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Aerobic cometabolic degradation of trichloroethene by methane and ammonia oxidizing microorganisms naturally associated with *Carex comosa* roots

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Abstract The degradation potential of trichloroethene by the aerobic methane- and ammonia-oxidizing microorganisms naturally associated with wetland plant (*Carex comosa*) roots was examined in this study. In bench-scale microcosm experiments with washed (soil free) *Carex comosa* roots, the activity of root-associated methane- and ammonia-oxidizing microorganisms, which were naturally present on the root surface and/or embedded within the roots, was investigated. Significant methane and ammonia oxidation were observed reproducibly in batch reactors with washed roots incubated in growth media, where methane oxidation developed faster (2 weeks) compared to ammonia oxidation (4 weeks) in live microcosms. After enrichment, the methane oxidizers demonstrated their ability to degrade 150 μg l^{-1} TCE effectively at 1.9 mg l^{-1} of aqueous CH_4. In contrast, ammonia oxidizers showed a rapid and complete inhibition of ammonia oxidation with 150 μg l^{-1} TCE at 20 mg l^{-1} of NH_4^+-N, which may be attributed to greater sensitivity of ammonia oxidizers to TCE or its degradation product. No such inhibitory effect of TCE degradation was detected on methane oxidation at the above experimental conditions. The results presented here suggest that microorganisms associated with wetland plant roots can assist in the natural attenuation of TCE in contaminated aquatic environments.

Keywords Trichloroethene · Methane oxidizers · Ammonia oxidizers · Cometabolism · Roots · Biodegradation

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Introduction

Trichloroethene (TCE) is one of the most common groundwater contaminants, and it poses a threat to human health as a suspected carcinogen (ATSDR 1997). Natural attenuation of TCE has been examined as a possible remediation practice as it is more advantageous, both economically and environmentally, than conventional options such as pump-and-treat that are time and money intensive (NRC 2000; Powers and Rubin 1996). However, natural attenuation of TCE is not always effective. In reducing environments, TCE biodegradation often leads to accumulation of less-chlorinated, more-toxic intermediate compounds, such as vinyl chloride. In comparison, oxidative biodegradation of TCE is more desirable

because it allows for TCE mineralization to carbon dioxide (CO_2), a nontoxic end-product. For this to occur, environmental conditions favoring TCE cometabolism are required, including aerobic conditions as well as suitable microorganisms, and access to their growth substrates.

TCE can be degraded cometabolically by aerobic microorganisms that utilize a wide range of growth substrates, such as methane (Fogel et al. 1986; Little et al. 1988), toluene (Shim et al. 2001), phenol (Chen et al. 2004), ammonia (Kocamemi and Cecen 2007), and several others. In particular, methane as a growth substrate has been successfully studied in a number of systems using both mixed (Fogel et al. 1986) and pure cultures (Little et al. 1988). In cometabolic degradation of TCE with methane, the methane-oxidizing microorganisms produce a non-specific enzyme, methane monooxygenase (MMO), that oxidizes methane as its substrate and can also fortuitously degrade TCE (Sullivan et al. 1998). In this process, TCE oxidation is initiated as an epoxidation reaction catalyzed by the MMO, with NADH as an immediate energy source (Chang and Alvarez-Cohen 1995). Similarly, ammonia can also serve as a growth substrate for cometabolic TCE degradation (Moran and Hickey 1997; Rasche et al. 1991; Yang et al. 1999), although it has not been studied as extensively as methane and such research has focused on laboratory studies mostly with pure cultures of *Nitrosomonas europaea*. The enzyme produced during ammonia oxidation, ammonia monooxygenase (AMO), can also degrade TCE cometabolically, but with limited success due possibly to inhibitory effects of TCE on ammonia oxidizers (Rasche et al. 1991; Yang et al. 1999).

The oxidative biodegradation of chlorinated ethenes with plant roots has been investigated at bench scale (Bankston et al. 2002; Tawney et al. 2008). Further, TCE biodegradation by the oxidative pathway has been suggested at the shallow depths of an experimental wetland, particularly due to the presence of wetland plant roots (Amon et al. 2007). The root zone of wetland plants can provide the appropriate conditions to support certain oxidizing microorganisms (King 1996). Oxygen is transported from the shoot to the root tissues of wetland plants for metabolic purposes, and a portion of that can diffuse out into the plant rhizosphere and the immediate soil environments (Armstrong et al. 2000; Colmer 2003). The specific aerobic microbial communities that inhabit the microaerobic environment in plant rhizosphere can oxidize species, for example methane (King 1996) and ammonia (Reddy et al. 1989), which are produced in the surrounding anaerobic soil and migrate toward plant roots (Bosse and Frenzel 1997). In microcosms planted with rice, approximately 90% of methane oxidation has been reported at a depth where roots were the only source of oxygen (Bosse and Frenzel 1997), and a significantly greater number of methane-oxidizing microorganisms were found in the soil immediately around roots (within 0.1–0.2 mm) than in the soil farther away from the roots. Similarly, the nitrification activities (i.e., ammonia oxidation) in rice plants decreased with increasing distance from the root zone, and they were positively correlated with the ammonia-oxidizing bacteria abundance (Li et al. 2007).

The main objective of this investigation was to provide a proof-of-concept that oxidative cometabolic TCE degradation can be facilitated by the methane- and ammonia-oxidizing microorganisms that are naturally associated with wetland plant roots. Recent studies have indicated that microbial processes can facilitate removal of chlorinated hydrocarbons in vegetated wetland settings (Amon et al. 2007; Imfeld et al. 2008). Prior to this, studies of chlorinated hydrocarbon degradation with wetland soils by reductive dehalogenation processes have been studied (Kassenga et al. 2004; Lorah and Olsen 1999; Lorah and Voytek 2004). Other studies have examined degradation of chlorinated hydrocarbons in soil/aquatic systems assisted by plants, including by rhizodegradation (James et al. 2009; Nzengung and Jeffers 2001), phytovolatilization (Bankston et al. 2002; Imfeld et al. 2009), and uptake and biotransformation, i.e., metabolic degradation within plant tissues (Newman et al. 1997; Nzengung and Jeffers 2001; Wang et al. 2004). While the studies describing the role of microbial processes in plant rhizosphere in chlorinated hydrocarbon degradation are limited (Bankston et al. 2002; Tawney et al. 2008), the synergistic activity of the plant–microbe system in the wetland in the removal of organic chemical removal has increasingly been recognized (Imfeld et al. 2009).

In the present study, the role of naturally occurring methane- and ammonia-oxidizing microbes associated with plant roots in oxidative cometabolic TCE degradation has been examined. This is the first study to our

knowledge that provides direct evidence for a mechanism of microbial TCE degradation with plant roots by studying the roots themselves. Bench-scale microcosm experiments were performed using soil-free roots from the wetland plant species *Carex comosa* (longhaired sedge). Live plants were not used to eliminate plant effects, such as uptake and volatilization, and to study the activity of bacteria directly associated with the roots. Methane and ammonia were evaluated as growth substrates for their role in TCE degradation by aerobic cometabolism since these compounds are often abundantly produced in anaerobic wetland soil. We expected to investigate the activities of root-associated methane- and ammonia-oxidizing microorganisms by measuring substrate and product concentrations over time when growth media was added to the microcosms. After the enrichment period, we also expected that the root-associated methane and ammonia oxidizers would demonstrate the ability to degrade TCE through aerobic cometabolism in the presence of methane and ammonia substrates, respectively. This approach enabled us to evaluate the potential of cometabolic TCE degradation by methane and ammonia oxidizers associated with wetland plant roots.

Materials and methods

Collection of wetland plants

Wetland plants (*Carex comosa*) were collected from a natural wetland located in Dayton, OH (USA) in September 2008. After collection, the plants were placed in individual pots containing a 50:50 mix of peat moss and wetland soil, and maintained in a university greenhouse until use (~25–40 weeks). In order to prepare for the bench-scale microcosm experiments described below, the roots from individual *Carex comosa* specimens were clipped, separated from the plant shoot, and washed with de-ionized water thoroughly to remove attached soil. The entire root was used so as to include the root tip, lateral root and primary root.

Growth media

Mineral media were prepared separately for methane and ammonia oxidizers for their optimal growth. The methane-oxidizing microorganisms associated naturally with plant roots were enriched with growth medium 'A' with a pH of 6.8 (described in Fogel et al. 1986). Ammonia-oxidizing microorganisms naturally-associated with plant roots were enriched with growth medium 'B' containing 94 mg $(NH_4)_2SO_4$, 400 mg $NaHCO_3$, 100 mg $MgSO_4$, 50 mg KH_2PO_4, 445 mg $Na_2HPO_4{\cdot}12H_2O$, 30 mg KCl, 50 mg $CaCl_2{\cdot}2H_2O$, 3 mg $FeSO_4{\cdot}7H_2O$, 0.02 mg $MnCl_2$, 0.7 mg $ZnSO_4{\cdot}H_2O$, 0.02 mg H_3BO_4, 0.01 mg $CuCl_2$, 0.03 mg $Na_2MoO_4{\cdot}2H_2O$, 0.02 mg $NiCl_2{\cdot}6H_2O$, 0.1 mg $CoCl_2{\cdot}6H_2O$ dissolved in 1 l of de-ionized water (final pH 7.8).

Experimental design

Microbial enrichment with methane and ammonia (in absence of TCE)

Six microcosms (160 ml borosilicate glass serum bottles; Wheaton, Millville, NJ) were used for the subset of experiments with methane as a substrate. Three bottles were prepared as live microcosms with 1 g of fresh roots and 100 ml of growth medium A, and the three remaining bottles were prepared as killed controls with 1 g of fresh roots, 100 ml of growth medium A, and 6.24 mg l^{-1} of sodium azide to inhibit gram-negative bacterial growth, including the growth of methane-oxidizing bacteria (Ginestet et al. 1998). After these additions, a headspace of 60 ml remained in each microcosm. All microcosms were bubbled with air for ~30 min to start the experiment with enough dissolved oxygen to maintain aerobic conditions. The microcosms were then capped with Teflon-lined grey butyl rubber stoppers (20 mm dia.; Wheaton, Millville, NJ) and sealed with aluminum crimps (Wheaton, Millville, NJ). Pure gaseous methane (CH_4) (5.25 ml, equivalent to 1.9 mg l^{-1} aqueous CH_4) were injected into each bottle using a gas-tight glass syringe (Hamilton, Reno, NV). The bottles were then wrapped in aluminum foil and placed on a rotary shaker (Glas-Col, Terre Haute, IN) at 30 rpm for gentle horizontal mixing in an upside down position at 22 ± 1°C.

There were four cycles of microbial enrichment with methane (called, ME1 through ME4) as a substrate over an 18-day period, where each cycle corresponding to a period during which methane degraded nearly completely in the live microcosms,

and needed to be replenished. At the end of each cycle, all microcosms were opened to carefully discard the aqueous growth medium without removing the roots and replaced with 100 ml of fresh growth medium (killed controls were amended with 6.24 mg l^{-1} of sodium azide), bubbled with air, sealed and wrapped in aluminium foil as described earlier. Each new cycle began by amending the microcosms with gaseous CH_4 (aqueous CH_4 = 1.9 mg l^{-1}), followed by their incubation on the rotary shaker. Six additional microcosms were set up (3 live and 3 killed controls) with ammonia as substrate with an experimental design similar to the microcosms set up with methane as the substrate (described above), but with the following variations: (a) growth medium 'B' was used, containing 20 mg l^{-1} of N-ammonium (NH_4^+), (b) CH_4 was not added, (c) 10 mg l^{-1} of allylthiourea was used in the killed control microcosms (instead of sodium azide) to inhibit the growth of ammonia-oxidizers, and (d) three microbial enrichment cycles (called, AE1 through AE3) were completed during a 32-day period.

Cometabolic TCE degradation with methane and ammonia substrates

Following the four cycles of microbial enrichment with methane (described above), four additional cycles (TM1 through TM4) were completed in triplicate microcosms with methane under identical experimental conditions, but with 150 μg l^{-1} of TCE added. Similarly, following the three cycles of microbial enrichment with ammonia (described above), two additional cycles (TA1 and TA2) were completed in triplicate microcosms with ammonia under identical experimental conditions, but with 150 μg l^{-1} of TCE added. A number of cycles equal to both sets of live microcosms were completed for the killed control microcosms.

Analysis

The following analyses were performed to determine the activity of methane- and ammonia-oxidizing microorganisms associated with roots in the microcosms. In microcosms with methane and TCE amendments, concentrations of CH_4, dissolved oxygen (DO), dissolved inorganic carbon (DIC), and TCE were measured daily in each cycle. Similarly, in the microcosms with ammonia and TCE amendments, [NH_4^+], [NO_2^-], [NO_3^-], [DO], and [TCE] were measured daily in each cycle. Aqueous samples were processed through 0.22 μm, 25 mm dia. syringe filters (Restek, Bellefonte, PA) before analysis; NO_2^- and NO_3^- were quantified by ion chromatography (DX2500, Dionex Corporation, Sunnyvale, CA), and NH_4^+ was quantified by the salicylate method (Hach Company, Loveland, Co) by spectrophotometry (Lambda 45 UV/Vis, Perkin Elmer, Waltham, MA). The measurements of N-NO_2^- and N-NO_3^- were combined and it is referred henceforth as NO_x. The pH measurements were made using a meter (AP10 pH/mV/temp, Denver Instrument, Bohemia, NY) by collecting 2–2.5 ml aqueous samples from each of the methane and ammonia microcosms, after gas sampling on day 1 and day 5 of each cycle. Headspace samples from the microcosms were analyzed by gas chromatography to estimate aqueous phase [CH_4], [DO], and [DIC] and [TCE] in each microcosm. CH_4 and TCE were analyzed by a HP 6890 series GC system with flame ionization (FID) and electron capture (ECD) detectors; CH_4 was separated on a capillary column (GS GasPro, 30 m × 0.32 mm; J&W Scientific) connected to an FID, and TCE was separated on a capillary column (HP-624, 30 m × 0.32 mm; Agilent Technologies), connected to an ECD. O_2 and CO_2 were analyzed by a HP 5890 series GC system with a thermal conductivity detector (TCD), and separated on a packed column (Shin Carbon 100/120, 2 m × 1 mm; Restek, Belefonte, PA) (see *Online Resource*).

The aqueous [CH_4] and [TCE] at equilibrium were calculated by the Gas law and Henry's law using a published approach (Burris et al. 1996). Based on measured partial pressures of O_2 and CO_2 in the microcosm headspace, [DO] was calculated using Henry's Law (Lide and Frederikse 1995), and [DIC] was calculated using Henry's Law and carbonate system equilibrium relationships at the measured pH (Pankow 1991). Detailed calculation approaches are available in the *Online Resource*.

Data treatment and statistical analysis

For each of the TCE cycles for methane and ammonia microcosms, the initial rates of TCE degradation and solute consumption/production (i.e., CH_4, DO, DIC, NH_4^+, and NO_x) were calculated using the difference between their respective measured values on day 2

subtracted from their measured values on day 1 for each treatment (with the exception of the second TCE cycle with ammonia, which was from day 0 to day 5). The initial rates were taken as day 1–2, rather than day 0–1, to avoid the data analysis during transient conditions caused by microcosm reset prior to each cycle. Similarly, TCE was not analyzed on the day it was injected in the microcosms (i.e., day 0), to allow for a 24 h equilibration of TCE between the liquid and headspace. Transformation yield (T_y) was calculated for the 4 TCE cycles with methane by subtracting the average loss in TCE and methane found in the killed controls from the live microcosm and then dividing TCE mass degraded by methane mass degraded.

For the methane- or ammonia-oxidizing microorganisms, the statistical differences in their treatments (i.e., control vs. live microcosms) were determined for each solute using a one-way repeated measures analysis of variance (RM ANOVA) with time as the repeated factor. If the differences between the live and control microcosms were found to be statistically significant, the Tukey post hoc tests were performed to determine the differences between control and live treatments for each time point. For the solute consumption/production rates of each cycle, the effects of treatments were examined using a one-way RM ANOVA and Tukey post hoc tests. As necessary, the data were log-transformed before statistical analysis to fit the assumption of homoscedasticity. Statistical analyses were performed using Statistica 9^{TM} (Statsoft, Tulsa, OK, USA).

Results and discussion

Association of methane and ammonia oxidizers with *Carex comosa* roots

Comparisons of live and killed microcosms amended with soil-free roots indicates that the increase in substrate metabolism was likely due to increase in population size of methane and ammonia oxidizers associated with the plant roots in their respective enrichment cycles. During the four enrichment cycles with methane (ME1 through ME4), [CH_4] and [DO] were significantly lower and [DIC] was significantly higher in the live microcosms compared to the controls through time (one-way RM ANOVAs, treatment effect, time effect, $P < 0.05$, Fig. 1). Likewise, during the three cycles of microbial enrichment with ammonia (AE1 through AE3), [NH_4^+] were significantly lower in the live microcosm compared to the controls through time (one-way RM ANOVAs, treatment effect, time effect, $P < 0.01$, Fig. 2). Further, [DO] were significantly lower and [NO_x] were significantly higher in the live microcosms in comparison to the controls for the ammonia system, but their statistical significance was not consistent between the cycles (See the Online Resource). The approach to replace the growth medium at the end of each cycle discarded the microorganisms growing in suspended form and favored the enrichment of microorganisms that were growing on the root surface and perhaps also inhabiting the interior of root tissues (Bosse and Frenzel 1997).

Comparison of results during enrichment cycles after initial set-up (ME1 vs. AE1) indicate that significant methane oxidation was observed quicker in comparison to ammonia oxidation in the live microcosms. This may be due to higher initial population of methane oxidizers associated with the roots that allowed them to become dominant over heterotrophic microorganisms more quickly, in comparison to ammonia oxidizers. Methane oxidizers are generally adaptable to a wide range of environmental conditions (Hanson and Hanson 1996). In ME1, oxygen and methane levels concomitantly decreased in the microcosms on day 4, indicating activity of methane oxidizers. By comparison, in AE1, the initial decrease in oxygen occurred without concomitant ammonia degradation in both control and live microcosms until day 14, suggesting initial oxygen uptake may have been due to heterotrophic activity. It appears that either the initial population of ammonia oxidizers in the root samples was low, or the ammonia oxidizers had physiological limitations requiring a longer enrichment period compared to methane oxidizers. Indeed, ammonia oxidizers are relatively slow growing organisms (Vaccari et al. 2006) and methane oxidizers are ubiquitous with consumption rates depending on ambient methane concentration (Rogers and Williams 1991).

During enrichment cycles, the initial methane degradation and oxygen consumption rates (d[CH_4]/dt and d[DO]/dt) increased from cycle ME1 to cycle ME3, and then decreased slightly or stabilized in cycle ME4, whereas initial DIC production rates (d[DIC]/dt) were significantly different between

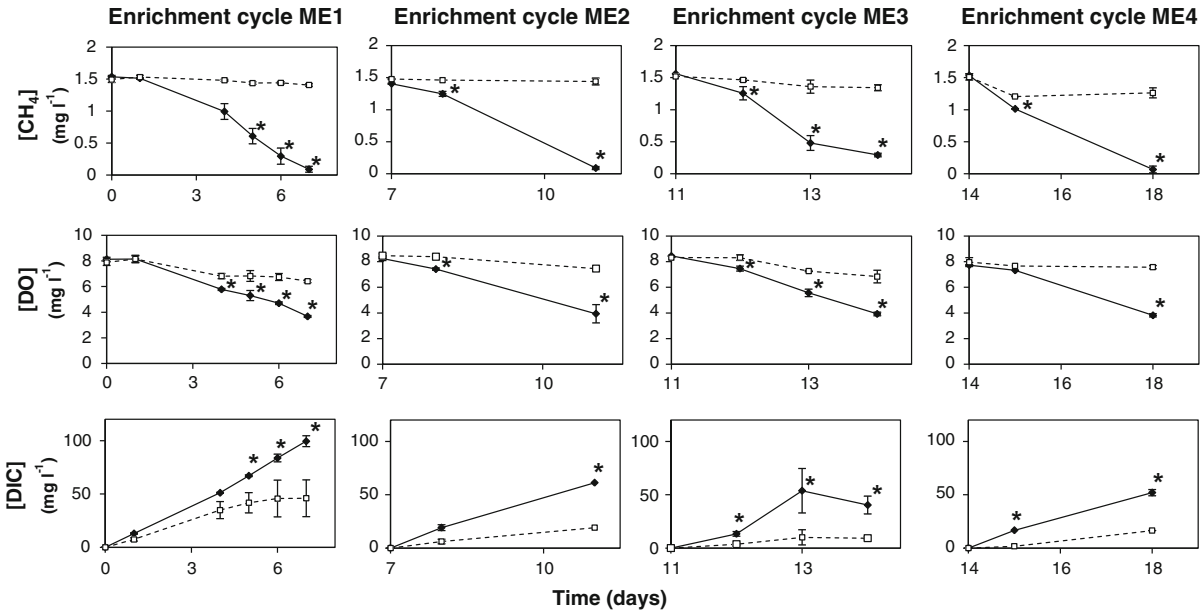

Fig. 1 Aqueous [CH_4], [DO], and [DIC] in the control (*white squares*) and the live (*black diamonds*) microcosms for the four enrichment cycles of the methane experiment (mean ± 1 SD, $n = 3$ per treatment). *Asterisk* (*) indicates a significant difference between control and live microcosms (Tukey HSD post hoc tests, $P < 0.05$)

treatments but not with time (one-way RM ANOVAs, treatment effect, $P < 0.001$, and cycle effect, $P > 0.05$; Table S1 in Online Resource). In comparison, the initial ammonia degradation and NO_x production rates (d[NH_4^+]/dt and d[NO_x]/dt) increased rapidly from cycle AE1 to AE3, whereas initial oxygen consumption rates did not show the same trend (one-way RM ANOVAs, treatment effect, cycle effect, $P < 0.01$; Table S1 in Online Resource).

The investigation demonstrated quite clearly that methane and ammonia oxidizers are associated with the roots of *Carex comosa*, a phenomenon that may be common among various wetland plants, which is consistent with the similar observations in other plant species (Bosse and Frenzel 1997; Li et al. 2007). A sustained degradation of methane and ammonia through the enrichment cycles in this study showed that wetland plant roots can provide a favorable environment for the growth of methane- and ammonia-oxidizing microorganisms. Wetland plants can overcome anaerobic environments by transporting oxygen from the atmosphere to their roots via the aerenchyma tissue (Armstrong et al. 2000; Colmer 2003). During this process, some oxygen typically leaks from the roots into the surrounding soil (i.e., rhizosphere), an otherwise anaerobic environment, creating a microaerobic niche suitable for growth of methane and ammonia oxidizers (Vaccari et al. 2006). The primary electron donors (methane and ammonia) for these organisms are produced in the anaerobic zone of wetland soils (Chanton et al. 1997; Neill 1995) and diffuse into the rhizosphere (King 1996; Reddy et al. 1989), and the juxtaposition of anaerobic and microaerobic environments can create an ideal niche for microorganisms that can cometabolize TCE.

TCE degradation potential by root-associated methane and ammonia oxidizers

The main objective of this study was to evaluate the TCE degradation potential of methane and ammonia oxidizers associated with *Carex comosa* roots. In experiments with methane, [TCE] were significantly lower in the live microcosms compared to the killed controls through time in four TCE cycles with methane, TM1 through TM4 (one-way RM ANOVAs, treatment effect, time effect, $P < 0.05$, Fig. 3). Also, during these cycles, [CH_4] and [DO] were significantly lower and [DIC] significantly higher in the live microcosms compared to the controls through time (one-way RM ANOVAs, treatment effect, time

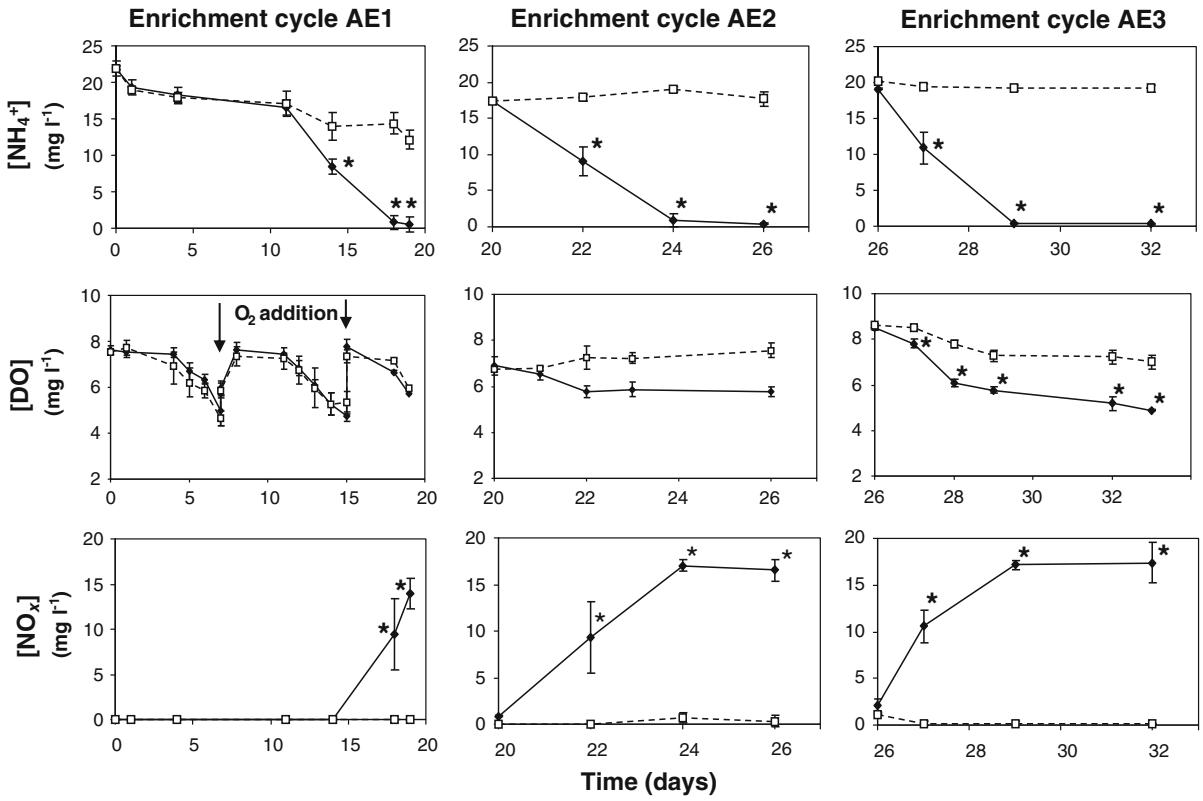

Fig. 2 Aqueous [NH_4^+], [DO], and [NO_x] in the control (*white squares*) and the live (*black diamonds*) microcosms for the three enrichment cycles of the ammonia experiment (mean ± 1 SD, $n = 3$ per treatment). *Asterisk* (*) indicates a significant difference between control and live microcosms (Tukey HSD post hoc tests, $P < 0.05$)

effect, $P < 0.05$, Fig. 3). Further, in experiments with ammonia, [TCE], [NH_4^+], and [DO] were significantly lower and [NO_x] were significantly higher in the live microcosms compared to the killed controls through time in cycle TA1 (one-way RM ANOVAs, treatment effect, time effect, $P < 0.05$, Fig. 4). In cycle TA2, [TCE] were not significantly different in the live microcosms compared to the controls (one-way RM ANOVA, treatment effect, time effect, $P > 0.05$, Fig. 4). However, for other analytes, significant differences were observed in [NH_4^+] between time and treatments (one-way RM ANOVA, treatment effect, time effect, $P < 0.01$, Fig. 4), [NO_x] between treatments (one-way RM ANOVA, treatment effect, $P < 0.05$, Fig. 4) on two days (day 13 and day 17), and [DO] with time (one-way RM ANOVAs, time effect, $P < 0.01$, Fig. 4). The [DO] did not exhibit a treatment effect (i.e., no significant differences between live microcosms and killed controls).

The results presented here have partially validated our initial hypothesis that root-associated methane and ammonia oxidizers have the ability to degrade TCE though aerobic cometabolic processes. Methane oxidizers were found to significantly cometabolize TCE, whereas the activity of ammonia oxidizers stalled soon after their exposure to TCE. This result is consistent with an earlier study (Bankston et al. 2002) that showed simultaneous TCE and methane degradation in microcosms containing soil from broad-leaf cattail roots. By using soil-free roots, this study provided a better understanding of the role of root-associated microorganisms in TCE degradation.

In this study, the average net TCE loss between live and control microcosms was ~40% for each TCE cycle of methane experiment. This is consistent with previous studies that demonstrated cometabolic TCE degradation with methane in bench-scale systems (Fogel et al. 1986; Little et al. 1988; Shukla et al. 2009). In a comparable study, a mixed culture

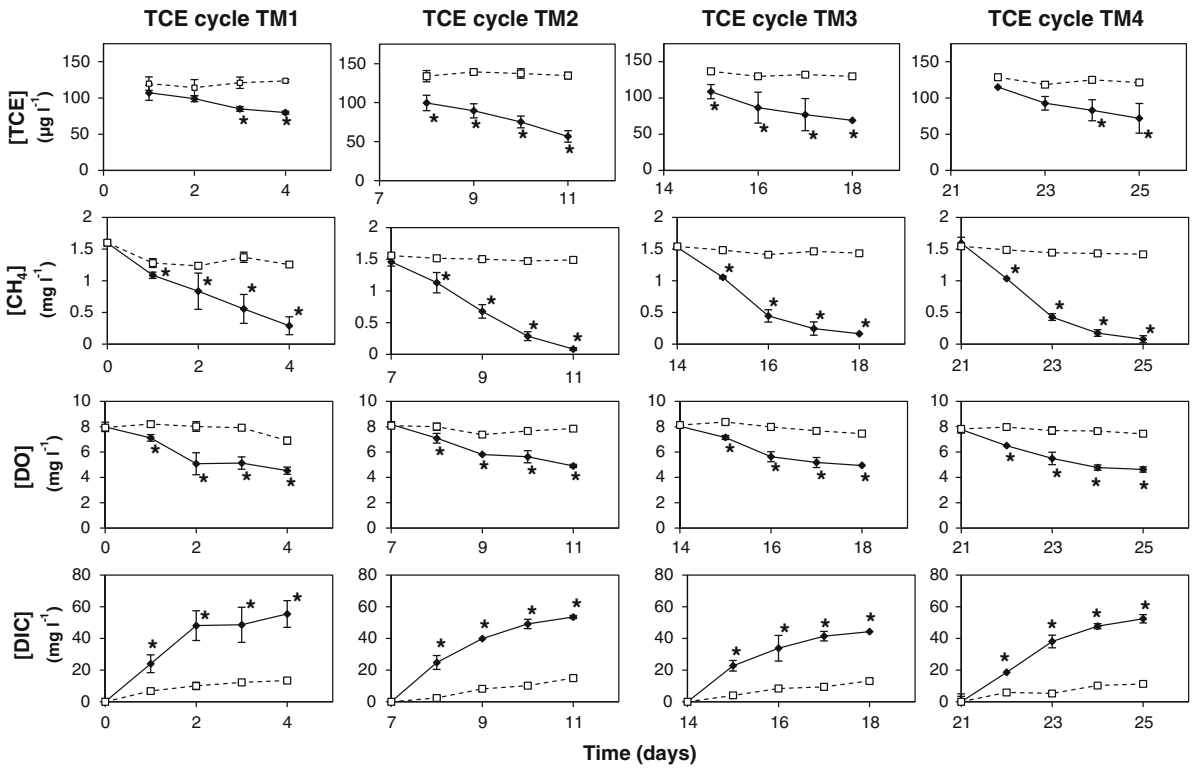

Fig. 3 Aqueous [TCE], [CH_4], [DO], and [DIC] in the control (*white squares*) and the live (*black diamonds*) microcosms for the four TCE cycles of the methane experiment (mean ± 1 SD, $n = 3$ per treatment). *Asterisk* (*) indicates a significant difference between control and live microcosms (Tukey HSD post hoc tests, $P < 0.05$)

obtained from sediment enriched for several weeks with 18% methane headspace demonstrated TCE degradation was 90% at initial [TCE] at 80 μg l^{-1} (Fogel et al. 1986). Another study with higher initial [TCE] and less methane (400 μg l^{-1} TCE, and 8% headspace, respectively) found 40% loss in TCE due to degradation after 10 days using a pure culture enriched from well water (Little et al. 1988). A direct comparison of our TCE removal rates with other studies is not particularly informative because rates depend on experimental parameters such as initial [TCE] and [CH_4], and microbial biomass. In the present study, d[CH_4]/dt shows a declining trend on days 2–4 of cycles TM3 and TM4 and pseudo first-order like kinetics, thus suggesting methane oxidation to become somewhat limited in later part of the cycles.

The average T_y for TM1 through TM4 cycles were 2.8, 3.6, 2.9 and 2.1 μg TCE/mg CH_4 respectively. This is less than those reported for a variety of cultures, ranging from 15 to 50 μg TCE/mg CH_4 (Alvarez-Cohen and Speitel 2001), possibly due to lower initial [TCE] in this study, and the potential for less efficient methane utilization at lower [TCE] (Chang and Criddle 1997). However, with an initial [TCE] of 150 μg l^{-1} and methane at 9% headspace used in the present study, a significant amount of TCE was degraded in the system with soil-free roots. Initial rates for CH_4, TCE and DO loss, and DIC production (d[C]/dt) were significantly different between treatments (one-way RM ANOVAs, treatment effect, $P < 0.05$, see Table 1) during all four TM cycles. CH_4 and TCE degradation rates were also significantly different between cycles (one-way RM ANOVAs, cycle effect, $P < 0.05$, Table 1) with the rates increasing from cycle TM1 to cycle TM3, and then remaining constant from cycle TM3 to cycle TM4, which suggests a lack of significant epoxide toxicity development during TM cycles (Ely et al. 1995; Hyman et al. 1995; Kocamemi and Cecen 2007).

The potential for cometabolic oxidation of TCE by ammonia oxidizers was not statistically significant because initial degradation was followed

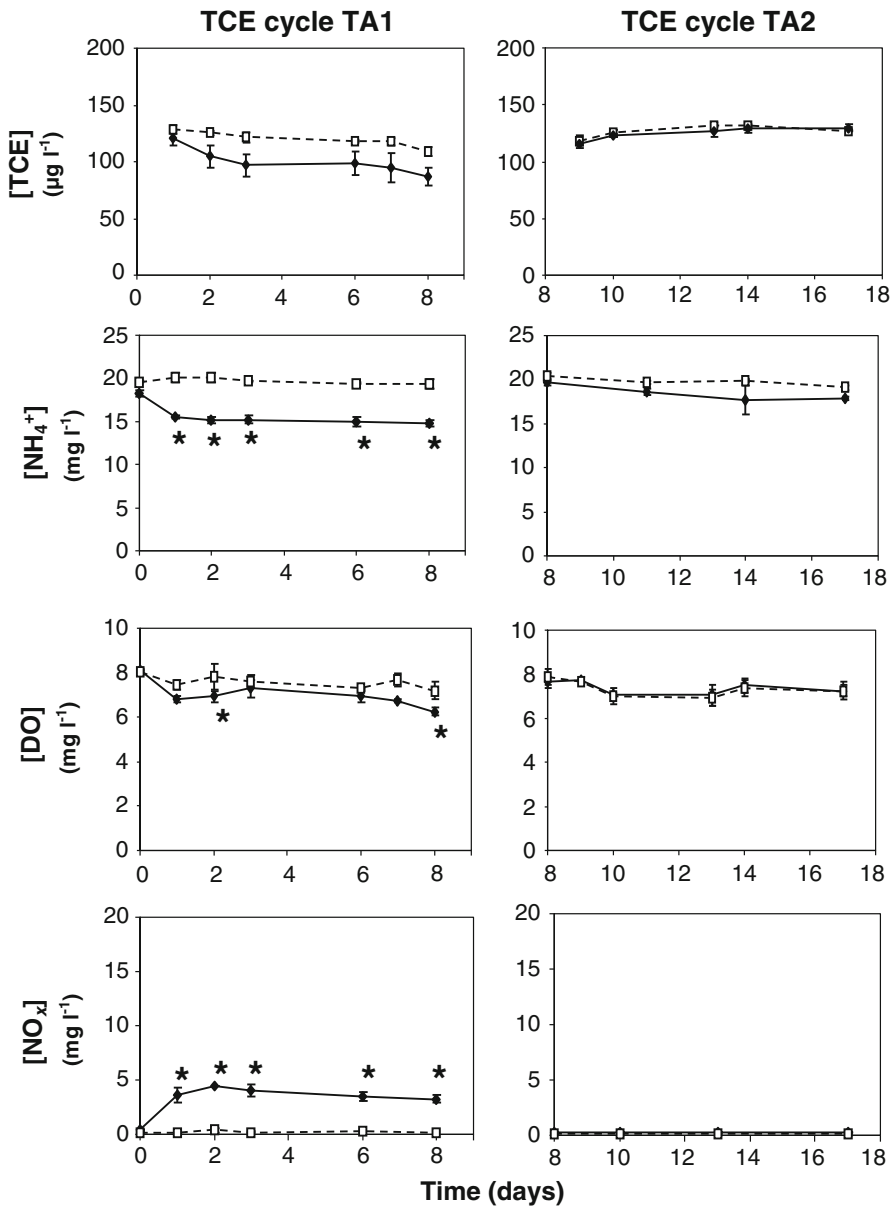

Fig. 4 Aqueous [TCE], $[NH_4^+]$, [DO], and $[NO_x]$ in the control (*white squares*) and the live (*black diamonds*) microcosms for the two TCE cycles of the ammonia experiment (mean ± 1 SD, $n = 3$ per treatment). *Asterisk* (*) indicates a significant difference between control and live microcosms (Tukey HSD post hoc tests, $P < 0.05$)

quickly by inhibition of both ammonia and TCE degradation under the experimental conditions. Several investigations have shown oxidative cometabolic TCE degradation using mixed nitrifying cultures (Kocamemi and Cecen 2005; Yang et al. 1999). In the present study, approximately 15% of initial [TCE] (150 µg l^{-1}) degraded in 2 days in live microcosms compared to the killed controls before complete inhibition of the system. In comparison, Yang et al. (1999) showed TCE cometabolism by a nitrifying culture enriched from a sewage treatment plant at initial [TCE] were between 125 and 200 µg l^{-1}, while higher initial TCE levels (>200 µg l^{-1}) induced inhibition of ammonium oxidation. Kocamemi and Cecen (2005) provided evidence for ammonia oxidation at a high initial [TCE] (4500 µg l^{-1}), and showed 68% ammonia removal in comparison to controls. The differences between the results of the present investigation and those of Yang et al. (1999) and Kocamemi and Cecen (2005) may be attributed to differences in nitrifying strains.

Table 1 Initial loss/production rates (d[C]/dt) of solutes (CH_4, NH_4^+, DO, NO_x, and DIC) in control and live microcosms for each cycle of TCE amendment (mean ± SD, $n = 3$) calculated over a 24 h period except for the cycle 2 of the ammonia experiment (96 h period)

Initial rate d[C]/dt	TCE cycles with methane (TM)				TCE cycles with ammonia (TA)	
	Cycle TM1	Cycle TM2	Cycle TM3	Cycle TM4	Cycle TA1	Cycle TA2[a]
TCE loss rate (μg l^{-1} day^{-1})						
Control	5.67 ± 5.32	−5.37 ± 4.78	6.97 ± 1.16	10.3 ± 2.04	3.92 ± 2.32	0.00 ± 0.00
Live	8.16 ± 6.35	10.1 ± 2.40	22.1 ± 11.9	22.3 ± 5.40	7.68 ± 1.94	0.00 ± 0.00
CH_4 loss rate (mg l^{-1} day^{-1})						
Control	0.04 ± 0.04	0.01 ± 0.02	0.07 ± 0.01	0.04 ± 0.02	–	–
Live	0.24 ± 0.24	0.45 ± 0.12	0.61 ± 0.08	0.61 ± 0.08	–	–
NH_4^+ loss rate (mg l^{-1} day^{-1})						
Control	–	–	–	–	0.05 ± 0.54	0.00 ± 0.00
Live	–	–	–	–	0.36 ± 0.19	0.22 ± 0.39
DO loss rate (mg l^{-1} day^{-1})						
Control	0.19 ± 0.55	0.62 ± 0.15	0.39 ± 0.20	0.28 ± 0.20	0.00 ± 0.00	0.15 ± 0.10
Live	2.04 ± 0.98	1.28 ± 0.29	1.52 ± 0.26	1.01 ± 0.30	0.00 ± 0.00	0.16 ± 0.03
DIC production rate (mg l^{-1} day^{-1})						
Control	3.24 ± 2.19	5.76 ± 1.38	4.31 ± 0.74	0.00 ± 0.05	–	–
Live	24.1 ± 8.06	15.1 ± 1.59	11.1 ± 7.11	19.5 ± 4.03	–	–
NO_x production rate (mg l^{-1} day^{-1})						
Control	–	–	–	–	0.18 ± 0.04	0.00 ± 0.00
Live	–	–	–	–	0.56 ± 0.86	0.00 ± 0.00

[a] Rates calculated over a 96 h period

The initial TCE degradation rates (d[TCE]/dt) during cycles TA1 and TA2 provide evidence of toxicity as no TCE degradation was observed in cycle TA2. The initial rates of [TCE], [NH_4^+] and [DO] loss, and [NO_x] production were not significantly different between treatments or with cycles (one-way RM ANOVAs, treatment effect, cycle effect, $P > 0.05$, Table 1). The inhibitory effect of TCE on ammonia oxidation has been attributed to two potential mechanisms: (a) AMO affinity for TCE may be equivalent (Hyman et al. 1995) or greater (Ely et al. 1995) in comparison to its affinity for ammonia, and (b) exposure of ammonia oxidizers to TCE (Kocamemi and Cecen 2005) or TCE epoxide (a cometabolism byproduct) (Rasche et al. 1991) may cause substantial cellular injury and AMO inhibition. The specific mechanism of AMO inhibition in the present study is unclear, yet there was evidence of toxicity in the precipitous decrease in ammonia oxidation rate that immediately followed TCE addition (compare d[NH_4^+]/dt in cycle AE2 vs. cycle TA1; refer to Table S1 in Online Resource, and Table 1). It is possible that toxic TCE epoxide induced significant damages to the enzyme system. After exposure to TCE, nitrifying cells are often slow to recover and show low rates of ammonia-oxidizing activity (Hyman et al. 1995). In this study, the root-associated ammonia oxidation did not recover or regained their activity. A longer enrichment period with greater initial [NH_4^+] may have been helpful in developing larger biomass to withstand toxic exposure of TCE. In summary, TCE cometabolism by methane oxidizers was clearly more effective than by ammonia oxidizers within the same initial root-associated microbial community under similar experimental conditions.

Conclusions

This bench-scale investigation clearly demonstrated that methane and ammonia oxidation can be facilitated by the naturally-occurring microorganisms that are associated with wetland plant (*Carex comosa*)

roots. Methane oxidation developed faster than ammonia oxidation during the enrichment period, possibly due to greater initial population of methane oxidizers with the roots, or their ability to better compete with other (heterotrophic) microorganisms. Methane oxidizers demonstrated significant TCE degradation reproducibly. For a shorter enrichment period (2 weeks), methane oxidizers were effective in TCE degradation at bench scale without an inhibitory effect due to TCE degradation on methane oxidation. However, a rapid and complete inhibition of ammonia oxidation was observed under similar experimental conditions in the nitrifying system. Such inhibition of ammonia oxidation may be attributed to a greater sensitivity of ammonia oxidizers towards TCE or its degradation product (TCE epoxide). Follow-up studies may include the role of TCE and substrate levels (methane and ammonia) on TCE degradation and toxicity will be determined. The results presented here provide key evidence for oxidative cometabolic TCE degradation in vegetated wetlands that has direct implications for the natural attenuation of TCE in impacted aquatic environments.

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