Journal of Hazardous Materials 187 (2011) 488–494

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

ETBE (ethyl *tert* butyl ether) and TAME (*tert* amyl methyl ether) affect microbial community structure and function in soils

Johanna Bartling[a,*], Jürgen Esperschütz[b], Berndt-Michael Wilke[a], Michael Schloter[b]

[a] Berlin University of Technology, Institute of Ecology, Franklinstr. 29, 10587 Berlin, Germany
[b] Helmholtz Zentrum München, Department of Terrestrial Ecogenetics, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

ARTICLE INFO

Article history:
Received 21 October 2010
Received in revised form
21 December 2010
Accepted 13 January 2011
Available online 20 January 2011

Keywords:
Oxygenates
Ecotoxicity
Functional redundancy
Soil ecology
PLFA
Gasoline additives

ABSTRACT

Ethyl *tert* butyl ether (ETBE) and *tert* amyl methyl ether (TAME) are oxygenates used in gasoline in order to reduce emissions from vehicles. The present study investigated their impact on a soil microflora that never was exposed to any contamination before. Therefore, soil was artificially contaminated and incubated over 6 weeks. Substrate induced respiration (SIR) measurements and phospholipid fatty acid (PLFA) analysis indicated shifts in both, microbial function and structure during incubation. The results showed an activation of microbial respiration in the presence of ETBE and TAME, suggesting biodegradation by the microflora. Furthermore, PLFA concentrations decreased in the presence of ETBE and TAME and Gram-positive bacteria became more dominant in the microbial community.

1. Introduction

Oxygenates such as ethyl *tert* butyl ether (ETBE), *tert* amyl methyl ether (TAME), and methyl *tert* butyl ether (MTBE) are widely used as gasoline additives in order to reduce emissions of carbon monoxide, ozone, and unburned hydrocarbons [1]. In the last ten years, in European countries ETBE has become more and more important, since it can be synthesized from bioethanol [2]. TAME is primarily used in Finland and Italy [3,4]. Gasoline additives enter the environment during storage, distribution, and handling of the pure chemical, but also as a constituent of oxygenated gasoline. Not surprisingly since the 1990's, oxygenates have been found in the subsoil. They have mainly been detected in groundwater, but also in surface waters in the USA, Denmark, Belgium, Great Britain, in the Netherlands, and in Germany among others [5–12].

Since ETBE and TAME are highly water soluble, a rapid leaching into the saturated zone is generally assumed [5,13–15]. Furthermore ETBE and TAME are highly volatile and may be emitted from contaminated soils to the atmosphere. Consequently, little impact on soil ecology had been expected so far. However, recent studies revealed clear effects of oxygenates on soil fauna and (micro-) flora. An [16] and An and Lee [17] observed restless behaviour of earthworms and significant changes in their morphology, particularly coiling, blooding, swelling and fragmentation into two pieces, after exposition to MTBE. In addition, reduced seed germination, seedling growth, and roots elongation have been reported for wild oats, sweet corn, wheat, and lettuce [18,19]. Also the soil microflora can be negatively influenced by MTBE as shown by Shahidi Bonjar [20] and Bartos et al. [21] in growth inhibition tests with pure cultures using *Fusarium solani*, *Erwinia carotovora*, *Streptomyces* spp. and *Pseudomonas* sp. as model organisms. In a recent study, we investigated the consequences of ETBE and TAME on nitrification rates at least in the first two weeks of soil contamination [22]. However, detailed data on the mode of action of oxygenates on microbial community structure and function in soil is still missing.

Therefore, the aim of the present study was to examine the impact of TAME and ETBE on the microbial community structure and function in soils without any contamination history. For this purpose, a standard soil was artificially contaminated with TAME and ETBE, respectively, and incubated for 42 d. At selected time points, respiration measurements and phospholipid fatty acid (PLFA) analysis were carried out to determine the microbial function and possible shifts in the community structure. According to Bartling et al. [22], we hypothesized a toxic effect on microbial communities linked with a reduction of microbial respiration activity (I). Those were assumed to be short-term effects followed by regression based on the high volatilization rates of oxygenates (II). Since biodegradation of TAME and ETBE by microorganisms that never had contact to any contamination with hydrocarbons

* Corresponding author. Tel.: +49 30 314 73682; fax: +49 30 314 736690.
E-mail address: johanna.bartling@tu-berlin.de (J. Bartling).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.01.058

has rarely been reported [23], we did not expect visible microbial degradation of TAME and ETBE (III).

2. Experimental

2.1. Test soil, contamination procedure and sampling

Experiments were carried out using "standard test soil 2.3" from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Germany). The soil has been characterized as follows: 9.4% clay, 31.9% silt; 58.7% sand (w/w); organic C content 0.98% (w/w), pH ($CaCl_2$) 6.4; maximum water holding capacity 34.4% at a bulk density of 1.3 kg l^{-1}. All experiments were performed in 500 ml glass flasks using 200 g m_d (dry mass) test soils, that has been adjusted to 50% of the maximum water holding capacity (WHC_{max}). Soil samples were contaminated with 5 ml (equivalent to 6.76 g) kg^{-1} m_d^{-1} ETBE (Merck, Germany), respectively 5 ml (equivalent to 6.41 g) kg^{-1} m_d^{-1} TAME (Sigma–Aldrich, Germany). These concentrations have been proven to affect soil microflora and fauna [16,22] and additionally, they reflect typical levels of contamination at field sites. Control soil samples received 5 ml water. Flasks were closed with screw taps and incubated at 20 ± 1 °C for 6 weeks. To ensure appropriate mixing, the flasks were shaken at 30 rpm in an overhead shaker during the first three days of incubation.

Soil samples for respiration measurements and PLFA analysis were taken 3, 7, 14, 28, and 42 d after contamination. For all treatments and incubation time points 4 replicates were prepared.

2.2. Respiration measurements

Soil respiration curves were measured according to ISO 17155 [24]. Briefly, soil samples equivalent to 20 g m_d were incubated in a respirometer at 20 ± 1 °C. The respiration rate was measured continuously as CO_2 production before and after addition of the substrate (composed of 80 g glucose, 13 g $(NH_4)_2SO_4$, and 2 g KH_2PO_4). After 24 h of incubation in the respirometer, basal respiration rate was constant in all treatments, so 0.2 g of the substrate mixture were added to each soil sample. In addition to the respiration curve progressions, the respiration parameters basal respiration (R_B) and substrate induced respiration (R_S) have been evaluated (Fig. 1). Respiration parameters were computed by SnoopPlotWin 2.x (PRW Electronics, Berlin, Germany).

2.3. PLFA analysis

PLFA analysis was based on Zelles et al. [25]. Aliquots equivalent to 20 g m_d were extracted with 125 ml methanol, 63 ml chloroform and 50 ml phosphate buffer (0.05 M, pH 7). After 2 h of horizontal shaking, 63 ml water and 63 ml chloroform were added for phase separation. After 24 h the water phase was removed and discarded. The total lipid extract was separated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI 2 g/12 ml; Bond Elut, Analytical Chem International, CA, USA). Phospholipids were separated into saturated (SATFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. A derivatisation of MUFA was performed prior to measurement to identify the position of the double bond (see Zelles et al. for details). PLFAs were analyzed as fatty acid methyl esters (FAMEs) on a gaschromatograph / mass spectrometry system (5973MSD GC/MS Agilent Technologies, Palo Alto, USA). FAMEs were separated on a polar column (BPX-70, SGE GmbH, Griesheim, Germany, 60 m × 0.25 mm × 0.25 ml, coated with 70% of cyanopropyl polysilphenylene–siloxane). The mass spectra of the individual FAME were identified by comparison with established fatty acid libraries (Solvit, CH 6500 – Luzern, Switzerland) using MSD Chemstation (Version D.02.00.237). For fatty acid description, standard nomenclature was used [26]. For data evaluation, PLFAs were classified into four major groups, Gram-positive bacteria (incl. actinomycetes), Gram-negative bacteria, fungi and microeukaryotes/protozoa according to the literature (Table 1).

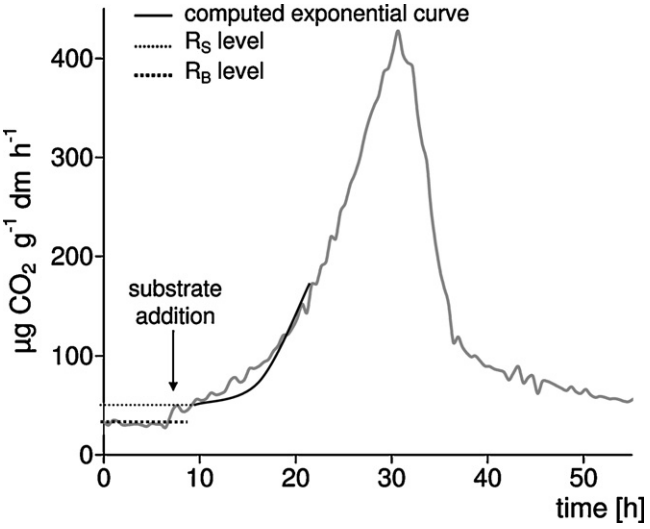

Fig. 1. Typical respiration curve with respiration parameters R_B and R_S.

2.4. Ether concentration measurements

Determination of the bioavailable fraction of ETBE and TAME was carried out after 3, 21, and 42 d of incubation using the method described by Bartling et al. [22]. In brief, soil samples equivalent to 5 g m_d and distilled water (1:2, w/v) were filled into 20 ml glass vials. The vials were closed immediately to prevent exorbitant volatilization. Samples were shaken for 24 h and stored at

Table 1
Classification of FA according to the given references.

Class	Underlying fatty acids	References
Gram-positive bacteria incl. actinomycetes	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 10Me17:0, 10Me18:0, br18:0, 10Me19:0, br20:0, br9,17:0	Gattinger [50] Leckie [51] Paterson et al. [52]
Gram-negative bacteria	cy17:0, cy19:0, 16:1ω5, 16:1ω9, 18:1ω7, 15:1?, 17:1ω8	Leckie [51] Paterson et al. [52]
Fungi	18:1ω9, 18:2ω6,9	Vestal and White [53]
Microeukaryotes/protozoa	n22:0, 18:3, 20:4	Gattinger [50] Leckie [51]
Unspecific	a14:0, n15:0, a16:0	Gattinger [50] Zelles [54]

Table 2
Ether concentrations after 3, 21, and 42 d of incubation; initial concentration at day 0: 6.41 mg g^{-1} m_d (TAME) and 6.76 mg g^{-1} m_d (ETBE); means of $n = 3 \pm$ standard deviation.

Days after contamination	TAME (mg g^{-1} m_d)	ETBE (mg g^{-1} m_d)
3	1.52 ± 0.15	2.14 ± 0.22
21	0.70 ± 0.26	0.56 ± 0.04
42	0.43 ± 0.22	0.08 ± 0.02

−15 °C until analysis. Ether concentrations were determined in the headspace of the glass vials by gas chromatography with flame ionization detector (GC-FID, Hewlett Packard 5890 Series II, Avondale, PA) equipped with a DB5 capillary column (0.25 mm × 30 m, J&W Scientific, Folsom, CA). The samples were preheated to 35 °C and transferred to the GC at 85 °C by the autosampler (PerkinElmer Headspace Sampler HS40, Waltham, USA). The initial GC temperature (40 °C) was hold for 6.5 min, increased with 40 °C min^{-1} for 4.0 min, finally hold at 200 °C for the remaining 5.5 min. Measurements were performed in triplicates which were considered as technical replicates for statistical analyses.

2.5. Statistics

All data were statistically analyzed using R 2.10.0 (The R Foundation for Statistical Computing 2009, ISBN 3-900051-07-0). Outliers were identified using the inter quartile range (IQR) according to Lorenz [27]. Values bigger than the upper quartile plus 1.5 × IQR, respectively smaller than the lower quartile minus 1.5 × IQR were not considered in further analyses. Data were log 10 transformed and analyzed using a Mann Whitney U test for comparing the means (one-sided, $\alpha = 0.2$).

3. Results

3.1. Ether concentration measurements

TAME and ETBE concentrations in the soils are presented in Table 2. Concentrations for both oxygenates decreased rapidly for more than 75% of initially added TAME respectively 60% of the added ETBE, even in the first 3 d of incubation. After 42 d, a TAME concentration of 0.43 mg g^{-1} m_d (7% of the initial concentration) was measured; for ETBE a concentration of 0.08 mg g^{-1} m_d (1% of the initial concentration) could be determined.

3.2. Respiration measurements

Respiration curves from the start and the end of the incubation period are presented in Fig. 2. Respiration curves of controls, ETBE contaminated, and TAME contaminated soils varied depending on the time of incubation.

At the beginning of the incubation period, in both contaminated soils the main exponential increase in the respiration rate started later compared to the control (Fig. 2A). However, in the ETBE contaminated soil samples obviously some microbial groups started respiration earlier, indicated by the visible shoulder 17 h after substrate addition, but reached the maximum respiration rate 6 h later compared to the soil samples that served as controls. The maximum respiration rate of 360 μg CO_2 h^{-1} g^{-1} m_d^{-1} was the highest in the ETBE contaminated soil samples. In TAME and ETBE contaminated soil samples, the respiration curves formed a so-called terrace (an elevated level of respiration rate) after the maximum respiration rate was reached.

After 42 d of incubation (Fig. 2B) in both, ETBE and TAME contaminated soil samples an exponential increase in respiration started 10 h after substrate addition whereas in the control an exponential increase was observed 4 h later. Furthermore, both maximum respiration rates (>420 μg CO_2 h^{-1} g^{-1} m_d) were higher compared to the beginning of the incubation as well as compared to the control (320 μg CO_2 h^{-1} g^{-1} m_d). Additionally after 42 d of incubation, the respiration curve of the control soil samples formed a shoulder before the maximum respiration rate was reached.

An evaluation of the respiration parameters R_B and R_S is presented in Fig. 3. Statistical analysis did not provide a level of significance better than $\alpha = 0.2$. At the beginning of the experiment, R_B was significantly lower in the control than in the contaminated soils (Fig. 3A). In the TAME and ETBE contaminated soils, R_B decreased after 14 d of incubation. After 42 d of incubation, the level of R_B was lowest in the ETBE contaminated soil samples and highest in the TAME contaminated soil samples. R_S was almost constant and on the same level in control and ETBE contaminated soils (Fig. 3B). In TAME contaminated soils, R_S remained on the same level until 14 d of incubation and then increased.

3.3. PLFA analysis

Results of the PLFA analysis are presented in Figs. 4 and 5. Again, statistical analysis did not provide a level of significance better than $\alpha = 0.2$. Overall total PLFA concentrations increased over the incubation period in all treatments. Nevertheless the total PLFA concentration was considerably lower in the contaminated soil samples compared to the control (Fig. 4), indicating a decreased microbial biomass in those samples.

Both PLFA, which have been used as indicators for Gram-negative bacteria and fungi were highly influenced by the contamination, indicated by the lower values compared to those from the control soil samples. This observation did not change over the whole incubation period (Fig. 5A). In contrast in the contaminated soil samples the absolute fatty acid concentrations (Fig. 5A) as well as the proportion of Gram-positive microorganisms increased from 3 d to 42 d (TAME contaminated soils) and from 3 d to 28 d (ETBE contaminated soils, Fig. 5B). Microeukaryotes were not affected by the contamination and were mainly influenced by the time of incubation as indicated by the concentration of the corresponding PLFA based indicators. The concentration of saturated fatty acids was constant in the control soil samples. In contrast in the contaminated soil samples, the concentration strongly increased until 28 d and slightly decreased afterwards, indicating the presence of microbial stressors at the beginning of the incubation period (data not shown).

4. Discussion

Respiration measurements clearly indicated an impact of TAME and ETBE on microbial community function. Exponential growth (i.e. the exponential slope in the respiration curve as defined in ISO 17155 [24]) started later in the contaminated soil samples at the beginning of incubation. It has been reported that prolonged lag-times may indicate a toxic effect of chemicals on the microbial communities [28–30]. The lag-time of microbial respiration (defined as time from substrate addition to start of exponential growth according to ISO 17155 [24]) may also reflect the quality of the previously available carbon source [31]. This is confirmed by the terrace formation (visible after the peak maximum in the TAME and ETBE related respiration curves), since that phenomenon has been linked to the utilization of an additive as additional carbon source in a previous study [28]. Furthermore, ETBE is considered to be more easily degradable compared to TAME, which could explain the differences in the respiration curve progressions, the early start of exponential growth and the higher respiration maximum [32–35].

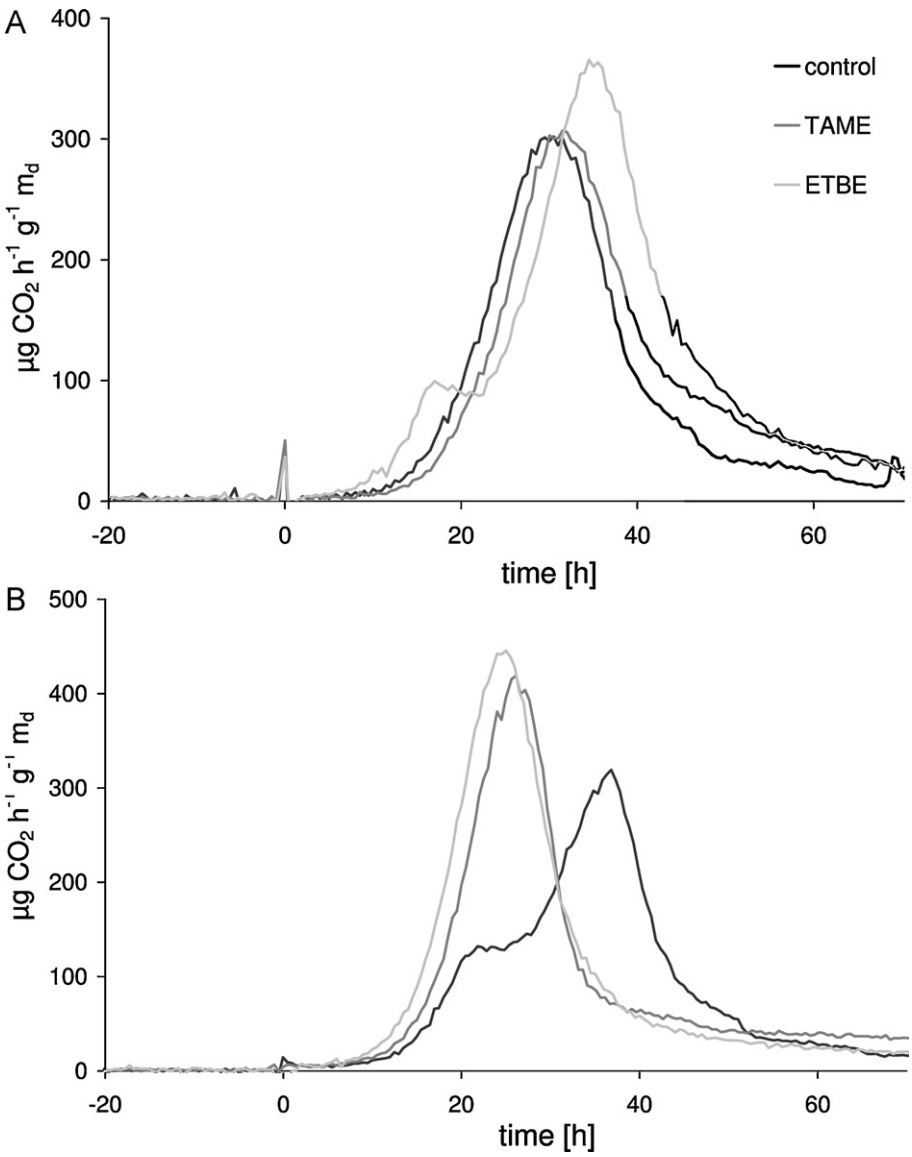

Fig. 2. Respiration curves of all treatments 3 d (A) and 42 d (B) after contamination; substrate addition at time zero.

Consistently, R_B was significantly higher in the presence of TAME or ETBE indicating an activation of the microbial community possibly as a carbon source [36–38]. R_B decreased during incubation and reached the level of the control after 42 d, probably because of decreasing ether concentrations in the soils. Due to the physicochemical properties of TAME and ETBE, only 7% and 1% of the initial concentrations remained in the soils after 42 d of incubation. These findings are consistent with volatilization rates from top soil reported in the literature [5,13]. The reason for the strong decrease is assumed to be mainly volatilization, since the incubation flasks were opened periodically to remove samples for analyses. However, as it is indicated by the results of the respiration measurements, biodegradation could be another reason, albeit to a lesser extend. Usually, the first step of biodegradation is the oxidation of the methyl respectively butyl moiety; therefore the first degradation products are *tert* amyl alcohol and *tert* butyl alcohol for TAME and ETBE, respectively [39,40]. The presence of both metabolites has been proven in our experiment by GC-FID measurements (data not shown), which confirms biodegradation in our study.

Despite the low concentrations of ETBE and TAME after 42 d of incubation, the influence of both chemicals on microbial activity was still visible. In the control soil samples, microbial activity overall declined. Consequently, a decelerated start of exponential growth and a lower respiration maximum indicated diminishing availability of easily degradable nutrients. In contrast, in the TAME and ETBA contaminated soil samples response to the substrate addition occurred earlier and maximum respiration rates exceeded those from the beginning of incubation. This indicated that ETBE and TAME maintained the microbial community active, particularly capable to adapt to glucose substrate utilization. Similarly, Degens [41] observed an accelerated turnover of organic carbon after addition of different organic substrates and suggested a modified metabolic state of the microbial community to be responsible.

Interestingly, R_S was almost constant over the incubation time, which might indicate that the microbial biomass did not change (ISO 14240-1 [42]). However, Wang et al. [43] observed a stronger correlation between R_S and substrate availability rather than between R_S and microbial biomass. Likewise, correlation between R_S and total extractable PLFA based microbial biomass is not necessarily strong [44]. This might explain the unchanged R_S values in the contaminated soil samples despite the clear decrease in microbial

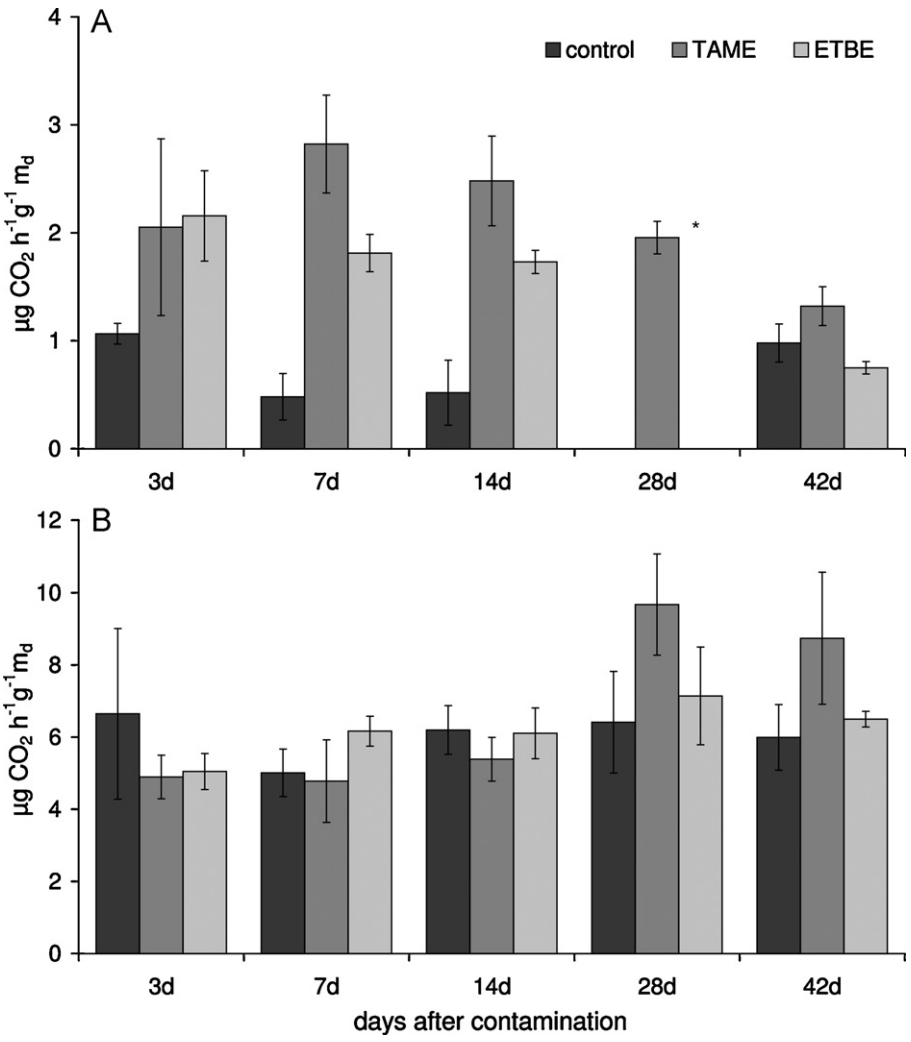

Fig. 3. Respiration parameters R_B (A) and R_S (B) ($n = 4$). Bars represent standard errors.

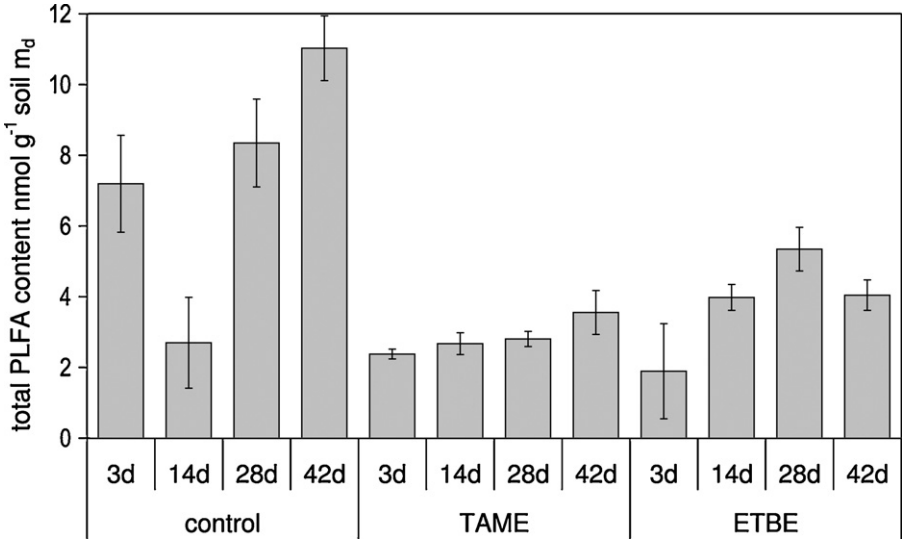

Fig. 4. Total PLFA content in soil samples from the different treatments 3 d 14 s, 28 d and 42 d after contamination during the incubation period ($n = 4$). Bars represent standard errors.

biomass in our experiment (Figs. 4 and 5). Therefore assuming the total PLFA concentration as an indicator for microbial biomass [45], the increased R_B was likely associated to an increased catabolism activity of the microbial community. A similar decrease in microbial biomass and a simultaneously increasing R_B in the presence of diesel have been reported by Peña et al. [46].

In addition, the decreased PLFA concentration and the increasing ratio of saturated to unsaturated fatty acids indicated a toxic

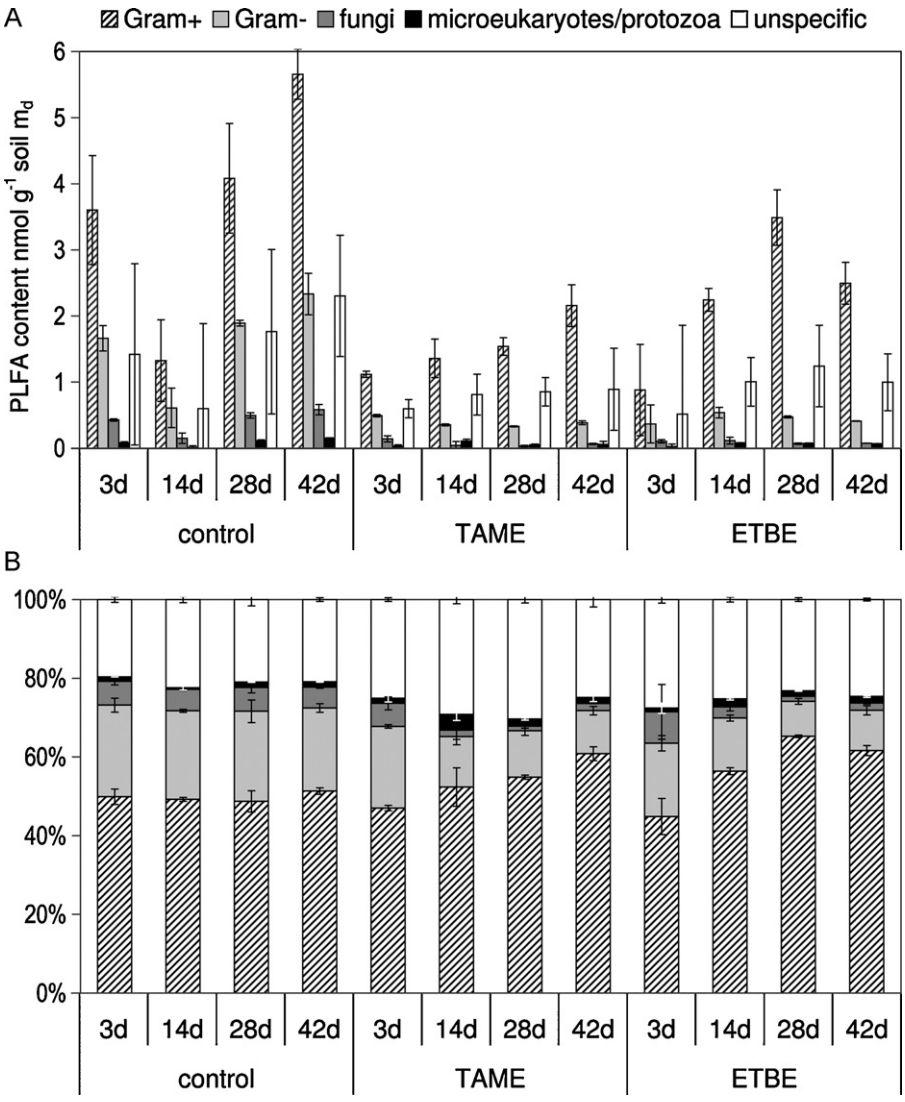

Fig. 5. Concentrations of indicator PLFA for selected microbial groups (A), and proportions of these groups (B) in different treatments during the incubation period. Bars represent standard errors.

effect of TAME and ETBE [47,48]. The sustainability of this effect in the contaminated soil samples over the whole experimental period is remarkable since the TAME and ETBE concentrations in the soils decreased. Focusing on the identified microbial groups, the results clearly indicated that mainly Gram-negative bacteria and fungi were negatively affected by TAME and ETBE. In contrast Gram-positive bacteria benefited from the disappearance of TAME and ETBE during incubation since the related PLFA concentrations and their proportion within all groups increased. This might be related to the more robust structure of the cell wall of Gram-positive compared to Gram-negative bacteria. Another reason might be the fact that many Gram-positve bacteria like bacilli are able to form spores, which are able to persist the toxic conditions in soil after contamination and can be transformed easily in active cells again, once the stressor disappears. Taking into account the biodegradation stated above, mainly Gram-positive bacteria might be capable to utilize TAME and ETBE as a carbon source in the present experimental setup, which is in line with previous studies where several representatives of Gram-positive bacteria have been identified to biodegrade oxygenates [32,33,49–51].

5. Conclusions

In the present study, the toxicity of gasoline additives like TAME and ETBE on the microbial community structure could be proven. However, reduced PLFA concentrations were not necessarily linked to a reduction of microbial respiration activity (hypothesis I). Accordingly, this indicated that microbial degradation of TAME and ETBE by microorganisms in soils without any contamination history occurred and our hypothesis (III) could not be corroborated. In addition, we were able to show that effects on structure and function of microbial communities maintained during the incubation period, so the high volatilization rates hardly attenuated the impact of TAME and ETBE (hypothesis II).

In future research will be needed to target microbial groups that are involved in TAME and ETBE degradation and responsible for functional redundancy in soils using isotope labelled compounds. In addition, experiments in the field have to be performed to confirm the data obtained in our study and to assess the impact of changing climatic conditions for the degradation of TAME and ETBE.

Acknowledgements

We are thankful to Ruth See for excellent technical assistance, as well as Wolfgang Rotard and Mandy Görnitz (both Berlin University of Technology) for providing gas chromatography equipment.

References

[1] R. Cataluña, R. da Silva, E.W. de Menezes, R.B. Ivanov, Specific consumption of liquid biofuels in gasoline fuelled engines, Fuel 87 (2008) 3362–3368.
[2] R.M. Villamañán, J.J. Segovia, M.C. Martína, D. Vega-Mazaa, C.R. Chamorroa, M.A. Villamañán, Thermodynamics of fuels with a bio-synthetic component (IV): (vapor + liquid) equilibrium data for the ternary mixture (ethyl 1,1-dimethylethyl ether + 1-hexene + toluene) at T = 313.15 K, J. Chem. Thermodyn. 41 (2009) 189–192.
[3] H.P.H. Arp, T.C. Schmidt, Air–water transfer of MTBE, its degradation products, and alternative fuel oxygenates: the role of temperature, Environ. Sci. Technol. 38 (2004) 5405–5412.
[4] F. Ancillotti, V. Fattore, Oxygenate fuels: market expansion and catalytic aspect of synthesis, Fuel Process. Technol. 57 (1998) 163–194.
[5] R.C. Borden, D.C. Black, K.V. McBlief, MTBE and aromatic hydrocarbons in North Carolina stormwater runoff, Environ. Pollut. 118 (2002) 141–152.
[6] CDHP, California Department of Public Health: MTBE: regulations and drinking water monitoring results. http://www.cdph.ca.gov/certlic/drinkingwater/Pages/MTBE.aspx, accessed 05/09 (2008).
[7] R.K. Juhler, G. Felding, Monitoring methyl tertiary butyl ether (MTBE) and other organic micropollutants in groundwater: results from the Danish National Monitoring Program, Water Air Soil Pollut. 149 (2003) 145–161.
[8] D. Moreels, L. Bastiaens, F. Ollevier, R. Merckx, L. Diels, D. Springael, Evaluation of the intrinsic methyl tert-butyl ether (MTBE) biodegradation potential of hydrocarbon contaminated subsurface soils in batch microcosm systems, FEMS Microbiol. Ecol. 49 (2004) 121–128.
[9] C. Guitart, J.M. Bayona, J.W. Readman, Sources, distribution and behaviour of methyl tert-butyl ether (MTBE) in the Tamar Estuary, UK, Chemosphere 57 (2004) 429–437.
[10] A. van Wezel, L. Puijker, C. Vink, A. Versteegh, P. de Voogt, Odour and flavour thresholds of gasoline additives (MTBE, ETBE and TAME) and their occurrence in Dutch drinking water collection areas, Chemosphere 76 (2009) 672–676.
[11] J. Klinger, C. Stieler, F. Sacher, H.J. Branch, MTBE (methyl tertiary-butyl ether) in groundwaters: monitoring results from Germany, J. Environ. Monit. 4 (2002) 276–279.
[12] C. Baus, H.-J. Brauch, M. Fleig, F. Sacher, Auswirkungen des Einsatzes von MTBE und ETBE auf Oberflächengewässer und Trinkwasserversorgung im Rheineinzugsgebiet - Eine IAWR-Studie, DVGW-Technologiezentrum Wasser. http://www.iawr.org/docs/publikation_sonstige/080711_mtbe-iawr.pdf; accessed 05/09 (2008).
[13] M. Matthies, Exposure assessment of environmental organic chemicals at contaminated sites: a multicompartment modelling approach, Toxicol. Lett. 140 (2003) 367–377.
[14] R. Gonzalez-Olmos, M. Iglesias, Study of fuel oxygenates solubility in aqueous media as a function of temperature and tert-butyl alcohol concentration, Chemosphere 71 (2008) 2098–2105.
[15] R. Gonzalez-Olmos, M. Iglesias, J.M. Goenaga, J.M. Resa, Influence of temperature on thermodynamic properties of methyl t-butyl ether (MTBE) plus gasoline additives, Int. J. Thermophys. 28 (2007) 1199–1227.
[16] Y.J. An, Assessing soil ecotoxicity of methyl tert-butyl ether using earthworm bioassay; closed soil microcosm test for volatile organic compounds, Environ. Pollut. 134 (2005) 181–186.
[17] Y.J. An, W.M. Lee, Comparative and combined toxicities of toluene and methyl tert-butyl ether to an Asian earthworm *Perionyx excavatus*, Chemosphere 71 (2008) 407–411.
[18] Y.J. An, D.H. Kampbell, M.E. McGill, Toxicity of methyl tert-butyl ether to plants (*Avena sativa*, *Zea mays*, *Triticum aestivum*, and *Lactuca sativa*), Environ. Toxicol. Chem. 21 (2002) 1679–1682.
[19] M. Vosahlikova, T. Cajthaml, K. Demnerova, J. Pazlarova, Effect of methyl tert-butyl ether in standard tests for mutagenicity and environmental toxicity, Environ. Toxicol. 21 (2006) 599–605.
[20] G.H. Shahidi Bonjar, Potential ecotoxicological implication of methyl tert-butyl ether (MTBE) spills in the environment, Ecotoxicology 13 (2004) 631–635.
[21] P. Bartos, M. Balazs, I. Kiss, Z. Bihari, O. Kelemen, I. Mecs, Toxic effect of methyl tert-butyl ether on growth of soil isolate *Pseudomonas veronii* T1/1, World J. Microbiol. Biotechnol. 24 (2008) 875–878.
[22] J. Bartling, M. Schloter, B.M. Wilke, Ethyl tert-butyl ether (ETBE) and tert-amyl methyl ether (TAME) can affect distribution pattern of inorganic N in soil, Biol. Fertil. Soils 46 (2010) 299–302.
[23] J. Landmeyer, P. Bradley, D. Trego, K. Hale, J. Haas, MTBE, TBA, and TAME attenuation in diverse hyporheic zones, Ground Water 48 (2010) 30–41.
[24] ISO 17155. Bodenbeschaffenheit - Bestimmung der Abundanz und Aktivität der Bodenmikroflora mit Hilfe von Atmungskurven. Beuth Verlag, Berlin (2002).
[25] L. Zelles, Q.Y. Bai, R. Rackwitz, D. Chadwick, F. Beese, Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structure, Biol. Fertil. Soils 19 (1995) 115–123.
[26] A. Frostegård, A. Tunlid, E. Bååth, Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals, Appl. Environ. Microbiol. 59 (1993) 3605–3617.
[27] R.J. Lorenz, Grundbegriffe der Biometrie, vol. 4, Spektrum Akademischer Verlag, Heidelberg, 1996 (Auflage).
[28] J. Bartling, A. Kotzerke, M. Mai, J. Esperschütz, F. Buegger, M. Schloter, B.-M. Wilke, Microbial community structure and function during abnormal curve development of substrate induced respiration measurements, Chemosphere 77 (2009) 1488–1494.
[29] B.M. Wilke, M. Mai, A. Gattinger, M. Schloter, P. Gong, Effects of fresh and aged copper contaminations on soil microorganisms, J. Plant Nutr. Soil Sci. 168 (2005) 668–675.
[30] A. Eisentraeger, G. Maxam, J.-P. Rila, W. Dott, A Stepwise Procedure for Assessment of the Microbial Respiratory Activity of Soil Samples Contaminated with Organic Compounds, Ecotoxicol. Environ. Safe. 47 (2000) 65.
[31] C. Palmborg, A. Nordgren, E. Bååth, Multivariate modelling of soil microbial variables in forest soil contaminated by heavy metals using wet chemical analyses and pyrolysis GC/MS, Soil Biol. Biochem. 30 (1998) 345–357.
[32] F. Fayolle, G. Hernandez, F. Le Roux, J.P. Vandecasteele, Isolation of two aerobic bacterial strains that degrade efficiently ethyl t-butyl ether (ETBE), Biotechnol. Lett. 20 (1998) 283–286.
[33] G. Hernandez-Perez, F. Fayolle, J.P. Vandecasteele, Biodegradation of ethyl t-butyl ether (ETBE), methyl t-butyl ether (MTBE) and t-amyl methyl ether (TAME) by *Gordonia terrae*, Appl. Microbiol. Biotechnol. 55 (2001) 117.
[34] R.H. Müller, T. Rohwerder, H. Harms, Degradation of fuel oxygenates and their main intermediates by *Aquincola tertiaricarbonis* L108, Microbiology 154 (2008) 1414–1421.
[35] C. Malandain, F. Fayolle-Guichard, T. Vogel, Cytochromes P450-mediated degradation of fuel oxygenates by environmental isolates, FEMS Microbiol. Ecol. 72 (2010) 289–296.
[36] A. Kotzerke, S. Sharma, K. Schauss, H. Heuer, S. Thiele-Bruhn, K. Smalla, B.-M. Wilke, M. Schloter, Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig-manure, Environ. Pollut. 153 (2008) 315.
[37] R. Margesin, F. Schinner, Bioremediation of diesel–oil-contaminated alpine soils at low temperatures, Appl. Microbiol. Biotechnol. 47 (1997) 462–468.
[38] J.A. Marin, T. Hernandez, C. Garcia, Bioremediation of oil refinery sludge by landfarming in semiarid conditions: influence on soil microbial activity, Environ. Res. 98 (2005) 185–195.
[39] T. Rohwerder, U. Breuer, D. Benndorf, U. Lechner, R.H. Muller, The alkyl tert-butyl ether intermediate 2-hydroxyisobutyrate is degraded via a novel cobalamin-dependent mutase pathway, Appl. Environ. Microbiol. 72 (2006) 4128–4135.
[40] F.E. Ahmed, Toxicology and human health effects following exposure to oxygenated or reformulated gasoline, Toxicol. Lett. 123 (2001) 89–113.
[41] B.P. Degens, Microbial fuctional diversity can be influenced by the addition of simple organic substrates to soil, Soil Biol. Biochem. 30 (1998) 1981–1988.
[42] ISO 14240-1: Soil quality—Determination of soil microbial biomass – Part 1: Substrate induced respiration method, International Organization of Standardization, Genf (1997).
[43] W.J. Wang, R.C. Dalal, P.W. Moody, C.J. Smith, Relationships of soil respiration to microbial biomass, substrate availability and clay content, Soil Biol. Biochem. 35 (2003) 273–284.
[44] V.L. Bailey, A.D. Peacock, J.L. Smith, H. Bolton Jr., Relationships between soil microbial biomass determined by chloroform fumigation-extraction, substrate-induced respiration, and phospholipid fatty acid analysis, Soil Biol. Biochem. 34 (2002) 1385–1389.
[45] A. Frostegård, E. Bååth, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fertil. Soils 22 (1996) 59–65.
[46] W. Peña, C. Trasar-Cepeda, F. Gil-Sotres, M.C. Leirós, Modification of the degradative capacity of a soil artificially contaminated with diesel, Chemosphere 67 (2007) 1057.
[47] S. Isken, J.A.M. de Bont, Bacteria tolerant to organic solvents, Extremophiles 2 (1998) 229–238.
[48] H.J. Heipieper, G. Neumann, S. Cornelissen, F. Meinhardt, Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems, Appl. Microbiol. Biotechnol. 74 (2007) 961–973.
[49] K. Mo, C.O. Lora, A.E. Wanken, M. Javanmardian, X. Yang, C.F. Kulpa, Biodegradation of methyl t-butyl ether by pure bacterial cultures, Appl. Microbiol. Biotechnol. 47 (1997) 69–72.
[50] J. Purswani, C. Pozo, M. Rodriguez-Diaz, J. Gonzalez-Lopez, Selection and identification of bacterial strains with methyl-*tert*-butyl ether, ethyl-*tert*-butyl ether, and *tert*-amyl methyl ether degrading capacities, Environ. Toxicol. Chem. 27 (2008) 2296–2303.
[51] C.A. Smith, K.T. O'Reilly, M.R. Hyman, Characterization of the initial reactions during the cometabolic oxidation of methyl tert-butyl ether by propane-grown *Mycobacterium vaccae* JOB5, Appl. Environ. Microbiol. 69 (2003) 796–804.
[52] E. Paterson, T. Gebbing, C. Abel, A. Sim, G. Telfer, Rhizodeposition shapes rhizosphere microbial community structure in organic soil, New Phytologist 173 (2007) 600–610.
[53] J.R. Vestal, D.C. White, Lipid Analysis In Microbial Ecology - Quantitative Approaches To The Study Of Microbial Communities, Bioscience 39 (1989) 535–541.
[54] L. Zelles, Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review, Biol. Fertil. Soils 29 (1999) 111–129.