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Recovery of in-situ methanotrophic activity following acetylene inhibition

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Abstract Methane (CH_4) is the second most important greenhouse gas after carbon dioxide (CO_2). To understand CH_4 cycling, quantitative information about microbial CH_4 oxidation in soils is essential. Field methods such as the gas push-pull test (GPPT) to quantify CH_4 oxidation are often used in combination with specific inhibitors, such as acetylene (C_2H_2). Acetylene irreversibly binds to the enzyme methane monooxygenase, but little is known about recovery of CH_4 oxidation activity after C_2H_2 inhibition in situ, which is important when performing several experiments at the same location. To assess recovery of CH_4 oxidation activity following C_2H_2 inhibition, we performed a series of GPPTs over 8 weeks at two different locations in the vadose zone above a petroleum hydrocarbon-contaminated aquifer in Studen, Switzerland. After 4 weeks a maximum recovery of 30% and 50% of the respective initial activity was reached, with a subsequent slight drop in activity at both locations. Likely, CH_4 oxidation activity and CH_4 concentrations were too low to allow for rapid recovery following C_2H_2 inhibition at the studied locations. Therefore, alternative competitive inhibitors have to be evaluated for application in conjunction with GPPTs, especially for sites with low activity.

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Abbreviations
GFC Gas flow controller
GPPT Gas push-pull test

Introduction

Microbial methane (CH_4) oxidation is a key process in the global CH_4 cycle, lowering emissions of this greenhouse gas by over 50% and acting as a sink for atmospheric CH_4 (Reeburgh 2003). Aerobic CH_4 oxidation is mediated by methanotrophic bacteria that contain the enzyme methane monooxygenase, allowing them to use CH_4 as their main source of carbon and energy (Hanson and Hanson 1996). To understand CH_4 cycling and predict responses to changing climate conditions it is important to quantify CH_4 oxidation. While detailed laboratory studies allow to control important parameters and to assess their influence on metabolic activity, in-situ quantification of processes provides activity estimates that are likely more representative for the studied environment (Madsen 1998; Scow and Hicks 2005).

The GPPT is a tracer test to quantify CH_4 oxidation in situ, which is based on the injection of a gas mixture containing the reactants CH_4 and O_2 and a non-

reactive tracer, e.g., neon (Ne) into the vadose zone. While the injected mixture migrates away from the injection point, reactants are consumed by indigenous microorganisms. The gas mixture is subsequently pumped back, i.e., extracted together with soil air from the same location (Urmann et al. 2005). First-order rate constants of CH_4 oxidation can be calculated from CH_4 and tracer concentration data provided that their transport behavior is similar (Schroth and Istok 2006). To verify the latter, a GPPT with co-injection of an inhibitor for CH_4 oxidation is usually performed. Alternatively, CH_4 during an active test can be directly compared with CH_4 during a test with an inhibitor as a substitute tracer (Urmann et al. 2007a). For an inhibitor to be effective during a GPPT, a concentration sufficient for inhibition has to be reached relatively fast within the test zone and maintained during the entire test.

Specific inhibitors are a traditional tool for the assessment of microbial processes that allows to verify microbial activity and distinguish between different processes (Oremland and Capone 1988). To quantify CH_4 oxidation in situ, inhibitors have been employed in conjunction with CH_4 emission measurements using chambers (e.g., Ding et al. 2004; King 1996; Kruger et al. 2001), and more recently in conjunction with tracer tests like the GPPT (Urmann et al. 2005; Urmann et al. 2007a).

Gaseous inhibitors for CH_4 oxidation currently available include the traditional inhibitor acetylene (C_2H_2) (Prior and Dalton 1985), fluoromethane (CH_3F) (Oremland and Culbertson 1992) and difluoromethane (CH_2F_2) (Miller et al. 1998). Acetylene effectively inhibited CH_4 oxidation at concentrations as low as 10 $\mu l\, l^{-1}$ in laboratory studies (Bodelier and Frenzel 1999; Chan and Parkin 2000) and was shown to be effective during GPPTs (Urmann et al. 2005; Urmann et al. 2007a). In contrast, higher concentrations of 100–1,000 $\mu l\, l^{-1}$ for CH_3F (Chan and Parkin 2000; King 1996) and 300–500 $\mu l\ l^{-1}$ for CH_2F_2 (Miller et al. 1998) were required for effective inhibition, and the required concentration may depend on CH_4 concentrations due to the competitive nature of inhibition (Matheson et al. 1997). Furthermore, both inhibitors (CH_3F and CH_2F_2) can be consumed by methanotrophic bacteria at low concentrations (Miller et al. 1998; Oremland and Culbertson 1992). Therefore, it may be difficult to achieve effective inhibition during a GPPT using CH_3F or CH_2F_2. Additionally, these inhibitors are greenhouse gases (Ramaswamy et al. 2001) and expensive, while C_2H_2 is cheap, readily available, and does not act as a greenhouse gas.

However, there are two major disadvantages of C_2H_2: First, at higher concentrations, it also inhibits methanogenesis (Chan and Parkin 2000). Therefore, the range of C_2H_2 concentrations that can be applied is limited when CH_4 oxidation and methanogenesis co-occur. Fluoromethane and CH_2F_2 also inhibit methanogenesis, but mainly acetoclastic methanogenesis and, in the case of CH_2F_2, only at higher concentrations (Frenzel and Bosse 1996; Miller et al. 1998). Secondly, in contrast to the competitive inhibitors CH_3F and CH_2F_2, C_2H_2 irreversibly binds to methane monooxygenase (Prior and Dalton 1985). Consequently, de-novo enzyme synthesis is required for activity to recover, as was shown for ammonia monooxygenase, a similar enzyme that is also inhibited by C_2H_2 (Hyman and Arp 1992). Therefore, recovery may not be immediate and knowledge about the rate of recovery is important to be able to perform several experiments at the same location in situ in conjunction with C_2H_2 inhibition. In laboratory experiments, recovery of CH_4 oxidation after C_2H_2 inhibition ranged from no recovery within 14 days to recovery within one day, indicating that recovery may depend on the physiological state of the cells (Bodelier and Frenzel 1999; Miller et al. 1998). However, to our knowledge, recovery has not been assessed in detail in situ at the field scale.

Therefore, the aim of this study was to perform a series of GPPTs to evaluate recovery of in-situ methanotrophic activity following C_2H_2 inhibition. Experiments were performed in the vadose zone above a methanogenic, petroleum hydrocarbon-contaminated aquifer with relatively low CH_4 oxidation activity (Urmann et al. 2005) similar to that of oxic soils, i.e., under relatively unfavorable conditions for recovery from inhibition.

Materials and methods

Field site

Recovery of microbial CH_4 oxidation after C_2H_2 inhibition was assessed in the vadose zone above a petroleum hydrocarbon-contaminated, anaerobic aquifer in Studen,

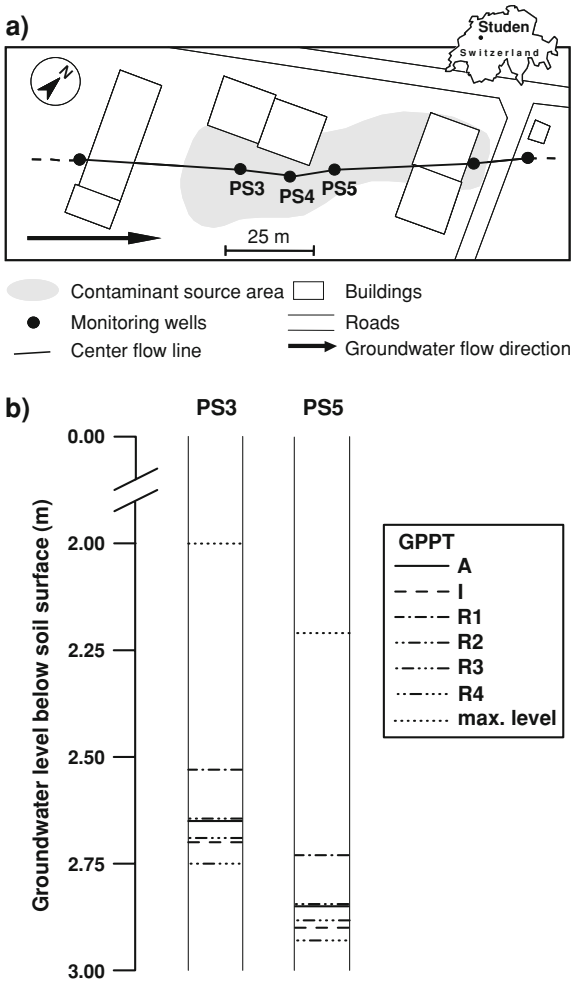

Fig. 1 Site map of the petroleum hydrocarbon-contaminated aquifer in Studen, Switzerland, showing (**a**) the contaminant source area and selected monitoring wells along a center flow line (adopted from Bolliger et al. 2000), and (**b**) groundwater levels in wells PS3 and PS5 during the time of the experiments. The maximum groundwater levels were observed between GPPT I and GPPT R1

Switzerland. Methane has been previously detected in groundwater of monitoring wells PS3, 4 and 5, all located within the contaminant source area at this site (Bolliger et al. 1999; Bolliger et al. 2000) (Fig. 1a). In earlier studies, CH_4 oxidation was assessed near monitoring well PS4 using GPPTs (Urmann et al. 2005, 2008). Higher activity was observed just above the groundwater table (at 2.7 m depth) compared to closer to the soil surface (at 1.1 m depth).

In this study, GPPTs were performed at 1 m depth below soil surface to avoid interference of changes in the groundwater level with the test zone. Monitoring wells PS3 and PS5 were chosen as test locations as highest CH_4 oxidation activity was previously observed near these wells (unpublished data), and activity had dropped near PS4 (Fig. 1a). Activity had to be high enough to be able to distinguish different levels of recovery with the currently available GPPT procedure, but was intended to be low to assess recovery under comparatively unfavorable conditions. Experiments were conducted in the annular space between each well and its surrounding 70-cm-diameter concrete casing, which was refilled with calcareous coarse sand and gravel in 1996. As a result of severe rainfall events, the groundwater level in well PS3 varied between 2.00 and 2.75 m and in PS5 between 2.21 and 2.90 m below soil surface during the time of the experiments (Fig. 1b). At 1 m depth, temperature remained stable around 17°C for the first 2 weeks of the experiments and then dropped to 12°C during the remaining 6 weeks.

Gas push-pull tests

A series of six GPPTs (GPPTs A, I, R1, R2, R3, R4—see below) was performed at 1 m below soil surface near each of the two wells (Table 1). The depth refers to the depth of the tip of the injection rod permanently installed at each location. The injection gas mixtures contained on average 0.43 ml l^{-1} and 226 ml l^{-1} of the reactants CH_4 and O_2, and 240 ml l^{-1} of each of the non-reactive gases Ne, He and Ar (Table 1). Helium and Ar were added as additional tracers to serve as a control for Ne transport behavior. As no further information was derived from He and Ar breakthrough curves, data for these gases are not shown. Prior to the first GPPT (GPPT A), CH_4 concentration profiles in soil air were measured following Urmann et al. (2005). Briefly, in the vicinity of each test location, a separate sampling rod was inserted to a maximum depth of 1.1 m below soil surface and 1-l gas samples were extracted in 10-cm vertical intervals using the GPPT equipment. An initial test, GPPT A, was subsequently performed to assess CH_4 oxidation activity at each location. Within one week of GPPT A, a second test (GPPT I) was performed, additionally containing C_2H_2 as an inhibitor (Table 1). Subsequently, four tests (R1–R4) were carried out to assess recovery of CH_4 oxidation activity during 8 weeks. GPPTs were performed as described earlier (Urmann et al. 2005) with slight

Table 1 Operational parameters for gas-push pull tests (GPPTs)

Well	GPPT	Time[a] (d)	Injection concentrations[b]		Injection		Extraction	
			CH_4 (ml l^{-1})	C_2H_2 (ml l^{-1})	Volume (l)	Pump rate (l min^{-1})	Volume (l)	Pump rate (l min^{-1})
PS3	A	−7	0.43	–	29.1	0.50	76.8	0.51
	I	0	0.35	8.73	29.0	0.50	75.3	0.50
	R1	7	0.47	–	28.1	0.49	76.3	0.51
	R2	14	0.47	–	28.2	0.49	74.7	0.50
	R3	27	0.49	–	29.2	0.50	77.5	0.52
	R4	56	0.38	–	29.2	0.50	76.7	0.51
PS5	A	−7	0.42	–	28.8	0.50	75.5	0.50
	I	0	0.33	8.12	27.3	0.49	75.1	0.50
	R1	7	0.47	–	28.5	0.49	73.5	0.49
	R2	14	0.47	–	28.4	0.49	75.8	0.51
	R3	27	0.49	–	28.6	0.49	74.4	0.50
	R4	56	0.38	–	29.1	0.50	79.7	0.53

[a] Days are given relative to the day of GPPT I

[b] Injection mixtures all additionally contained 210–263 ml l^{-1} He, 221–256 ml l^{-1} Ne, 207–243 ml l^{-1} Ar and 217–234 ml l^{-1} O_2 and were prepared in N_2

modifications. Initially, two replicate background samples of soil air were collected at 1 m depth through the permanently installed injection rod prior to each test. During subsequent GPPTs, between 27 and 29 l of gas mixture was injected with an average flow rate of 0.49 l min^{-1} through the injection rod (Table 1). Within 2 min from the end of injection, flow was reversed and between 74 and 80 l were extracted from the same location with an average flow rate of 0.51 l min^{-1}. Total test duration was 3.5 h. To remove C_2H_2 after the end of GPPTs I, extraction was continued for 2.5–3 h at a flow rate between 0.7 and 1.3 l min^{-1} and occasional samples were taken to measure C_2H_2 concentrations. For injection and extraction, a gas flow controller (GFC) was used. The core equipment of the GFC was a diaphragm pump and a mass flow meter (Urmann et al. 2005). Note that units of l and ml of gas in this paper all refer to volumes normalized to 0 C. Deviating from previous procedures, injection and extraction samples were collected with +0.6 bar pressure in gas-tight 20-ml GC-autosampler vials with butyl rubber stoppers. Samples were analyzed for CH_4 and C_2H_2 by gas chromatography with a FID detector and a Hayesep-N column at 85 C (Urmann et al. 2005). Acetylene was quantified down to a concentration of 0.01 µl l^{-1}, the detection limit was around a factor of 10 lower. Furthermore, samples for noble gases and O_2 were analyzed by gas chromatography with a TCD detector and a molecular-sieve column (10-m long, 2-mm i.d., packed with Molsieve 5A) at 35 C with a back-flushed pre-column to remove CO_2 and H_2O (Gonzalez-Gil et al. 2007). As O_2 concentrations were two orders of magnitude higher than CH_4 concentrations during all tests, O_2 was considered non-limiting. Therefore, O_2 data were not further analyzed and are not shown.

Estimation of kinetic parameters

To obtain breakthrough curves of the different gases, relative concentrations (C^*) were calculated by dividing concentrations in extraction samples by the concentration in the respective injection gas mixture (Table 1) and plotted versus time since end of injection. Prior to these calculations, concentrations in GPPT extraction samples were corrected for their background concentrations measured in soil air (CH_4 0.27–1.12 µl l^{-1}, Ne below detection) (Urmann et al. 2005). From this point forward corrected values will be referred to as CH_4. A simplified method was used to evaluate GPPTs, which accounts for reaction (in this case CH_4 oxidation) during both injection and extraction phases of a GPPT even when only a segment of the GPPT extraction breakthrough curve is evaluated (Schroth and Istok 2006). In this method, the gas

mixture is imagined to consist of individual "parcels" that are sequentially injected into the soil. It is further assumed that no mixing occurs between individual parcels during gas transport in soil. To apply this method, a residence time t_R was calculated for each parcel (i.e., sample) j collected during extraction, which is the time from its injection until its extraction (Eq. 1):

$$t_R^j = t^{*j} + \frac{\int_{t_{ext=0}}^{t_{ext}^j} Q_{ext} C_{Ne}(t) dt}{M_{Ne}} T_{inj} \quad (1)$$

where t^* is time since end of injection, Q_{ext} is the extraction pump rate, t_{ext} is time since extraction began, M_{Ne} is the total mass of the tracer Ne injected, C_{Ne} is the Ne concentration at time t_{ext} and T_{inj} is the injection time. Subsequently, the natural logarithm of the ratio of relative concentration C^* of CH_4 and Ne was plotted versus residence time t_R (Eq. 2). Neon thereby accounts for dilution of the injected gas mixture with soil air.

$$\ln\left(\frac{C^*_{CH_4}(t^*)}{C^*_{Ne}(t^*)}\right) = -k_{app} t_R + c \quad (2)$$

Apparent first-order rate constants k_{app} and corresponding 95% confidence intervals were calculated by linear regression from the segment of the data that showed a ln-linear relationship according to Eq. 2 with c as an arbitrary constant. In those cases where the entire dataset was linear, c was set to 0 (Schroth and Istok 2006).

Results

Methane gas concentrations in depth profiles at both locations were similar to or below atmospheric CH_4 concentrations. Concentrations decreased with depth indicating uptake of atmospheric CH_4 into the soil (data not shown).

Relative CH_4 concentrations near both wells during the initial GPPT A were considerably lower than relative concentrations of the tracer Ne (Fig. 2), indicating CH_4 oxidation occurring at both locations. The almost linear relationship between $\ln\left(C^*_{CH_4}/C^*_{Ne}\right)$ and t_R showed that CH_4 oxidation approximately followed apparent first-order kinetics throughout the entire GPPT A at both locations (Fig. 3). At PS5, the apparent first-order rate constant k_{app} for CH_4 oxidation determined from GPPT A was 0.67 h^{-1} compared to 1.16 h^{-1} at PS3 (Fig. 4).

In contrast to GPPT A, breakthrough curves of CH_4 and Ne, as well as C_2H_2, nearly coincided during both GPPT I, the tests with the co-injection of C_2H_2 as an inhibitor (Fig. 2). This confirmed similar transport behavior of reactant and tracer under the test conditions applied during all GPPTs, which is a pre-requisite for rate calculations (Urmann et al. 2005). Only in the first third of extraction of GPPT I at PS5, a slightly higher CH_4 breakthrough curve compared to the Ne breakthrough curve was observed (Fig. 2). These slight deviations indicated a larger influence of diffusion at the beginning of extraction at PS5. Under diffusion-dominated transport conditions, relative CH_4 concentrations in breakthrough curves were previously found to be higher than relative Ne concentrations (Gonzalez-Gil et al. 2007; Urmann et al. 2007a). As Ne cannot be used as a tracer for CH_4 under these conditions, only breakthrough curves from the later part of extraction ($t_R > 1.35$ h) were used for data analysis of all GPPTs (A, I and R1-4) at PS5 (Fig. 3). Small apparent first-order rate constants, computed from GPPT I at both locations (Fig. 4) were in accordance with similar breakthrough curves of Ne and CH_4 and inhibition of CH_4 oxidation activity (Fig. 2).

At the end of extraction of both GPPT I, C_2H_2 concentrations of 0.52 and 0.60 ml l^{-1} were observed at PS3 and PS5, respectively. Remaining C_2H_2 was extracted at a higher pump rate for 2.5–3 h, which decreased C_2H_2 concentrations by a factor of 10. During additional GPPTs two days after inhibition (data not shown), maximum C_2H_2 concentrations of 0.4 and 4.4 µl l^{-1} were detected at PS3 and PS5, respectively. After 1 week, during GPPT R1, no C_2H_2 was detected at PS3 while up to 0.03 µl l^{-1} was detected at PS5, which was gone one week later, during GPPT R2.

In the 8 weeks following inhibition, CH_4 oxidation activity partially recovered, as indicated by lower relative CH_4 concentrations compared to relative Ne concentrations (see data from GPPT R4 in Fig. 2). Accordingly, the slopes of rate plots increased again in comparison to GPPT I (Fig. 3). In contrast to GPPT A, CH_4 oxidation followed apparent Michaelis–Menten kinetics in the first part and apparent first-order kinetics only in the later part of extraction in all GPPT R as indicated by curved rate plots at the beginning of extraction (Fig. 3). Apparent first-order

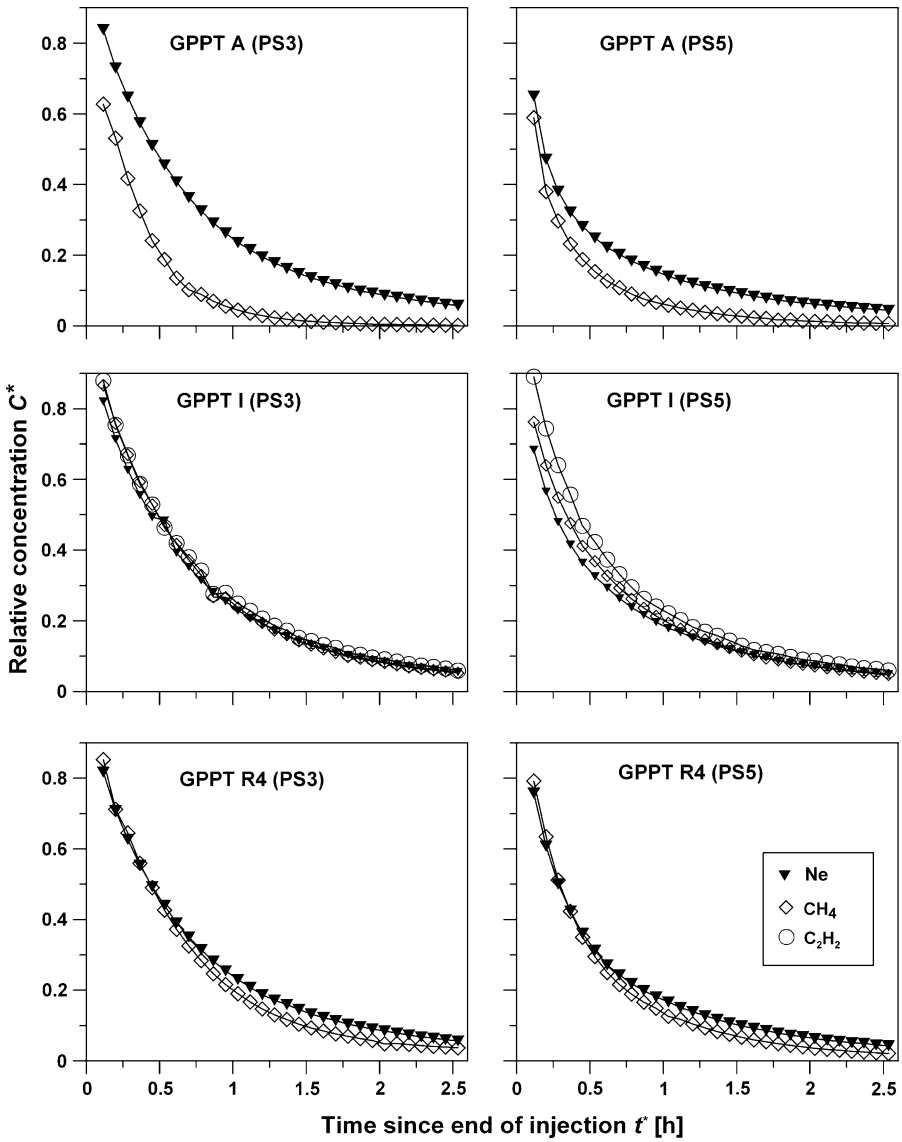

Fig. 2 Neon and CH_4 breakthrough curves at PS3 and PS5 during gas push-pull tests (GPPTs) before inhibition (GPPT A), during the GPPT with the inhibitor C_2H_2 (GPPT I) and 8 weeks after inhibition (GPPT R4). For GPPT I, C_2H_2 breakthrough curves are shown in addition

rate constants, computed from linear parts of rate plots, increased during the first week leading to a recovery of 28% of the initial activity at both PS3 and PS5 (Fig. 4). At PS5, activity continued to recover at a lower rate during the following three weeks with recovery reaching 50% 4 weeks after inhibition. However, after 8 weeks, activity dropped to 43% of initial activity. In contrast, at PS3, activity did not recover any further between 1 and 4 weeks after inhibition and then dropped to 22% of initial activity 8 weeks after inhibition. Despite the different rates and percentages of recovery, apparent first-order rate constants were very similar at both locations four and eight weeks after inhibition (Fig. 4).

Discussion

We studied recovery of CH_4 oxidation activity at two locations above a petroleum-hydrocarbon contaminated aquifer after inhibition with C_2H_2. In accordance with previous experiments (Urmann et al. 2005), effective inhibition was confirmed in GPPTs at both test locations by similar Ne and CH_4 breakthrough curves resulting in small apparent first-order rate constants (Fig. 4) and by sufficient inhibitor concentrations throughout the entire tests. Similar C_2H_2 and CH_4 breakthrough curves furthermore confirmed that the inhibitor was distributed in the test zone similar to the reactant (Fig. 2, Schroth et al. 2001). In previous experiments, effective C_2H_2

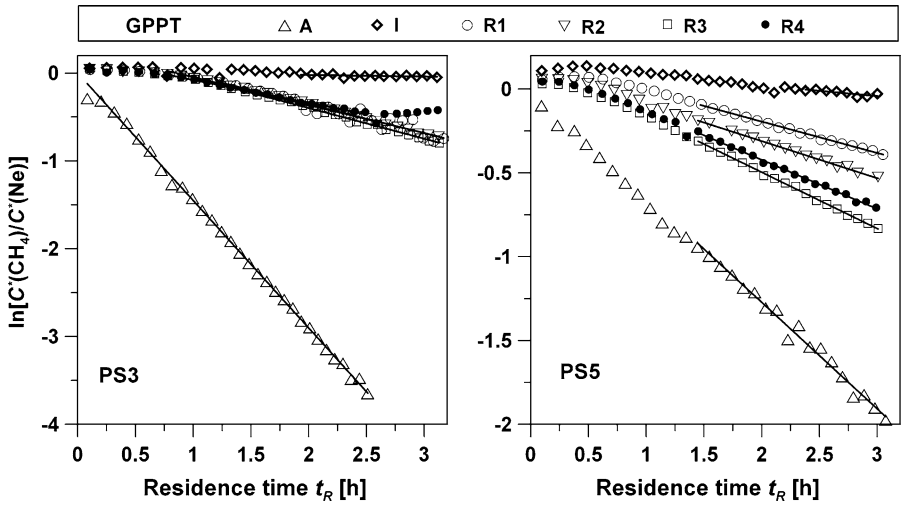

Fig. 3 Plots for rate calculations from gas push-pull tests (GPPTs) at PS3 and PS5. Apparent first-order rate constants were derived from the slopes by linear regression (solid lines). At PS5, data were only evaluated for $t_R > 1.35$ h due to deviating transport behavior of CH_4 and Ne at the beginning of extraction

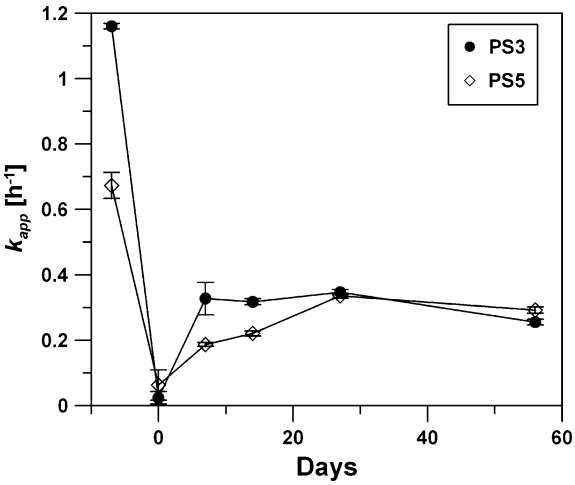

Fig. 4 Apparent first-order rate constants k_{app} for CH_4 oxidation at PS3 and PS5 before, during, and up to 8 weeks after inhibition with C_2H_2. Day 0 is the day of inhibition. Error bars represent 95% confidence intervals

inhibition during a comparable GPPT was additionally confirmed by CH_4 stable carbon isotope data (Urmann et al. 2005). However, when applying this method in future studies, it should be noted that the amount of C_2H_2 necessary for effective inhibition may vary between different environments.

In this study, we report apparent first-order rate constants k_{app} as a measure of activity. According to Michaelis–Menten kinetics, k is defined as the ratio of maximum activity V_{max} over the affinity constant K_m for substrate concentrations much smaller than K_m. Assuming that K_m remained constant during our experiments, a higher k means a higher V_{max}, which in turn implies the presence of more enzyme for CH_4 oxidation (Dunn et al. 1992). Considering GPPT results, apparent first-order rate constants contain more information about the intrinsic activity of the cells or enzymes than CH_4 turnover rates, calculated by multiplying k_{app} with CH_4 concentrations, as the latter would be influenced by variations in CH_4 test concentrations. However, to directly compare apparent first-order rate constants, k_{app}-values should be obtained under the same conditions, as values may depend on the physical conditions under which they were determined (Urmann et al. 2007b). As all tests were performed under nearly the same test conditions at the same site, this is valid for the presented experiments.

Comparing the k_{app}-values with previous GPPTs at the same site, the apparent first-order rate constant for CH_4 oxidation at PS5 during GPPT A was similar to rate constants previously determined at a similar depth at PS4 (Urmann et al. 2005, 2008) (Fig. 1a). During a GPPT 3 months prior to GPPT A, a similar k_{app} of 0.63 h^{-1} was also determined at PS3. This may indicate that the higher observed rate constant at PS3 during GPPT A (1.16 h^{-1}), i.e., the higher activity, was induced by high CH_4 concentrations of up to 3 ml l^{-1} observed in the test zone during a significant rise in the water table 12 days prior to GPPT A. A second rise in the water table occurred 2

days after inhibition (max. groundwater level in Fig. 1b) leading to a high CH_4 background concentration of 0.68 ml l^{-1} at PS3 and a slightly enhanced CH_4 background concentration at PS5. This impeded quantitative analysis of additional GPPTs performed on this day (data not shown).

The high water solubility of C_2H_2 (Wilhelm et al. 1977) and its strong adsorption to surfaces made it difficult to totally remove C_2H_2 from the soil. However, despite small concentrations still being observed after 2 days and at PS5 even after 1 week, recovery was fastest during the first week after inhibition at both locations. At PS3 maximum recovery was already reached after 1 week, while at PS5 recovery continued until 4 weeks after inhibition. As de-novo enzyme synthesis is assumed to be required for recovery from C_2H_2 inhibition (Hyman and Arp 1992), it was proposed that the physiological status of methanotrophic cells at the time of C_2H_2 addition determines their ability to recover (Bodelier and Frenzel 1999). For example, in laboratory incubations of rice field soils, CH_4 oxidation activity recovered from 24-h-long exposure to 10–10,000 µl l^{-1} C_2H_2 within one day when cells were activated by incubation with 1,000 µl l^{-1} CH_4 for 24 h prior to inhibition. In contrast, without pre-incubation with CH_4, activity did not recover at all from the same exposure to C_2H_2 within 90 h (Bodelier and Frenzel 1999). Similarly, CH_4 oxidation did not recover within 14 day after exposure to 10 ml l^{-1} C_2H_2 for 24 h in soil that was not pre-incubated with CH_4 (Miller et al. 1998). In a field study, CH_4 oxidation was quantified in a freshwater marsh by comparing CH_4 emissions of a 50 cm × 50 cm plot covered by a chamber after 20 h of incubation with 40 ml l^{-1} C_2H_2 with CH_4 emissions without C_2H_2 addition. Comparison of a time series of these experiments with an alternative method suggested that CH_4 oxidation fully recovered in less than a month at this field site (Ding et al. 2004). In contrast to the freshwater marsh, in-situ CH_4 concentrations at our site were several orders of magnitude lower. Nonetheless, methanotrophic bacteria were active before addition of C_2H_2 in our experiments, which may explain the observed partial recovery from C_2H_2 inhibition. However, the low level of activity, due to exposure to near atmospheric CH_4 concentrations at most times, together with continued low availability of CH_4 after inhibition likely slowed down recovery and prevented cells from reaching their initial activity within the 8 weeks of our experiments. Exposure to higher CH_4 concentrations at PS3 before and/or after inhibition might explain why at PS3 the maximum rate constant after inhibition was reached faster. The slight drop in activity between 4 and 8 weeks after inhibition may have been a seasonal effect or natural fluctuations overlaying the recovery process. Temperature dropped by 5 C during the duration of the experiments and even though temperature effects on CH_4 oxidation under substrate-limited conditions were usually found to be small (Mosier et al. 1996; Whalen and Reeburgh 1996), temperature could have played a role in the slight decrease in activity. Similarly, although not measured, soil moisture might have played a role as it likely varied during the time of the experiments as a result of the severe rainfall events.

Conclusions and implications

Using a series of GPPT field experiments, we showed that recovery of CH_4 oxidation activity following C_2H_2 inhibition was slow and activity only recovered by up to 50%. At the studied locations, caution should therefore be exercised when performing a series of experiments to assess CH_4 oxidation with a method comprising C_2H_2 inhibition, as the recovery process may mask natural trends. However, recovery time will likely vary between different environments and the studied locations may represent relatively unfavorable conditions for recovery as in situ CH_4 concentrations and activities were low. Recovery may be significantly faster at sites with high CH_4 concentrations and high CH_4 oxidation activity as observed in a laboratory study (Bodelier and Frenzel 1999) and indicated by results from a field study (Ding et al. 2004). However, this requires further investigation. To overcome the problem of slow recovery from C_2H_2 inhibition, alternative inhibitors will be evaluated in conjunction with GPPTs, especially for sites with low CH_4 oxidation activity.

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