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Demethylation of Dimethylarsinic Acid and Arsenobetaine in Different Organic Soils

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Abstract Methylation and demethylation of arsenic may change substantially the toxicity and mobility of arsenic in soils. Little is known about demethylation of organic arsenic species in organic soils. We incubated dimethylarsinic acid (DMA) and arsenobetaine (AsB) in soils and aqueous soil extracts from a forest floor and fen, in order to investigate demethylation processes. Incubations were conducted at 5°C in the dark under oxic or anoxic conditions. Arsenobetaine demethylated rapidly in all soil extracts with half-lives of 3.6–12 days, estimated from first order kinetic. Demethylation of DMA was relatively slow with half-lives of 187 and 46 days in the forest floor extracts and oxic fen extracts, respectively. In comparison, DMA was stable for 100 days in anoxic fen extracts. The apparent half-lives were much shorter in soils for DMA (1.3–12.6 days) and AsB (0.5–1.9 days) than in soil extracts, suggesting also irreversible AsB and DMA adsorption to soils beside demethylation. An unknown arsenic species and DMA were detected as metabolites of AsB demethylation. The results indicate rapid demethylation of AsB probably via the pathway AsB → Dimethylarsenoylacetate → DMA, followed up by slow demethylation of DMA → monomethylarsonic acid → inorganic As species.

Keywords arsenic · methylation · demethylation · forest floor · fen

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1 Introduction

Arsenic (As) is one of the most toxic elements and is ubiquitous in the environment. Concentrations of As in non-polluted soils are typically well below 10 μg g^{-1}. Elevated concentrations in soils is due to both anthropogenic and natural inputs. Anthropogenic sources include mining and smelting processes besides application of As-based insecticides, herbicides, fungicides, algicides, sheep dips, wood preservatives, dyestuffs, feed additives and chemicals for the eradication of tapeworm in sheep and cattle (Adriano, 2001). Geochemical sources of As-contaminated soils include As-rich bedrock material as As easily substitutes for Si, Al or Fe in silicate minerals (Bhumbla & Keefer, 1994).

Arsenic occurs mainly as inorganic species in soils (Geiszinger, Gössler, Kühnelt, Francesconi, & Kosmus, 1998; Helgesen & Larsen, 1998). Arsenate (As(V)) predominates under oxic conditions, whereas most of As may be present as arsenite (As(III)) under anoxic conditions (Sadiq, 1997). Organic As species

are usually the minor component in soils with monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO) as the most usually found organic As species (Helgesen & Larsen, 1998; Takamatsu, Aoki, & Yoshida, 1982; Tlustoš, Gössler, Száková, & Balík, 2002). In comparison, the occurrence of arsenobetaine (AsB) was only shown in some cases (Geiszinger, Gössler, & Kosmus, 2002; Huang & Matzner, 2006; Pongratz, 1998). Chemical forms of As influence their toxicity and mobility. In general, inorganic As species are more toxic and less mobile than organic As species (Chiu & Hering, 2000; Lafferty & Loeppert, 2005; Mandal & Suzuki, 2002). Thus, methylation and demethylation may have substantial influence on toxicity and biogeochemical cycling of As in soils.

Demethylation of AsB in the aquatic environment has been broadly investigated (Hanaoka, Nakamura, Ohno, Tagawa, & Kaise, 1995a; Hanaoka, Uchida, Tagawa, & Kaise, 1995b; Hanaoka, Ueno, Tagawa, & Kaise, 1989, Hanaoka, Yamamoto, Kawashima, Tagawa, & Kaise, 1988). However, knowledge about AsB demethylation in soils is rare. For DMA, demethylation was indicated as the major pathway related to formation of arsine (Gao & Burau, 1997). The rate of DMA demethylation in soils increased with increased soil moisture and temperatures, but addition of cellulose depressed DMA demethylation (Gao & Burau, 1997). Under field conditions, the half life of DMA in soils was estimated to be 20 days. Demethylation of DMA was followed by the formation of As(V) and small amounts of MMA (Woolson, Aharonson, & Iadevaia, 1982). Organic As species may reach to forest soils via throughfall, probably as consequence of biomethylation of As in the environment (Mukai, Ambe, Muku, Takeshita, & Fukuma, 1986). The forest floor is the biologically most active part of forest soils and may act as a natural biogeochemical barrier that suppresses the percolation of As species and thus strongly accumulates deposited As (Matschullat, 2000). Wetland soils are of special importance for transformation reactions in the case of As: large amounts of organic As species were found in soil solution of a fen soil in the growing season which disappeared in the dormant season, indicating the potential methylation and demethylation (Huang & Matzner, 2006). Arsenobetaine and DMA were shown to be the major organic As species in the forest floor and wetland soils.

The aim of the present study was to determine the role of organic soils, including the forest floor (Oa) and fen soils, in As demethylation. Two most dominant organic As species, namely AsB and DMA found in our forest floor and fen, were spiked to soils and soil extracts for incubation. We hypothesize that DMA and AsB demethylated rapidly in the forest floor and fen soils under oxic condition but much more slowly in fen soils under anoxic conditions. Demethylation of AsB should occur via the pathway: AsB → TMAO → DMA → MMA → inorganic As species.

2 Materials and Methods

2.1 Site Description

The sampling site "Coulissenhieb" is located in the Lehstenbach catchment (4.2 km^2 size) in the German Fichtelgebirge Mountains, at an elevation of 700 to 880 m a.s.l. at 50° 08′ N, 11° 52′ E. The catchment is dominated by Norway spruce (*Picea abies* [L.] Karst.), and 30% of the area are covered with wetland soils of bog and fen type. Upland soils are mainly Dystric Cambisols and Haplic Podzols (FAO classification) of sandy to loamy texture, developed from deeply weathered granitic bedrock. The forest floor is a well stratified mor type of approximately 9 cm depth with total As concentrations up to 24 μg g^{-1}. The fen at "Schlöppnerbrunnen I" is classified as Fibric Histosol (FAO) covered with patches of *Sphagnum* mosses and Norway spruce trees. The concentrations of total As range between 3.3 and 4.2 μg g^{-1} and vary little with depth in the fen soil (Huang & Matzner, 2006). The fen is slightly acidic with soil $pH_{(H_2O)}$ of approximately 5 and the carbon content is close to 40% down to 70 cm depth.

2.2 Reagents and Standards

Arsenate (As(V)), arsenite (As(III)) and dimethylarsinic acid (DMA) were purchased from Merck. Arsenobetaine (AsB) was obtained from Fluka and monomethylarsonic acid (MMA), arsenocholine (AsC), trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA) as iodide from Argus Chemicals, Italy. De-ionized water used throughout the work was purified in a Milli-Q system (Millipore, Milford, MA).

Individual stock solutions (50 mg As L^{-1}) of As (III), As(V), MMA, DMA, AsB, AsC, TMAO and TETRA were prepared in Milli-Q water and stored at 4°C in the dark. A multi-compound working solution with a total concentration of 40 μg As L^{-1} was prepared before each use by dilution of the stock solutions with Milli-Q water.

2.3 Sampling and Sample Preparation

The soil material used for incubation was sampled at the "Coulissenhieb" site in summer 2004. The forest floor (Oa) was taken as 2 kg mixture from an 2 m^2 area. Fen materials were taken from 10–20 and 40–50 cm depth as 1.5 kg mixture of three samples at 1 m distance from one another. All samples were sieved to 2 mm and directly used for incubation.

Slow rates of irreversible contaminant sorption may occur in soils and sediments after an initial rapid phase, which is often called as "aging effect" (Hatzinger & Alexander, 1995). The "aging effect" may lead to overestimation of demethylation of organic As species in soil incubations because the extractability of the contaminant decreases with time. Thus, additional incubations experiments were conducted with soil extracts to avoid the "aging effect". For incubations in soil extracts, the forest floor (Oa) and fen soils were sampled in "Coulissenhieb" in August 2005 and "Schlöppenbrunnen I" in May 2006 in the same way as described above. The method of soil extraction was modified from Riis, Lorbeer, and Babel (1998). Each soil sample was mixed with 0.1% pyrophosphate solution with 1:3 (v/v) soil-to-solution ratio. The mixture was then sonicated for 10 min and subsequently shaken for 2 h at room temperatures. After 10 min centrifugation (99 G), the supernatants were taken for incubation. The biochemical activities and cell counts in soil extracts were expected to be about 50% of the corresponding soils. This estimation was based on the results from Riis et al. (1998).

For analysis of C content, 30 mg of soil material was analyzed by CHN–O Rapid, Elementar, Germany. Soil pH was detected using a pH electrode in a 1:2.5 soil/0.01 M $CaCl_2$ solution mixture. For determining concentrations of total As in soils, parts of the sieved soils were freeze-dried and then grounded. Samples (0.5 g) were first digested with 3 ml nitric acid (65%) and 1 ml hydrochloric acid (30%) by High Pressure Accelerated Solvent (HPA-S, Anton Paar, Austria). In the 3-step program, a first heating to 80°C, is followed by heating to 170°C and finally to 270°C, lasting for 90 min. The supernatant was then filtered to 0.45 μm with membrane filter, diluted to 257 ml with Milli-Q water for further analysis with ICP-MS (Agilent 7500c, Japan).

Total As concentrations, pH and carbon contents in the investigated organic soils are shown in Table 1.

2.4 Incubation Experiments

Anoxic incubations with fen soil and fen soil extract from 40–50 cm depth were accomplished by flushing N_2 in bottles. For oxic incubations with Oa and 10–20 cm fen soils and their extracts, the air in the headspace was refreshed, when the CO_2 concentrations in bottles exceeded 5%. All incubations were carried out in gastight borosilica bottles (120 ml, Schott) in the dark at 5°C. At least three replicates were prepared for each sampling date.

Generally, 10–20 g of soils were used for incubation. For demethylation experiments, DMA was spiked to 100 and 73 ng As g^{-1} and AsB was spiked to 250 and 730 ng As g^{-1} to fen soils and to the Oa, respectively. The spiked amounts were kept as low as possible but also high enough to allow detection during incubation. The spiked DMA and AsB were first diluted with 0.2 ml Milli-Q water and dispensed homogeneously onto the soils. The As species were analyzed by merging the incubated soils with 10–20 ml Milli-Q water and subsequent sonication for 15 min. After two step centrifugation (1,200 and 8,800 G), 1 ml supernatant was filtered to 0.2 μm for HPLC-ICP-MS analysis. Since the demethylation of AsB in different environmental mediums is generally rapid (e.g., Khokiattiwong, Gössler, Pedersen, Cox, & Francesconi, 2001), the first analysis of water extractable As species was conducted 3 h after spiking.

Table 1 Concentrations of total arsenic, pH values, water and carbon content of the soil sample

	pH_{CaCl_2}	C (%)	Total As (μg g^{-1})
Oa	3.1	43	13.2
Fen $_{10–20\ cm}$	4.1	43	3.60
Fen $_{40–50\ cm}$	4.2	45	3.44

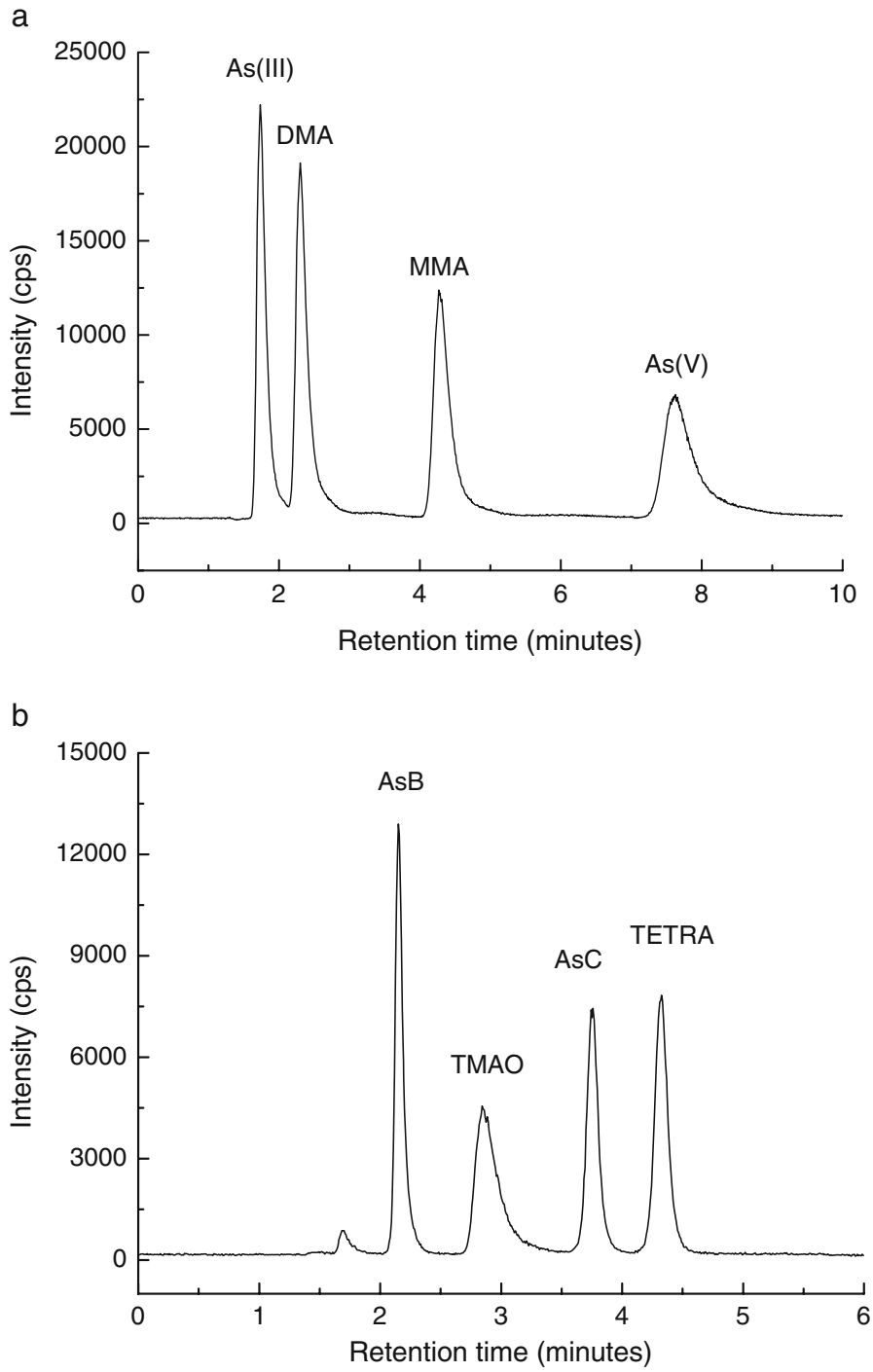

Fig. 1 **a** Anion exchange and **b** cation exchange chromatogram (HPLC-ICP-MS) of arsenic standards each 5 μg As L^{-1} (100 μl injection). As(III): arsenite, DMA: dimethylarsinic acid, MMA: methylarsonic acid, As(V): arsenate, AsB: arsenobetaine, TMAO: trimethylarsine oxide, AsC: arsenochorine, TETRA: tetraarsonium ion

For the incubations with soil extracts, 1 μg As as DMA or as AsB was spiked to 50 ml soil extracts. Analysis of As species was done using HPLC-ICP-MS after filteration to 0.2 μm.

2.5 Arsenic Speciation

A high performance liquid chromatograph (HPLC) instrument (BIOTEK Instruments, USA), consisting

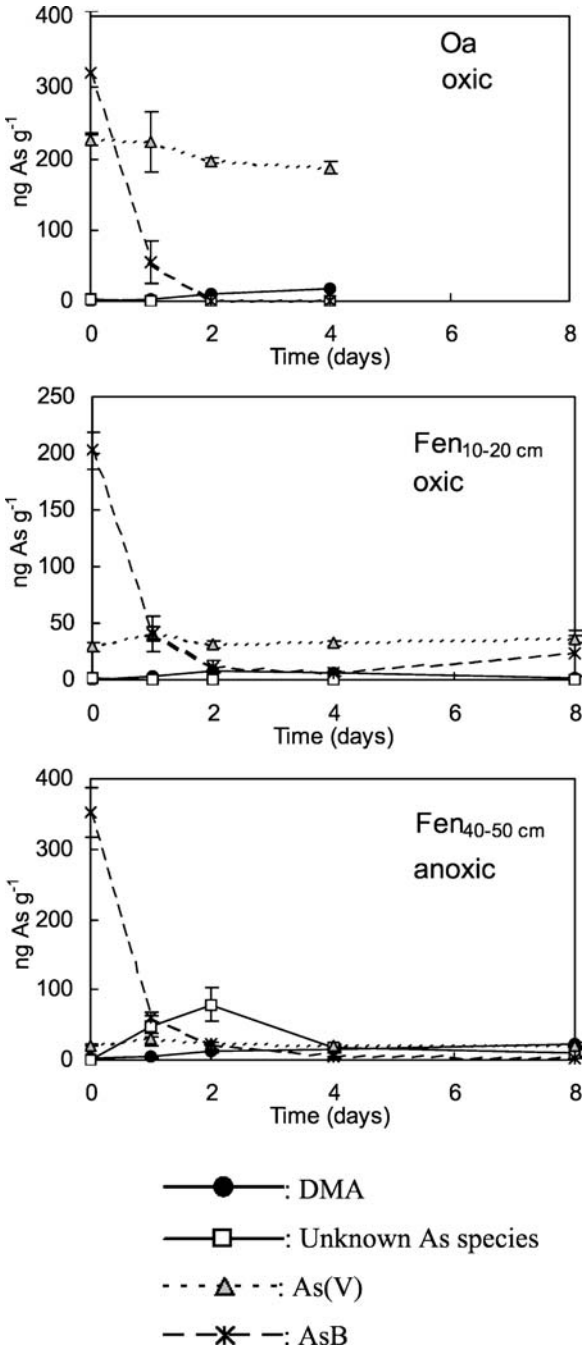

Fig. 2 Water extractable concentrations of spiked arsenobetaine (AsB) incubated in Oa and fen soil at 5°C in the dark under oxic or anoxic conditions. Mean values and SDs of three replicates are shown

of a gradient pump (System 525), capillary PEEK tubing (0.25 mm i. d.) and a 200-µl injection loop (Stainless Steel), and a HPLC autosampler 465 (Kontron Instruments, Germany) was connected to an ion exchange column and coupled to an ICP-MS (Agilent 7500c, Japan), equipped with a concentric nebulizer (Glass Expansion, Australia) and a Scott-type glass spray chamber.

Speciation of As(III), As(V), MMA and DMA were performed on a PRP-X100 anion-exchange column from Hamilton Company (Reno, NV, USA) at 40°C with a mobile phase (1.5 ml min^{-1}) of 20 mM (NH_4) H_2PO_4 pH 5.6 adjusted with aqueous NH_3 (Francesconi, Visoottiviseth, Sridokchan, & Gössler, 2002). Speciation of AsB, AsC, TMAO and TETRA was conducted on a Zorbax 300-SCX cation-exchange column at 30° C with mobile phase (1.5 ml min^{-1}) of 20 mM pyridine pH 2.6 adjusted with phosphoric acid. Arsenic species were quantified by external calibration with standard solutions of As(III), As(V), MMA, DMA, TMAO, TETRA, AsB and AsC (Fig. 1).

3 Results

The loss of water extractable AsB during soil incubation under both oxic and anoxic conditions was at least 50% in the first day (Fig. 2). The concentrations of water extractable AsB decreased further during incubation, however, to a less extent. The half-lives of AsB calculated with first order kinetic based on the results from soil incubations ranged from 0.3 to 0.4 days (Table 2). Increasing concentrations of DMA and of an unknown As species (up to 80 ng As g^{-1}) were observed in all soil incubations spiked with AsB, while the concentrations of AsB declined (Fig. 2). The retention time of the unknown As species in the anion exchange chromatogram was close to that of MMA. However, spiking MMA in the extract showed clearly that the unknown As species is not MMA (Fig. 3). The concentrations of water extractable DMA also decreased during soil incubations but less steeply compared to those of AsB (Fig. 4). The estimated half-lives of DMA based on the results from soil incubations were between 1.3–7.7 days. Trace amounts of MMA (up to 3.3 ng As g^{-1}) were detected in all soil incubations. Large losses of water extractable As species were shown during soil incubations of DMA and AsB compared to the starting amounts (up to 70 and 90%, respectively) (Figs. 2 and 4).

Table 2 First order degradation rate constants and half-lives of arsenobetaine and dimethylarsinic acid spiked in organic soils and extracts of organic soils

	Soils			Soil extracts		
	Apparent k (day^{-1})	R^2	Apparent $t_{1/2}$ (days)	k (day^{-1})	R^2	$t_{1/2}$ (days)
Dimethylarsinic acid						
Oa	0.09	0.85	7.7	0.004	0.83	187
Fen $_{10–20\ cm}$	0.26	0.89	2.67	0.007	0.87	46
Fen $_{40–50\ cm}$	0.55	0.66	1.26	–	–	–
Arsenobetaine						
Oa	1.81	0.99	0.38	0.006	0.99	12.4
Fen $_{10–20\ cm}$	1.86	0.98	0.37	0.19	0.85	3.6
Fen $_{40–50\ cm}$	1.69	0.99	0.41	0.08	0.93	8.7

–:not determined

A decrease of AsB concentrations during incubation of soil extracts of Oa and fen soils was also observed (Fig. 5), but was not as rapid as in soil incubations. The calculated half-lives ranged from 3.6 to 12.4 days, which were an order of magnitude higher than those from the soil incubation (Table 2). The half-lives of AsB in fen extracts were higher under anoxic than under oxic conditions. About 75% of demethylated AsB in Oa and fen extracts could be found as DMA and as an unknown As species under oxic incubation (Fig. 5). The As(V) concentrations remained constant during the incubation. Under anoxic incubation, 10% of demethylated AsB was found as DMA and as an unknown As species and the As(V) concentrations decreased. The concentrations of DMA in anoxic fen extracts varied little during 100 days incubation (Fig. 6). However, DMA underwent demethylation in Oa and fen extracts under oxic conditions. Small amounts of MMA were detected during oxic incubations. The estimated half-lives of DMA calculated from first order kinetic were 187 and 46 days, respectively (Table 2). An almost complete transformation of DMA into As(V) was found in the soil extract incubations (Fig. 6).

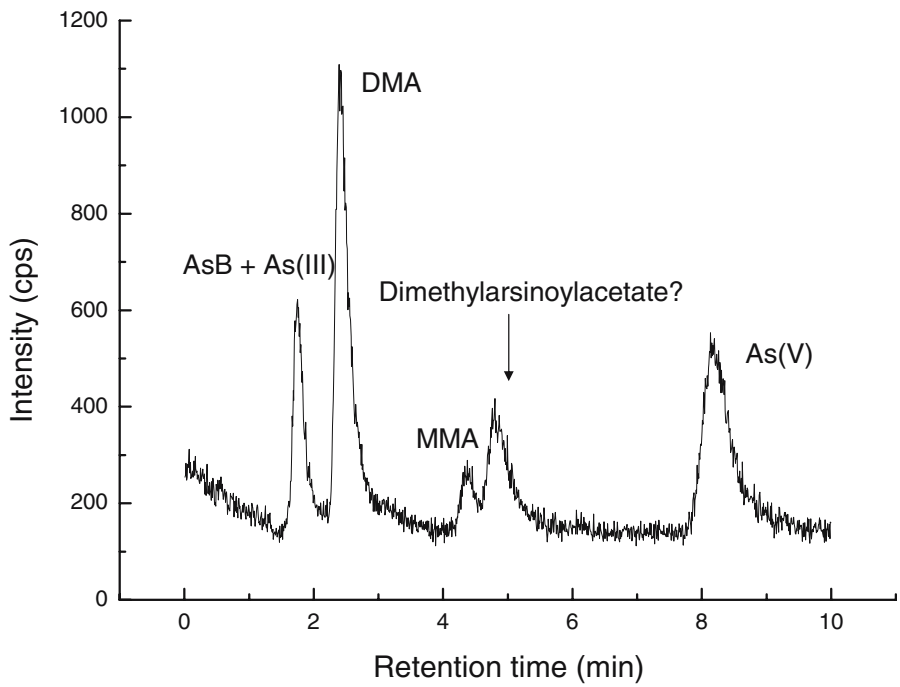

Fig. 3 Anion exchange chromatogram of metabolites from arsenobetaine demethylation in fen soils. Monomethylarsonic acid (MMA) was spiked to 0.5 μg As L^{-1} in the extract

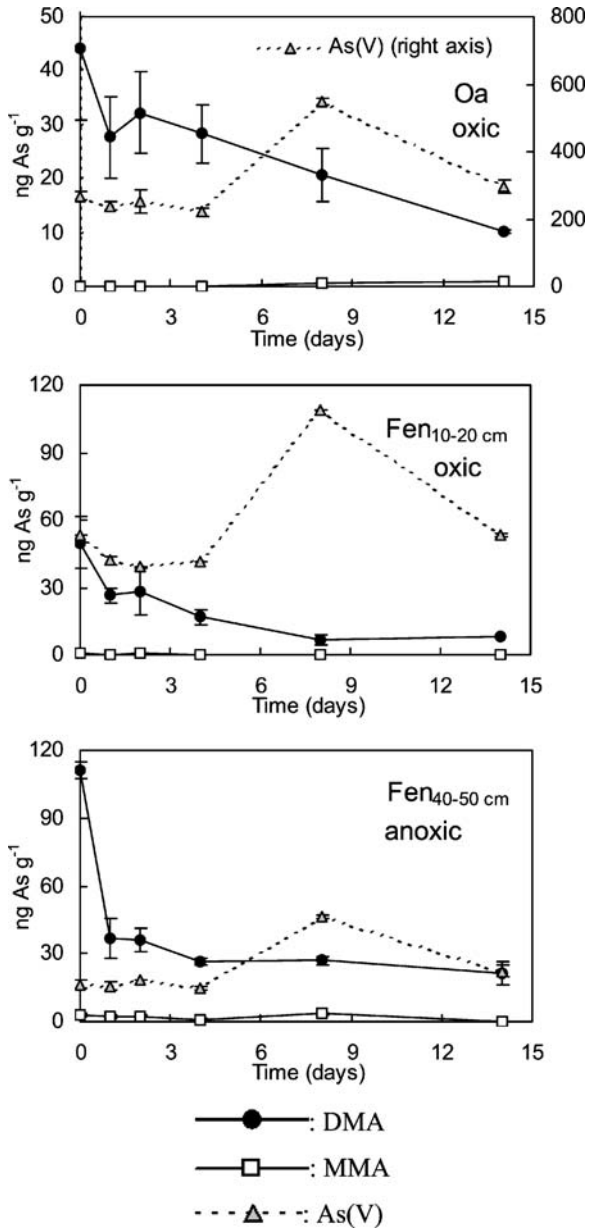

Fig. 4 Concentrations of spiked dimethylarsinic acid (DMA) incubated in Oa and fen soil at 5°C in the dark under oxic or anoxic conditions. Mean values and SDs of three replicates are shown

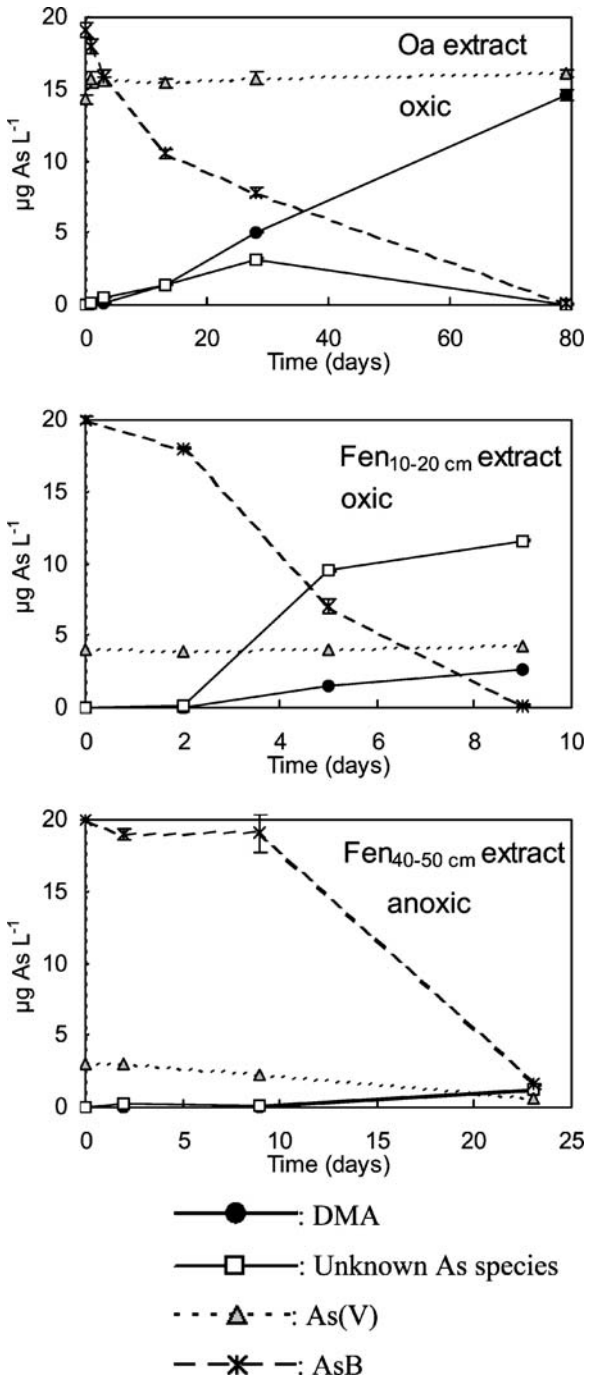

Fig. 5 Concentrations of arsenic species in soil extracts spiked with AsB. Each incubation was spiked with 20 µg As L^{-1} AsB. Mean values and SDs of three replicates are shown

4 Discussion

The estimated half-lives of DMA and AsB were one to two orders of magnitude higher in incubations with soil extracts than in soils (Table 2). Since the microbial activity in the soil extracts were estimated to be about 50% of the corresponding soils (Riis et al., 1998), the decrease of water extractable DMA and AsB in soils seems to be affected by the irreversible adsorption of DMA and AsB to organic soils ("aging effect"). The "aging effect" may be enhanced by generally low

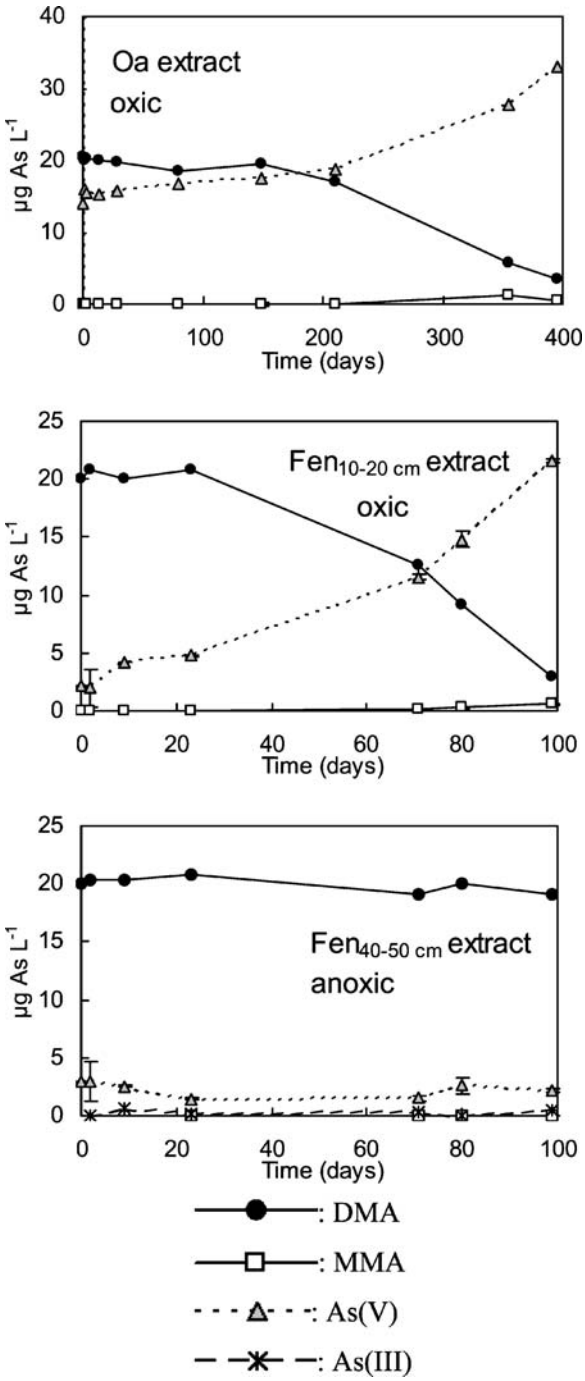

Fig. 6 Concentrations of arsenic species in soil extracts spiked with DMA. Each incubation was spiked with 20 µg As L^{-1} of DMA. Mean values and SDs of three replicates are shown

recoveries of As species using water extraction, which recovers typically <5% of total As in soils (Francesconi & Kuehnelt, 2004). Probably, the true half-lives of DMA and AsB in the forest floor and fen soils should range between the values estimated from incubation in soils and soil extracts. Demethylation of DMA and AsB in Oa and fen extracts was evidenced by both decreased concentrations of DMA and AsB and apparently increased concentrations of metabolites e.g., DMA for AsB and MMA for DMA demethylation (Figs. 5 and 6). In comparison, small amounts of metabolites related to the spiked DMA and AsB were observed during incubations in Oa and fen soils (Figs. 2 and 3). Demethylation of DMA and AsB cannot easily be seen from the soil incubations. Therefore, we preferred to discuss the AsB and DMA demethylation based on the results from the incubation in soil extracts.

The half-life of DMA was about 20 days in mineral soils (Matapeake silt loam) under field conditions during the growing period (Woolson et al., 1982). The half-lives of DMA obtained from our incubations of soil extracts were apparently higher. This may be due to the lower microbial activity in soil extracts than in soils (ca. 50%, Riis et al., 1998). Additionally, our incubation was conducted at 5°C. Gao and Burau (1997) reported the rates of DMA demethylation five times higher at 25°C than at 5°C. Besides, high amounts of organic matter may increase the value of DMA half-life. Woolson et al. (1982) indicated that addition of organic matter may increase of DMA half-lives from 20 to 31 days in mineral soils. Demethylation of DMA in fen extracts was slower under anoxic than under oxic conditions. Inorganic As species were the dominant metabolites of DMA and MMA was only occasionally detectable in trace amounts. It is therefore suggested that DMA first demethylated to MMA and then to inorganic As species in organic soils. Demethylation of MMA seems to be much more rapid than DMA so that little MMA could be observed during incubations. An almost complete transformation of DMA to MMA and inorganic As was found in the incubations of soil extracts. This reflects the hypothesis that demethylation of DMA to inorganic As species was the major pathway of DMA (Gao & Burau, 1997; Sierra-Alvarez et al., 2006; Woolson et al., 1982).

For the first time, the demethylation of AsB in terrestrial soils was demonstrated. The demethylation of AsB in extracts of all organic soils was generally quick and complete under formation of DMA and an unknown As species as metabolites. The demethylation of AsB has been previously shown in seawater (Hanaoka, et al., 1995a), marine sediments (Hanaoka, et al., 1988; 1995b), sea weed (Hanaoka, et al., 1989),

animal tissues (Kaise et al., 1998) and microflora (Devesa et al., 2005). The proposed demethylation pathway starts with the cleavage of the carboxymethyl-As bond of AsB, generating TMAO, with subsequent cleavage of methyl-As bonds to form DMA and MMA. The process ended with the formation of inorganic As (Hanaoka, Tagawa & Kaise, 1992). Khokiattiwong et al. (2001) showed an alternative pathway for AsB demethylation, which begins with the cleavage of methyl-As bonds, generating dimethylarsenoylacetate ($Me_2As(O)CH_2COO^-$) (DMAA) and then DMA. The demethylation of AsB in organic soils and extracts is more likely via the later pathway because no TMAO as a metabolite of AsB was found. The unknown metabolite seemed to be DMAA as indicated by the quite similar behaviour of retention in the anionic chromatography in Jenkins et al. (2003).

Dimethylarsenoyl acetate and DMA seem the major metabolites of AsB in our short-term incubations of soil extracts. Constant concentrations of inorganic As species concentrations in soil extracts may reflect the much slower rates of DMA demethylation. However, a loss of 15−20% As from the extracts of Oa and fen in oxic incubations and 70% of As from the extracts of fen in anoxic incubations were indicated. Loss of As in incubations may be due to formation of As species undetectable with our HPLC-ICP-MS method, sorption of As species to particles (Cullen & Reimer, 1989) or incorporation of AsB into microorganisms (Khokiattiwong et al., 2001), which needs to be further investigated. Undetectable As species here may include volatile As hydrides (Woolson & Kearney, 1973), thioarsenite (Hollibaugh et al., 2005; Wilkin, Wallschläger, & Ford, 2003) and organic As(III) species (Hasegawa, 1997; Sohrin, Matsui, Kawashima, Hojo, & Hasegawa, 1997). The formation of thioarsenite in incubations seems of little importance, since thioarsenite occurs only in highly reducing and sulphide rich waters (Hollibaugh et al., 2005; Wilkin et al., 2003). Organic As(III) species were mostly found as intermediates of As methylation in humans and rats (Mandal, Ogra, & Suzuki, 2001; Okina et al., 2004; Styblo, Del Razo, & Vega, 2000). In the environment, organic As(III) species were seldom detected and the concentrations were very low (<0.2 nM, Hasegawa, 1997; Sohrin, Matsui, Kawashima, Hojo, & Hasegawa, 1997). If organic As(III) species play a significant role during demethylation of organic As species in soils is an open question.

Arsenobetaine is seldom detected in natural soils probably due to its rapid demethylation. In forest ecosystems, AsB in soils may originate from throughfall (Huang & Matzner, 2006). The rapid AsB demethylation to DMA and high stability of DMA may lead to accumulation of DMA in the forest floor and fen. While methylation of As is generally regarded as detoxification for microorganisms (Turpeinen, Pantsar-Kallio, Haggblom, & Kairesalo, 1999), transformation of AsB to DMA may represent an elevating toxicological risk of As in the forest floor and fen. Nevertheless, the mobility (or bioavailability) of AsB and DMA in organic soils is not known, and we are not able to evaluate the potential ecotoxicological effect of AsB demethylation in the forest floor and fen.

5 Conclusion

The results of our incubation experiments point to the rapid demethylation of AsB in the forest floor and fen, probably via the pathway AsB → DMAA → DMA. In comparison, DMA as a metabolite of AsB may demethylate much slower via the pathway DMA → MMA → inorganic As species.

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