect of CH_2F_2 in intact soil columns has not been reported before (although CH_2F_2 was applied in a few in situ studies; Krüger et al., 2001, 2002; Shrestha et al., 2008).

Miller et al. (1998) used bacterial cultures and 5 g dry soil in serum bottles for their experiments, and concluded that a headspace concentration of about 0.03% CH_2F_2 was sufficient to inhibit the CH_4 oxidation completely. However, we expected that higher concentrations would be needed when complete soil columns are investigated, because the inhibitor has to diffuse into the soil to

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reach the sites of CH_4 oxidation. Furthermore, the depth of WL is expected to affect the inhibitory effect, because dilution of the CH_2F_2 concentration in the aerobic soil is expected to increase with increasing WL depth. In an incubation experiment, we therefore exposed peat soil cores to two different water levels and treated these soil cores with different CH_2F_2 concentrations. Our objectives were (1) to verify which CH_2F_2 concentration is needed to inhibit the CH_4 oxidation completely at the two water levels and (2) to assess whether CO_2 and N_2O emissions were affected by CH_2F_2 concentrations needed to inhibit CH_4 oxidation.

2. Materials and methods

2.1. Soil collection

In September 2006, we collected peat soil from a fen in nature reserve 'Het Wik' (Genk, Belgium; 50° 57′ N, 5° 25′ E). The vegetation on this soil was dominated by *Erica cinerea*, *Pieris* sp. and *Sphagnum* sp. Other species occurring were *Eriophorum angustifolium*, *Drosera* sp. and *Typha latifolia*. After peat collection, all vegetation was removed from the soil and, in order to keep the variation among soil samples as small as possible, we homogenized the peat by hand and packed it into PVC columns (inner diameter 10 cm) to a height of 28 cm. In 12 columns with homogenized peat the water level (WL) was set at 5 cm below the soil surface (high water level, WL = −5 cm). In 12 other soil columns, we maintained a WL of 15 cm below the soil surface (low water level, WL = −15 cm). In order to control the water level in the soil columns, each column (of which the lower 5 cm were perforated) was placed in a water bath to obtain communicating water levels between soil column and water bath. Water levels in the soil columns were controlled via the water level in the water bath.

2.2. Gas flux measurements

The columns were closed by PVC lids (diameter 10 cm; height 10 cm) and placed in automated incubators, set at 17 °C. A fresh air supply unit continuously flushed the headspaces with a controlled fresh air flow of 10.0 ml min^{-1}. Net CO_2, CH_4 and N_2O fluxes were measured with a GC that contained a flame ionization detector for CH_4 and a ^{63}Ni electron capture detector for CO_2 and N_2O analysis (Shimadzu, Duisburg, Germany; for details see Loftfield et al., 1997 and Flessa and Beese, 2000). Every 3 h, the input air and the exhaust air of each soil column were analyzed for CO_2, CH_4 and N_2O. Multi-component calibration gases covering the concentrations of 300–3000 μl l^{-1} CO_2, 1–200 μl l^{-1} CH_4 and 0.35–2 μl l^{-1} N_2O were measured at the same regular intervals. Flux rates were calculated from the airflow rate through the microcosm headspace and the difference in the CO_2, CH_4, and N_2O concentrations between the input air and the exhaust air.

2.3. Experimental set-up

The inhibition experiment started after the greenhouse gas emissions reached a stable flux rate (after 3 months). In order to verify whether CH_2F_2 inhibited the CH_4 oxidation, we injected different amounts of CH_2F_2 (Spectra Gases, Germany) into the headspace of the soil columns (via tubes in the headspace). Subsequently, we closed the headspaces and stopped the airflow for 2 h (no measurements were made during these 2 h). Then, the fresh air supply through the column headspaces was resumed. Due to a technical problem, we have no results from the third inhibition treatment in the high water level columns. To remove the dissolved CH_2F_2 from the system, water baths were completely refreshed between the sequential additions of different CH_2F_2 concentrations. Prior to injecting the next CH_2F_2 concentration, care was taken that the fluxes had readjusted to their initial values. The CH_2F_2 concentration sequence that was applied is given in Table 1.

After these inhibition treatments, we tested whether the inhibition of the CH_4 oxidation by CH_2F_2 was complete. To this end, we added $^{13}CH_4$ (99at% ^{13}C, Spectra Gases, Germany) to the headspaces and checked whether this was oxidized to $^{13}CO_2$. In detail, we used the following methodology. We first sampled air from the headspaces four times within 1 h after closing the headspaces. Gas samples were collected in 12 ml vacuum vials and were analyzed for $\delta^{13}C$-CO_2 by GC-IRMS (Finnigan MAT, Delta plus; reference standard, PDB). After shortly opening the headspaces for equilibration with the atmosphere, we closed the headspaces again and injected 6.4 ml of pure CH_2F_2 to achieve a headspace CH_2F_2 concentration of 1% [vol/vol] (the lowest concentration that significantly affected the CH_4 emissions; see Section 3) in half of the columns (six per WL, randomly selected). We also stopped the airflow for 2 h, the time needed to ensure complete inhibition at 1% [vol/vol] CH_2F_2. Subsequently, for each WL, we injected 5.0 ml $^{13}CH_4$ in three inhibited samples and in three samples without CH_2F_2 addition, to obtain a headspace concentration of 0.6% [vol/vol] $^{13}CH_4$ and then sampled air for $^{13}CO_2$ analysis (three samples within 1 h after $^{13}CH_4$ injection). With the $\delta^{13}CO_2$ data, we determined the $\delta^{13}C$ of the CO_2 produced in each soil column as the *y* axis intercept of a Keeling plot. We are aware that the number of data points was small for constructing a Keeling plot, increasing the uncertainty of the $\delta^{13}CO_2$ to several per mil. However, our goal was not to study the $\delta^{13}CO_2$ of the decomposition or to determine natural isotope abundance, but to find an indication for CH_4 oxidation. If CH_4 was oxidized, an increase of $\delta^{13}CO_2$ in the order of hundreds per mil of $\delta^{13}CO_2$ would be observed after $^{13}CH_4$ injection. Hence, the increased uncertainty in the Keeling plots was irrelevant for this study. The substantially higher $\delta^{13}CO_2$ in the plots treated with $^{13}CH_4$ in the absence of CH_2F_2 (see Section 3.2) demonstrates that our goal was achieved.

2.4. Data analysis

In order to verify whether CH_2F_2 inhibited CH_4 oxidation, we measured CH_4 emissions before and after CH_2F_2 injection. For quantification of the inhibitory effect on the CH_4 emissions, we opted to exclude the CH_4 release via ebullition. Methane emitted via ebullition is only partially affected by CH_4 oxidation, because most of the CH_4 transported via bubbles quickly bypasses the sites of CH_4 oxidation. To exclude CH_4 emissions via ebullition, we considered only the basal CH_4 flux. To this end, we computed the median flux (unlike the average, the median is less affected by CH_4 emissions via ebullition; see Fig. 1 for more details on this issue) over the last 5 days before the inhibition and the median flux of 24 h following the injection of CH_2F_2 (after 24 h, the inhibitory effect on the CH_4 emissions started to decline). The inhibitory effect

Table 1
Experimental sequence of inhibition treatments with various headspace CH_2F_2 concentrations (% [vol/vol])

DOY	WL = −5 cm			WL = −15 cm		
	T1	T2	T3	T1	T2	T3
150	0	0.2	1	0	0.2	1
194	0	1	3	0	1	3
239	0	–	–	0	3	5
289	0	1	2	0	5	–

Each treatment (T) consisted of four replicates. Due to a technical problem, we have no data for the inhibitions performed on day of year (DOY) 239 for the high water level (WL).

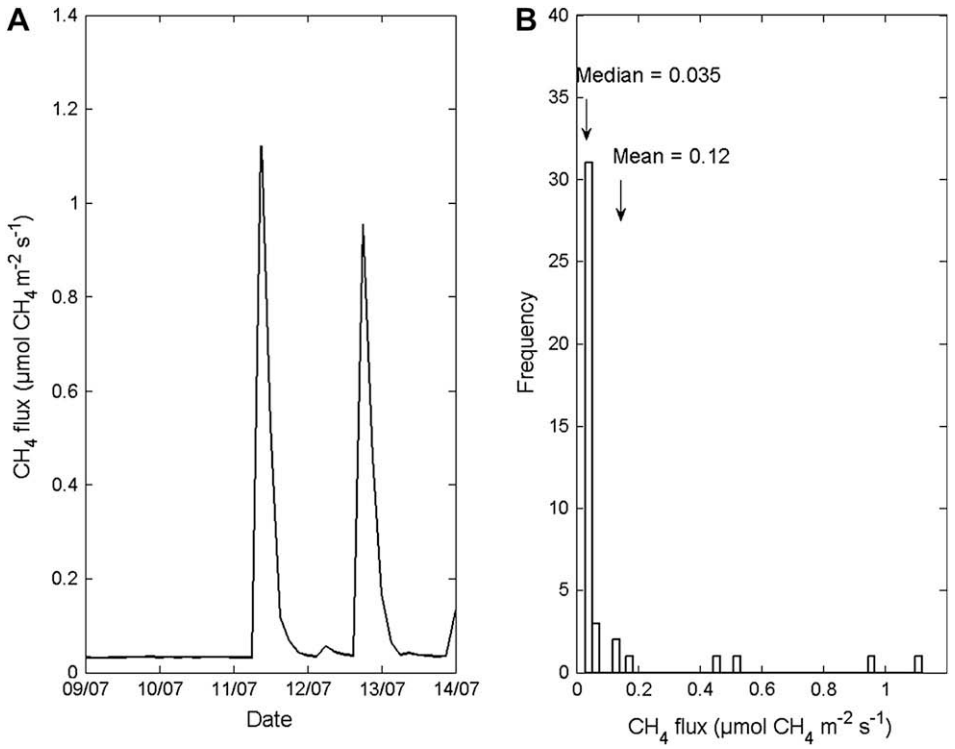

Fig. 1. An example of the CH_4 fluxes of one replicate over 5 days before an inhibition (A) and the histogram showing the distribution of these CH_4 fluxes (B). The median and mean CH_4 flux computed from these data are also shown. In this figure, it can be seen that CH_4 release via ebullition can substantially affect the mean CH_4 flux, whereas the effect on the median CH_4 flux is small or inexistent (adding or removing a few ebullition events will not affect the median flux, but will enhance or reduce the mean CH_4 flux substantially).

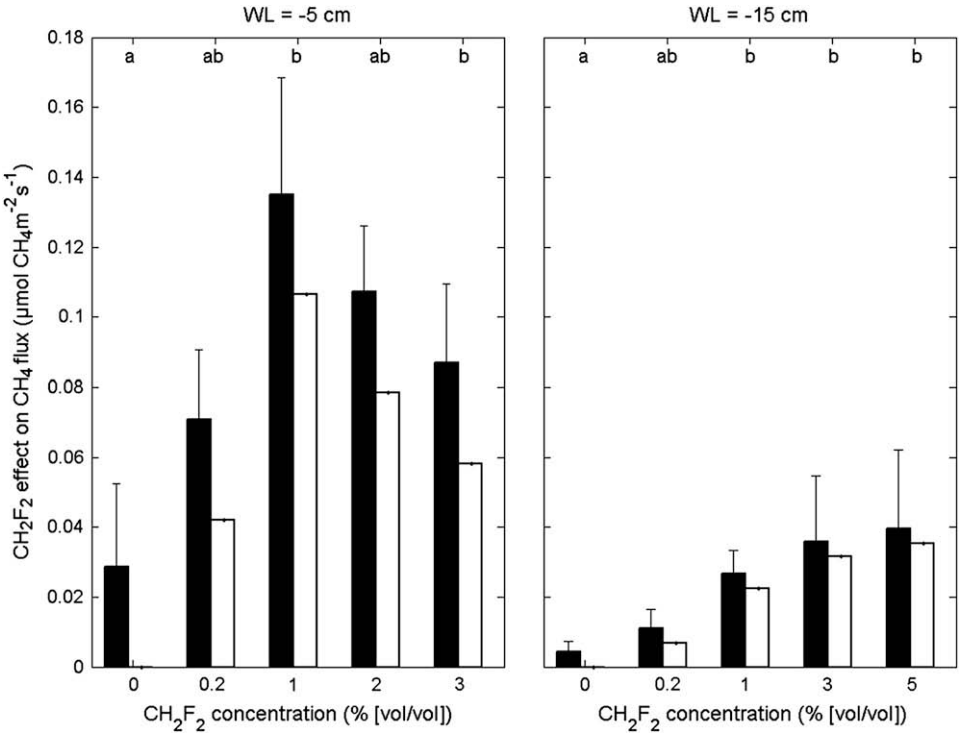

Fig. 2. Inhibitory effect on the net CH_4 fluxes at different CH_2F_2 concentrations and for the two water levels (WLs). Black bars were calculated as the average difference between the median CH_4 flux before the inhibition (over 5 days) and after the inhibition (over 24 h). White bars represent the CH_2F_2 effect corrected for changes in the control columns (i.e., we subtracted the effect at 0% [vol/vol] from the effect at each inhibition treatment). Error bars represent the standard error and for each WL, letters indicate the differences significant at $P < 0.10$. Note that the lack of a significant inhibitory effect at 2% [vol/vol] CH_2F_2 for WL = −5 cm is very likely due to loss of statistical power, as we could use only three of the four replicates at this CH_2F_2 concentration.

was calculated as the difference between the median flux rate before and after inhibition. The average inhibitory effect presented in Figs. 2, 3 and 4 was computed over all dates on which an inhibition treatment was applied (e.g., the inhibitory effect at 1% [vol/vol] CH_2F_2 represents the average over the T3 replicates inhibited on day 150 and the T2 replicates inhibited on days 194 and 289; see Table 1).

In order to ensure that the use of the median flux rate did not confound our results, we determined the median absolute deviation (MAD) before and after each inhibition. As expected, MAD was somewhat higher for CH_4 emissions at high WL than at low WL (one-way ANOVA for CH_4 emissions over the 5 days before the first inhibition: $P = 0.08$; Table 1), which reflects the higher abundance of ebullition events at WL = −5 cm. More importantly, however, we found similar MADs before and after the inhibitions (paired *t*-test: $P = 0.19$). Hence, CH_2F_2 addition did not enhance bubble ebullition (which would increase MAD) and therefore, we are confident that the median flux was an appropriate measure to determine the inhibitory effect on CH_4 oxidation.

For determining the recovery of the CH_4 oxidation after inhibition, we calculated for each soil column a 'running median' flux over clusters of 10 consecutive data points (i.e., a first median flux calculated from data point one to data point 10, a second median flux calculated from data point two to data point 11, etc.). In this way, we were able to reduce the influence of CH_4 release via ebullition. Subsequently, we determined the time span between CH_2F_2 addition and the time at which the CH_4 fluxes showed 75 and 90% recovery. Note that CH_4 fluxes were similar at the beginning and end of the experiment (May 2007 and November 2007). Emissions of CO_2 were stable throughout the experiment as well, whereas N_2O emissions, in particular at the low WL, increased over the course of the experiment (data not shown).

The second objective was to verify whether CH_2F_2 affected CO_2 and N_2O emissions. Similar to the CH_4 emissions, we calculated the average difference between the median CO_2 and N_2O flux before and after the inhibition. For the CO_2 emissions, we used the same time span as for the CH_4 fluxes, i.e., 5 days before the inhibition and 1 day after the inhibition. For the N_2O emissions, we opted for a longer time span after the inhibition, because for high CH_2F_2 concentrations, we observed an inhibitory effect that lasted 1 week after addition of the inhibitor. Therefore, we computed the average N_2O flux after CH_2F_2 injection over the 5 days following the inhibition.

We performed all analyses in Matlab (7.2.0.232, The Mathworks, US). Effects of WL and CH_2F_2 addition on CH_4, CO_2 and N_2O emissions were tested using ANOVA. Differences are reported significant at $P < 0.10$.

3. Results

3.1. Emissions and inhibitory effects

As expected, CH_4 emissions were higher at WL = −5 cm than at WL = −15 cm (Table 2). Note that Table 2 presents the median fluxes. The CH_4 emissions in this table thus approximate the CH_4 emission via diffusion. Average CH_4 emissions (including CH_4 release via ebullition) were 0.08 (±0.01) and 0.03 (±0.01) μmol CH_4 $m^{-}2$ $s^{-}1$, for WL = −5 and WL = −15 cm, respectively. These emissions were well within the range observed for undrained fens in temperate regions (Jungkunst and Fiedler, 2007). Average CO_2 and N_2O emissions were nearly identical to the median fluxes (data not shown) and both gases exhibited the highest emissions at WL = −15 cm (Table 2).

Addition of 0.2% [vol/vol] CH_2F_2 enhanced the CH_4 emissions, suggesting that CH_4 oxidation was being inhibited, but the increase

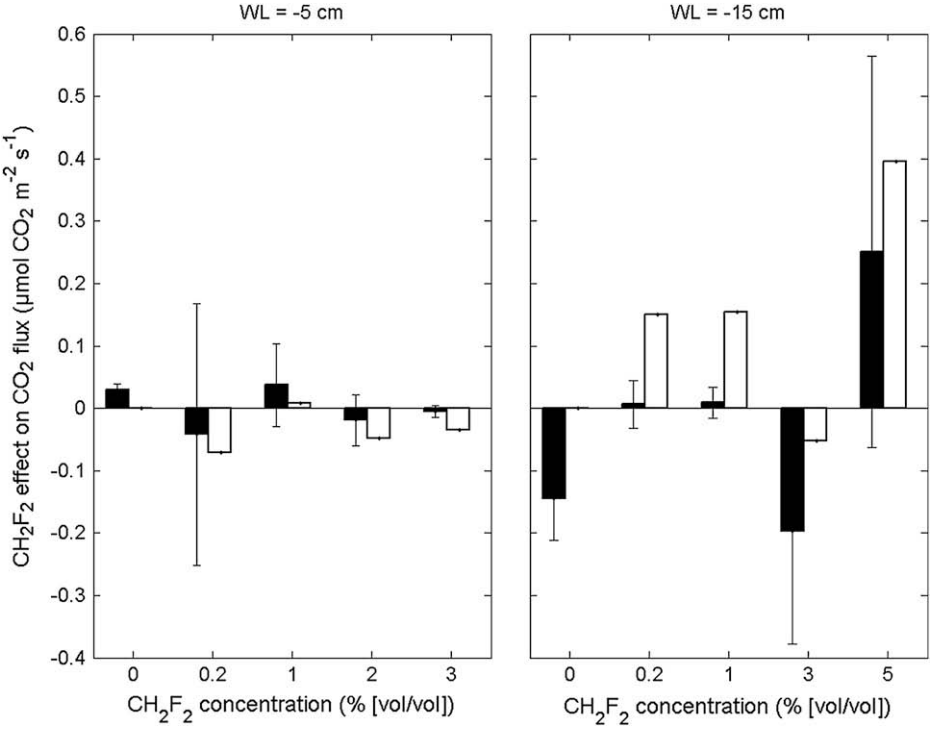

Fig. 3. Difluoromethane effect on the net CO_2 fluxes at different CH_2F_2 concentrations and for the two water levels (WLs). Black bars were calculated as the average difference between the median CH_4 flux before the inhibition (over 5 days) and after the inhibition (over 24 h). White bars represent the CH_2F_2 effect corrected for changes in the control columns (i.e., we subtracted the effect at 0% [vol/vol] from the effect at each inhibition treatment). Error bars represent the standard error. None of the differences are statistically significant at $P < 0.10$.

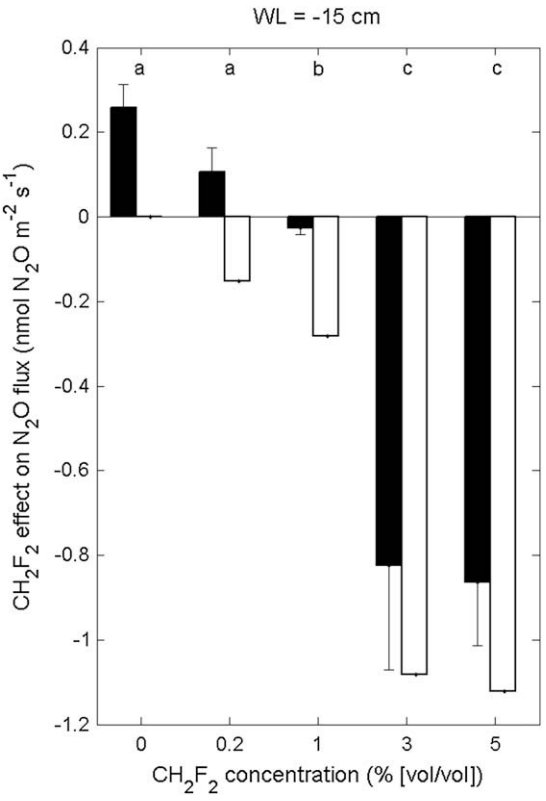

Fig. 4. Difluoromethane effect on the net N_2O fluxes at different CH_2F_2 concentrations for WL = −15 cm. Black bars were calculated as the average difference between the median CH_4 flux before the inhibition (over 5 days) and after the inhibition (over 24 h). White bars represent the CH_2F_2 effect corrected for changes in the control columns (i.e., we subtracted the effect at 0% [vol/vol] from the effect at each inhibition treatment). Error bars represent the standard error and for each WL, letters indicate the differences significant at $P < 0.10$.

was not statistically significant at $P < 0.10$, for either water level (Fig. 2). A CH_2F_2 concentration of 1% [vol/vol] or higher always resulted in a significant inhibitory effect (i.e., an increase of the CH_4 emission), except for 2% [vol/vol] CH_2F_2 at WL = −5 cm. For this concentration, however, we could use only three replicates due to a technical problem with the fourth replicate, resulting in a loss of statistical power. Even though the inhibitory effect was not constant across CH_2F_2 concentrations of 1% [vol/vol] and higher, the differences between these concentrations were never significant (Fig. 2).

Note that the inhibitory effect (and thus the CH_4 oxidation) at 1% [vol/vol] represented the equivalent of 75 and 64% of the basal CH_4 flux rate, for WL = −5 cm and WL = −15 cm, respectively. Hence, in this study, CH_4 oxidation consumed the majority of the CH_4 released via diffusion. When also taking into account the CH_4 release via ebullition, CH_4 oxidation removed only 58 and 43% of the total CH_4 emitted, for WL = −5 cm and WL = −15 cm, respectively.

3.2. Completeness of inhibition and recovery of CH_4 emissions

In order to verify whether CH_4 oxidation was inhibited completely, we sampled air for $\delta^{13}CO_2$ analysis before and after injection of CH_2F_2 and/or $^{13}CH_4$. Prior to the additions, the $\delta^{13}CO_2$ values of the produced CO_2 were similar for all treatments, ranging from −25.63 to −27.12‰. Addition of $^{13}CH_4$ in the absence of CH_2F_2 resulted in a two-orders-of-magnitude increase of the $\delta^{13}CO_2$ (Table 3). The $\delta^{13}CO_2$ produced in the soil columns treated with CH_2F_2 and $^{13}CH_4$ differed neither from the controls, nor from the soil columns treated only with CH_2F_2. On the other hand, we did observe some differences between the $\delta^{13}CO_2$ before and after addition of CH_2F_2 and $^{13}CH_4$ (for all treatments). These differences were expected given the small number of samples used to construct the Keeling plots ($n = 3$) and the associated low precision. Because these differences were inconsistent and always two orders of magnitude smaller compared to those in the soil columns treated only with $^{13}CH_4$, we considered these differences irrelevant.

Inhibitions remained complete from at least 2 h after headspace injection until ca. 24 h after the injection and then started to decline. We determined the time span (at a concentration of 1% [vol/vol] CH_2F_2) required for 75 and 90% recovery of the CH_4 emissions. At WL = −5 cm, roughly 2 days were needed for 75% recovery and about 3 days for 90% recovery (Table 4). At WL = −15 cm, the CH_4 oxidation showed 75% recovery only after 8 days and 90% recovery after 12 days (Table 4), which was significantly longer than for the high WL ($P < 0.001$ for both 75 and 90% recovery).

3.3. Inhibitory effects on CO_2 and N_2O emissions

We further studied whether CH_2F_2 affected CO_2 and N_2O emissions. The CO_2 emissions exhibited no significant response to CH_2F_2 addition (Fig. 3). Moreover, we observed no trend in the inhibitory effect on the CO_2 emissions over the different CH_2F_2 concentrations. The N_2O emissions, in contrast, did respond to CH_2F_2 addition (Fig. 4). For WL = −5 cm, net N_2O emissions were too low (near detection limit) for a reliable evaluation of a possible inhibitory effect. Hence, we determined the response of the N_2O emissions to CH_2F_2 addition only for WL = −15 cm. For this WL, CH_2F_2 concentrations equal to or higher than 1% [vol/vol] significantly decreased the net N_2O flux. Difluoromethane concentrations of 1% [vol/vol] reduced the N_2O emissions by about 6% and concentrations of 3 and 5% [vol/vol] CH_2F_2 roughly halved N_2O emissions (Fig. 4). We remark that the N_2O emissions increased over the course of the experiment (see also the positive CH_2F_2 effect for the control columns in Fig. 4), which implies that the values in Fig. 4 and Table 2 should not be compared.

Last, the recovery of the N_2O production at 1% [vol/vol] CH_2F_2 was remarkably faster than recovery of CH_4 oxidation, with complete recovery of the N_2O emissions after, on average, 2 days (SD = 0.9). Because of the fast recovery and the increase of the N_2O emissions over time, we did not determine the time needed for 75 and 90% recovery.

Table 2
Median net CO_2, CH_4 and N_2O fluxes (μmol m^{-2} s^{-1}) for the two water levels (WLs), calculated from the fluxes over the last 5 days before the first inhibition (day of year 146–150) and the standard deviation (SD) over the 12 replicates for each WL

	WL = −5 cm			WL = −15 cm			*P* value
	Median	SD	MAD	Median	SD	MAD	
CH_4	0.036	0.028	0.02	0.013	0.013	0.005	0.013
CO_2	1.41	0.28	0.043	3.27	0.47	0.040	<0.001
N_2O	1.2×10^{-5}	8.6×10^{-5}	1.2×10^{-5}	3.3×10^{-4}	3.5×10^{-4}	2.3×10^{-5}	0.006

Median absolute deviation (MAD) is a measure of the dispersion of the median fluxes over the 12 replicates. The *P* value represents the significance level of the difference between the two water levels.

Table 3
Isotopic signature ($\delta^{13}CO_2$ in ‰) of the CO_2 produced in the soil columns before and after addition of CH_2F_2 and/or $^{13}CH_4$, calculated via a Keeling plot and averaged over the three replicates used for each treatment of the two water levels (WLs). We also present the 95% confidence interval (95% CI) of this average.

	WL = −5 cm				WL = −15 cm			
	Before injection	95% CI	After injection	95% CI	Before injection	95% CI	After injection	95% CI
Control, no $^{13}CH_4$	−26.39	1.01	−27.61	3.02	−26.89	0.99	−19.56	4.09
Control, $^{13}CH_4$	−26.38	1.10	825.17	51.47	−27.12	0.65	688.77	26.88
Inhibited, no $^{13}CH_4$	−26.11	0.98	−19.94	1.81	−25.63	0.83	−29.15	1.53
Inhibited, $^{13}CH_4$	−26.62	0.80	−21.00	3.78	−25.84	0.87	−24.07	3.21

4. Discussion

4.1. Emissions and inhibitory effects

In accordance with many other studies (see review by Jungkunst and Fiedler, 2007), we observed higher CH_4 emissions at high WL than at low WL, whereas CO_2 emissions were higher at WL = −15 cm as compared to WL = −5 cm. Also for N_2O, we observed higher net flux rates at WL = −15 cm than at WL = −5 cm. This is in agreement with the conceptual model of Davidson et al. (2000), predicting highest N_2O production at intermediately high water-filled-pore space, where nitrification and denitrification can proceed simultaneously at aerobic and anaerobic microsites interspersed close to each other in the soil matrix.

Matheson et al. (1997) reported that CH_2F_2 inhibits methane monoxygenase (MMO), the enzyme produced by methanotrophic bacteria to oxidize CH_4. Miller et al. (1998) found that headspace concentrations as low as 0.03% CH_2F_2 were sufficient to completely inhibit CH_4 oxidation in 5 g of dry soil. In contrast, we observed that in our reconstructed soil columns a headspace CH_2F_2 concentration of 0.2% [vol/vol] was insufficient for complete inhibition of CH_4 oxidation. Concentrations equal to or above 1% [vol/vol] CH_2F_2, on the other hand, did enhance the CH_4 emissions significantly and thus inhibited the CH_4 oxidation. Moreover, headspace injection of $^{13}CH_4$ followed by $\delta^{13}CO_2$ analyses verified that this CH_2F_2 concentration of 1% [vol/vol] inhibited the CH_4 oxidation completely. In contrast to Miller et al. (1998), we approached natural conditions more closely, using 28 cm high soil columns in which the inhibitor had to diffuse into the soil, to the sites where CH_4 oxidation occurs. Also Krüger et al. (2001, 2002) and Shrestha et al. (2008) applied 1% [vol/vol] CH_2F_2 to inhibit CH_4 oxidation in situ, but no evaluation of the inhibitory effect was reported in these studies.

Besides effects of CH_2F_2 on CH_4 oxidation, Miller et al. (1998) found that, in some cases, CH_2F_2 also (partially) inhibited CH_4 production via acetate fermentation, albeit at concentrations considerably higher than those needed to inhibit CH_4 oxidation. In our experiment, we could not prove that CH_4 production was unaffected by CH_2F_2. Nonetheless, we believe that inhibitory effects on methanogenesis were very small or absent, because we observed no significant decrease of the CH_4 emission with increasing CH_2F_2 concentrations. At WL = −15 cm, net CH_4 fluxes even showed a slight (but not significant) increase with increasing CH_2F_2 concentration.

Table 4
Mean time (days) after addition of 1% [vol/vol] CH_2F_2 needed to reach 75% and 90% recovery from the inhibition for the two water levels (WLs), and the standard deviation (SD) on this mean

	75% reduction of inhibitory effect		90% reduction of inhibitory effect	
	Mean	SD	Mean	SD
WL = −5 cm	1.9	2.1	2.9	2.0
WL = −15 cm	7.8	3.7	11.6	5.5

4.2. Recovery of the CH_4 emissions

In accordance with Miller et al. (1998), who reported that the inhibition of CH_4 oxidation by CH_2F_2 was reversible, we observed a decrease of the inhibitory effect (starting about 24 h after CH_2F_2 addition). We found that the inhibitory effect diminished significantly more slowly at low WL than at high WL. Possibly, CH_2F_2 was more easily removed from the few air-filled pores at WL = −5 cm than from the numerous air-filled pores (at greater depth) at WL = −15 cm. Moreover, CH_2F_2 is very soluble in water (Horvath, 1982) and was thus more quickly diluted at WL = −5 cm than at WL = −15 cm, potentially clarifying the differences in recovery time. Further, the time needed for 75% recovery of the CH_4 emissions at WL = −5 cm agrees with the observations of Eller and Frenzel (2001), who reported 70% recovery of the CH_4 emissions 1 day after CH_2F_2 addition of a headspace concentration of 1% [vol/vol] to flooded rice soil samples.

4.3. Inhibitory effects on CO_2 and N_2O emissions

In addition to CH_2F_2 effects on CH_4 emissions, we also determined the response of the CO_2 and N_2O emissions to CH_2F_2. Concentrations up to 5% [vol/vol] CH_2F_2 did not affect CO_2 emissions. Apparently, the contribution of CH_4 oxidation to the CO_2 emissions (less than 10%, on average; see Fig. 2 and Table 2) was less than the inherent variation of the CO_2 emissions.

Net N_2O emissions (at WL = −15 cm), on the other hand, decreased at CH_2F_2 concentrations equal to or above 1% [vol/vol], to reach a minimum after approximately 24 h. Subsequently, N_2O emissions increased again and reached their initial flux rate several days after the CH_2F_2 injection (data not shown). At CH_2F_2 concentrations higher than 1% [vol/vol], the inhibitory effect on the N_2O emissions became larger (Fig. 4) and also the recovery time increased (data not shown). In accordance with our results, Miller et al. (1998) reported inhibition of NH_4^+ oxidation and of NO_3^- and NO_2^- production after CH_2F_2 addition. This was not surprising, as ammonium and methane monoxygenases are similar in function (Bédard and Knowles, 1989). Hence, CH_2F_2 can reduce N_2O production by affecting both N_2O producing processes, nitrification and denitrification. Nitrification is inhibited by the effects on ammonium monoxygenase, while denitrification is reduced because nitrate production via nitrification decreases.

4.4. Conclusions

Our results indicated that CH_2F_2 is capable of completely inhibiting CH_4 oxidation in a reversible manner also in soil columns, but that considerably higher CH_2F_2 concentrations are needed to inhibit CH_4 oxidation in soil columns than previously reported for small soil samples. Furthermore, we demonstrated that CH_2F_2 addition can substantially decrease N_2O emissions. The

CO_2 emissions remained unaffected by CH_2F_2, because, for both water levels, the contribution of CH_4 oxidation to the CO_2 flux was smaller than the natural variation in the latter flux.

With regard to future experiments, we conclude that CH_2F_2 can be a useful tool for studying controls on CH_4 production and/or oxidation. However, as highlighted in this experiment, CH_4 release via ebullition can considerably obscure the inhibitory effect and hence also the determination of CH_4 production and oxidation rates (especially at high water levels). This problem can be encountered by making continuous measurements and using median instead of mean flux rates.

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