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Impact of organic carbon and iron bioavailability on the magnetic susceptibility of soils

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Abstract

Microorganisms are known to couple the degradation of hydrocarbons to Fe(III) reduction leading to the dissolution and (trans)formation of Fe minerals including ferro(i)magnetic Fe minerals such as magnetite. The screening of soil magnetic properties, in particular magnetic susceptibility (MS), has the potential to assist in locating and assessing hydrocarbon (e.g. gasoline) contamination in the environment. In order to evaluate this, it must be understood how changes in soil geochemistry and hydrocarbon input impact MS. To this end, we incubated microcosms with soils from six different field sites anoxically and followed the changes in soil MS. In parallel we simulated hydrocarbon (i.e., gasoline) contamination in the same soils under anoxic conditions. We found that in microbially active microcosms both with or without added gasoline, average changes in MS of 6.9 ± 2.6% occurred, whereas in sterile controls the changes were less than 2.5% demonstrating that microbial metabolism played a major role in the (trans)formation of ferro(i)magnetic minerals. The microcosms reached stable MS values after a few weeks to months in four out of the six soils showing an increase in MS while in two soils the MS decreased over time. After stable MS values were reached, further addition of labile organic carbon (i.e., lactate/acetate) did not lead to further changes in MS, but the addition of Fe(III) oxyhydroxides (ferrihydrite) led to increases in MS suggesting that the changes in MS were limited by bioavailable Fe and not by bioavailable organic carbon. In the control experiments without carbon amendment, we observed that natural organic matter was mobilized from the soil matrix by water or microbial growth medium (0.33-0.47 mL/g field moist soil) added to the microcosms, and that this mobilized organic matter also stimulated microbial Fe metabolism and thus also led to a microbially driven change in MS. This study shows that changes in MS after an increase of the amount of bioavailable organic carbon can occur in a variety of soils. It also suggests that whether MS increases or decreases depends on the initial MS of the soil and the extent of the MS change seems to depend upon the amount of bioavailable Fe(III).

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1. INTRODUCTION

Crude oil and its products such as gasoline and diesel fuel are used worldwide. Soil and sediment contamination by these compounds represents a severe environmental threat (Readman et al., 1992; Masak et al., 2003; Zachara et al., 2004; Mendelssohn et al., 2012). For effective remediation of such sites, the contamination must be located and the spatial extent of the affected area assessed. Soil samples are usually taken from the potentially contaminated area and analyzed in the laboratory, however, this is time consuming and cost intensive. Therefore, rapid and inexpensive methods for assessment of hydrocarbon contamination in the field are necessary.

Measurement of soil magnetic susceptibility (MS) has been used to localize anthropogenic heavy metals in soils based on the fact that heavy metals and magnetic phases (mostly magnetite) are emitted from identical sources (combustion processes, steel industries, mining activities, traffic) and have similar transport pathways into and in the environment (Petrovský and Ellwood, 1999). Since MS can be measured within seconds in the field, this parameter can be used for fast and cost effective surveys of large areas. MS describes how strong a substance is magnetized in an external magnetic field. Diamagnetic materials (e.g. quartz, water) have a small negative MS, whereas paramagnetic minerals (e.g. siderite, ferrihydrite) and antiferromagnetic minerals with spin-canting (hematite) or defect moments (goethite) have a small positive MS. Ferromagnetic elements (e.g. metallic Fe) and ferrimagnetic minerals (e.g. magnetite, maghemite, greigite) have a very high or moderately high (e.g. pyrrhotite) positive MS (Dunlop and Özdemir, 1997). For simplicity the term ferro(i)magnetic minerals is used in this study and refers to ferrimagnetic minerals and antiferromagnetic minerals with spin-canting or defect moments. Although crude oil and its products such as gasoline have a low or even negative MS (Ivakhnenko and Potter, 2004), the measurement of soil MS has also the potential to serve as a proxy for the presence of hydrocarbons.

Ferro(i)magnetic phases are known to form in oil as secondary products during oil biodegradation (McCabe et al., 1987). Additionally, crude oil components like n-alkanes and polycyclic aromatic hydrocarbons can be degraded by soil microorganisms (Hamamura et al., 2006; Borch et al., 2010) including different Fe(III)-reducing microorganisms (Lovley et al., 1989; Lovley and Anderson, 2000). Fe(III)-reducers were shown to be able to reduce poorly crystalline ferrihydrite as well as more crystalline Fe(III) minerals such as goethite, hematite, and magnetite (for reviews see Kappler and Straub, 2005; Weber et al., 2006; Konhauser et al., 2011). Depending on the geochemical conditions present during Fe(III) reduction (e.g. pH, presence of carbonate, sulfide and phosphate) and depending on the Fe mineral transformation pathway (reductive dissolution, dissolution-reprecipitation or solid-state conversion), different Fe-phases can form. Ferrihydrite was shown for example to be converted into Fe(II)-carbonate, -sulfide, -phosphate or goethite as well as mixed Fe(II)-Fe(III) minerals such as magnetite or green rusts (Roden and Zachara, 1996; Fredrickson et al., 1998; Hansel et al., 2005; Borch et al., 2007; Piepenbrock et al., 2011; Amstaetter et al., 2012). In turn, Fe(II) can be oxidized by anaerobic and aerobic Fe(II)-oxidizing microorganisms (Kappler and Straub, 2005; Weber et al., 2006; Konhauser et al., 2011) which can also lead to the formation of magnetite (Chaudhuri et al., 2001; Jiao et al., 2005; Dippon et al., 2012) or green rust (Pantke et al., 2012).

Since some Fe(III)-reducers are able to metabolize hydrocarbons, the input of hydrocarbons into soils has a direct influence on microbial Fe(III) reduction and indirectly (via Fe(II) formation) on microbial Fe(II) oxidation. Additionally, hydrocarbons, especially lipophilic ones, are toxic for many microorganisms due to their interaction with microbial membranes (Sikkema et al., 1995) and thus hydrocarbon input into soils can also decrease microbial activity including Fe-metabolizing microorganisms. Therefore, hydrocarbon input may lead to changes in soil MS by changing Fe mineralogy including ferro(i)magnetic minerals. As a consequence, similar to heavy metal contamination hydrocarbon contamination may also be localized rapidly by screening soil MS in the presumably contaminated area in comparison to MS values at uncontaminated reference sites.

Only a few studies have tried to correlate the amount of hydrocarbons in soils with soil MS (see references in Schumacher, 1996). Guzman et al. (2011) and Aldana et al. (2011), studied the magnetic signature of oil fields from Venezuela and identified magnetite and Fe-sulfides (e.g. greigite) as the main magnetic phases causing increased MS values in oil wells. In two recent field studies, we also observed increased MS values in hydrocarbon contaminated soils (Rijal et al., 2010, 2012). However, no detailed studies exist that answer the key questions whether microbial activity is involved in the changes of soil MS after hydrocarbon input and how the changes in MS are influenced by the geochemical conditions of the soil. Therefore, we incubated soils from different field sites anoxically in laboratory microcosm experiments, simulated hydrocarbon contamination, and followed the change of soil MS over time. The objectives of this study were (i) to determine the importance of microbial processes for changes in MS and (ii) to determine the influence of geochemical conditions, including the amount of bioavailable organic carbon and bioavailable Fe, on the extent and temporal development of the changes in MS.

2. METHODS

2.1. Field sites, soil sampling and soil pre-treatment

Soil samples from six different field sites were collected. The field sites were chosen due to their minimal anthropogenic influence especially regarding combustion pollution that is known to release ferro(i)magnetic particles. The first five sampling sites Holzgerlingen (HG), Waldenbuch (Wabu), Fraeulinsberg (FB), Allemendwald (AW) (all four grassland) and Schoenbuch (Sbu) (forest) are located in Southwest Germany. At the grassland sites the top ~20 cm of soil was sampled including the sward, whereas the forest soil Sbu was sampled without leaf litter. During

a field study (Rijal et al., 2012), a sixth soil sample was collected in the Haenigsen area (Northeast of Hanover, Northern Germany), a region where crude oil is leaking naturally to the surface. For the microcosm experiments presented here a sample was taken in this region from an uncontaminated surface soil of farmland (Hclean). Large organic particles (e.g. plant matter) were removed from the soils either with tweezers (HG, Wabu, FB, AW, Hclean) or by pressing the field moist soil through a 2 mm sieve (Sbu). Soils were stored for up to four months in plastic bags at 4 °C in the dark before use. Soil stored at 4 °C that did not receive any amendments is named "original soil" in the following text.

2.2. Experimental setup of microcosms

In order to determine the processes influencing soil MS after addition of organic carbon, three microcosm experiments with six soils and three different carbon amendments (no carbon, lactate and acetate, and gasoline) were set up in 60 mL glass bottles under anoxic conditions (Table 1). Six different soils were used to determine if the geochemistry of soils had an influence on the rate or extent of MS change. Setups without carbon amendment served as a negative control to quantify the change of soil MS by the incubation of the soil alone. Gasoline (95 octane) containing a diverse mixture of aliphatic and aromatic hydrocarbons was added as model compound representing a hydrocarbon contamination arising from spills. Bottles with lactate/acetate addition served as control for setups containing easily biodegradable organics.

In order to determine the impact of microbial activity for the changes in soil MS, two sets of microcosms were set up, one with sterile soil and one with non-sterile soil with at least two replicates per setup. For setups with sterile soil, the soil was autoclaved (121 °C, 25 min, 1 bar). All solutions added to the soils were sterile and anoxic.

Either microbial growth medium (for composition see Straub et al., 2005) or high-purity water (to determine if the nutrients present in the medium had an influence on the MS changes) was added to the autoclaved and non-autoclaved soil (5–7 mL to 15–21 g of soil; see Table 1). The medium was buffered at pH 7 with 20 mM sodium bicarbonate. The headspace of the bottles was exchanged with N_2:CO_2 (90:10) to obtain anoxic conditions. Microcosms with three different carbon amendments were set up: (i) no carbon source added, (ii) lactate/acetate (each 15 mmol/L; referring to the volume of added microbial growth medium or water), and (iii) unleaded gasoline (3.60 μL/g field moist soil) obtained from a gasoline station. The lactate/acetate concentrations were chosen based on a previous study that indicated stimulation of Fe(III) reduction at these concentrations (Porsch et al., 2010). Bottles were closed with viton stoppers. Microcosms were homogenized directly after preparation and weekly thereafter by shaking for a few seconds on a vortexer. The microcosms were incubated at 28 °C in the dark. MS was measured weekly, until the MS was constant, then every other week.

In order to examine whether carbon or Fe limitation was responsible for cessation of changes in MS, selected microcosms were used for two additional experiments (Table 1). Since only two to four replicate bottles were available per setup, different soils were chosen for these two experiments. The importance of carbon limitation for the cessation of changes in MS, was determined by adding lactate/acetate (both 15 mmol/L, referring to the volume of added microbial growth medium) again to the corresponding setups of soils HG and Wabu (experiment I) after 14.5 weeks of incubation. In order to determine if bioavailable Fe(III) limitation led to cessation of changes in MS, microcosms with soil Hclean containing medium and no additional carbon (experiment II) were amended with ferrihydrite after 51 weeks of incubation. Soil Hclean was selected as this soil had the lowest total Fe (Fe_{tot}) concentration as determined by XRF (see below). Ferrihydrite was chosen since it is considered to be a source of bioavailable Fe(III) and magnetite is one of the possible products of its reduction (Porsch et al., 2010). 2 mL of a 0.5 M ferrihydrite suspension (synthesized according to Raven et al., 1998) was added to half of the parallel experiments in each setup. In order to follow changes in Fe mineralogy during incubation in more detail, sub-samples of sterile and microbial active microcosms without carbon amendment and with lactate/acetate addition with soil Sbu were analyzed by Mössbauer and EXAFS spectroscopy (see below) (Table 1, experiment III).

After incubation, one microcosm from each setup and experiment was opened under oxic conditions and sampled immediately for the quantification of Fe(II) and Fe_{tot} in the different Fe fractions (i.e., adsorbed plus Fe carbonates, poorly crystalline Fe, and crystalline Fe) and for the analysis of dissolved organic carbon, dissolved inorganic carbon, and organic acids.

2.3. Analytical methods

2.3.1. Soil analysis

The water content of the original soils was determined by drying the soil at 105 °C (Blume et al., 2000). The soil pH was measured 24 h after addition of 0.01 M $CaCl_2$ solution (Blume et al., 2000). All soils were finely ground and dried at 105 °C prior to the following analyses: Total organic carbon and total nitrogen was determined with a CN analyzer (Vario EL, Elementar, Germany) after carbonate removal with 1 M HCl. The $CaCO_3$ content was quantified by mixing soil with 1 M HCl and determining the consumed HCl by titration with 1 M NaOH. The total Fe and total sulfur content of the soils was quantified by X-ray fluorescence analysis (Bruker AXS S4 Pioneer X-ray spectrometer, Bruker AXS GmbH, Germany). Fe extractions from two sub-samples of the soil before and after incubation were performed according to Moeslund et al. (1994) and Roden and Zachara (1996) with a soil:extractant ratio (w:v) of 1:50. Prior to extraction, microcosms with soils FB, AW and Sbu were centrifuged (10 min, 2000 rpm) and the supernatant was removed for dissolved carbon and organic acid analyses (see below). The first sub-sample of soil was extracted with Na-acetate (pH 5) for 24 h and the second sub-sample with

Table 1
Experimental setup of microcosm experiments. Three microcosm experiments (I–III) with six soils were set up. The microcosms consisted of two sets: one with autoclaved (sterile setups) and one with non-autoclaved soil (microbially active setups). Either microbial growth medium or high-purity water was added as liquid. Microcosms were either not amended with organic carbon or amended with lactate/acetate or with gasoline.

Soil	Microcosm experiment/ replicates per setup	Measuring time (before + after 2nd C or Fe addition) [weeks]	Ratio Soil [g]:Liquid [mL]	Carbon source added to sets of sterile and microbially active microcosms					
				No carbon		Lactate/acetate (15 mM each)[a]		Gasoline (3.6 µL/g field moist soil)	
				Medium	Water	Medium	Water	Medium	Water
HG	I/2	11 + 10	15:5	x		x, 2nd C[b]		x	
Wabu	I/2	11 + 10	15:5	x		x, 2nd C[b]		x	
FB	II/4	46	15:7	x	x			x	
AW	II/4	46	15:7	x	x			x	
Hclean	II/4	46 + 73	15:7	x, Fe[c]	x			x, Fe[c]	
Sbu	III/4	48	21:7		x, MB + EXAFS[d]		x, MB + EXAFS[d]		x

[a] 15 mM final concentration in the microcosms referring to the volume of added liquid.
[b] After 14.5 weeks (MS values of microcosms were stable) lactate/acetate was added a second time.
[c] After 51 weeks (MS values of microcosms were stable) 2 mL of 0.5 M ferrihydrite suspension were added to two replicates per setup.
[d] Soil Fe mineralogy was analyzed after incubation by Mössbauer and EXAFS spectroscopy.

0.5 M HCl for 1 h, both under oxic conditions at room temperature on a shaker. For soil Sbu a third sub-sample was extracted with 1 M HCl at 70 °C under oxic conditions in a water bath for 24 h (Porsch and Kappler, 2011). From all extracts 1.8 mL were centrifuged (15 min, 20,817*g*) to remove soil particles. Fe(II) and Fe_{tot} of the supernatant were quantified by the ferrozine assay (Stookey, 1970) in microtiter plates as described by Hegler et al. (2008). The properties of the original soils are given in Table 2.

2.3.2. Magnetic susceptibility measurements

Low-field MS of the microcosms was measured as described in Porsch et al. (2010). In order to determine the mass specific MS of the original soil samples, 5–10 sub-samples of each soil were packed in 10 cm^3 plastic containers and the results of the volume specific MS were divided by the overall density of the contents of the container. The extent of MS change in soil microcosms was calculated as the % difference between the initial MS value and the final MS value which was calculated as average of the last four to five measured MS data points during the plateau phase.

2.3.3. Dissolved carbon and organic acid analysis

Microcosm bottles with soils FB, AW and Sbu were centrifuged (10 min, 2000 rpm) and the supernatants were frozen and stored in sterile plastic cups at −28 °C until analysis of dissolved organic and inorganic carbon, and organic acids. For analysis, the supernatant was thawed and remaining soil particles were removed either by centrifugation (soils FB and AW, 10 min at 20,817*g*) or by centrifugation and filtration (soil Sbu, centrifugation for 10 min at 5000*g* followed by filtration with a 0.22 µm mixed cellulose ester filter). Dissolved organic and inorganic carbon (DOC, DIC) contents were determined using a carbon analyzer (high TOC, Elementar, Germany). Organic acids (acetate, butyrate, formate, lactate, and propionate) were quantified by HPLC using a diode array detector (absorption at 210 nm) and a refractive index detector. The acids were separated on a Bio-Rad Aminex HPX-87H Ion Exclusion Column (300 * 7.8 mm) with two pre-columns, a Bio-Rad Micro guard Cation H Cartridge and a Dionex IonPac NG1 Guard column (2 * 50 mm). 5 mM H_2SO_4 was used as eluent (0.6 mL/min). The column heater temperature was 60 °C.

2.3.4. Mineral analysis by Mössbauer and extended x-ray absorption fine structure (EXAFS) spectroscopy

Initial attempts to separate magnetic mineral particles from the soil by magnetic separation failed (data not shown), probably due to their small size, coatings of the magnetic particles with organics or associations with non-magnetic mineral particles. Therefore, minerals were analyzed directly from soil samples.

Preparation of samples from microcosms for Mössbauer spectroscopic analysis was done anoxically in a glovebox (100% N_2), whereas the original soil was prepared under oxic conditions. Soil samples were sealed between two layers of Kapton tape. Mössbauer spectra were recorded at room temperature for up to 19 days and analyzed as described by Hohmann et al. (2010).

To prepare samples for EXAFS, microcosms of soil Sbu were opened in a glovebox (100% N_2) and a sub-sample of the soil was dried at ambient temperature and finely ground in an agate mortar. Ground samples were packed in Teflon sample holders for XAS analyses and sealed with Kapton tape to prevent oxidation. The structural environment of Fe was determined using EXAFS spectroscopy at the Stanford Synchrotron Radiation Lightsource (SSRL) on beamlines 11-2 (26-pole wiggler) and 4-1 (20 pole wiggler). The storage ring was operated at 3.0 GeV and at currents between 60 and 100 mA. The Fe EXAFS analytical procedures used here were similar to those described previously by Borch et al. (2007). Energy selection was accomplished

Table 2
Selected properties of original soils used for microcosms experiments.

Soil properties	HG	Wabu	FB	AW	Sbu	Hclean
pH[a]	5.4[c]	7.1[c]	5.2 ± 0.0	3.6[b] ± 0.0	7.0 ± 0.0	4.8 ± 0.0
Water content [wt.%][b,d]	32 ± 0	40 ± 0	23 ± 1	30 ± 1	48 ± 0	15 ± 1
$CaCO_3$ [wt.%][a,d]	4.7 ± 0.7	1.5 ± 0.0	1.2 ± 0.2	0.2 ± 0.2	12.0 ± 1.0	0.2 ± 0.2
TOC [wt.%][a,d]	2.8 ± 0.0	4.1 ± 0.1	3.3 ± 0.2	4.6 ± 0.3	3.5 ± 0.1	2.0 ± 0.2
N_{total} [wt.%][a,d]	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Fe_{total} 0.5 M HCl [wt.%][b,d]	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
Fe_{total} XRF [wt.%][a,d,e]	3.8 ± 0.0	2.2 ± 0.0	2.1 ± 0.1	2.0 ± 0.0	2.6 ± 0.0	0.6 ± 0.0
S_{total} XRF [ppm][a,d,e]	778 ± 120	1083 ± 51	n.d.[f]	n.d.	850 ± 2	n.d.
Mass specific magnetic susceptibility (MS) [10^{-8} m^3/kg][b]						
MS Average	19.9 ± 1.4	17.3 ± 5.3	27.1 ± 0.6	45.5 ± 4.7	13.9 ± 1.3	11.6 ± 0.5
MS Minimum	17.7	13.5	26.4	39.7	12.3	11.0
MS Maximum	21.3	26.7	27.8	51.1	16.1	12.3

[a] Average of duplicates ± difference to the minimum and maximum.
[b] Average of three or more replicates ± standard deviation.
[c] single measurement.
[d] wt.% and ppm refers to 105 °C dried soil.
[e] XRF = X-ray fluorescence analysis.
[f] Not determined.

with a Si (220) monochromator and spectra were recorded by fluorescent X-ray production using a Lytle-detector. A set of Fe reference compounds was used for linear combination k^3-weightened EXAFS spectral fitting using the SIXPACK interface to IFEFIT (Webb, 2005). Linear combinations of the reference compounds were optimized and the only variable parameters were the fractions of each reference compound (see Fig. EA1 of the Electronic Annex for one example of linear combination fitting). Reference compounds were chosen based on their likelihood of being present in the soil or being a reaction product and were included in the fit only if they contributed 5% or more. The detection limit for minor constituents is approximately 5 mol.%. Mössbauer spectroscopy, with a detection limit of approximately 5 wt.%, was used to constrain EXAFS analysis as described earlier by Borch et al. (2007).

2.4. Quantification of Fe(III)-reducing microorganisms

Anaerobic Fe(III)-reducing microorganisms of the original soil Sbu were quantified by the most probable number (MPN) method as described in Emmerich et al. (2012) with the following changes: the soil was diluted in 1:10 dilution series with the microbial growth medium, the electron donor mix contained 5 mM Na-acetate, 5 mM Na-lactate, 10 mM Na-formate, 2 mM Na-propionate and 2 mM Na-butyrate, and the MPN plates were incubated for 18 weeks.

2.5. Statistical analysis

A one sample *t*-test was used for setups with gasoline amendment and for setups without additional carbon for which there were more than two replicates (Table 1) to determine if the MS of each microbially active setup as well as each sterile setup changed significantly over time. The setups for which this test was conducted, as well as the data used for the tests, are shown in Table EA1 of the Electronic Annex. The Pearson correlation coefficient *r* for linear correlations between MS dependent parameters (maximum extent of MS change, absolute value of the maximum extent of MS change, time within stable MS values were reached (Fig. 1)) and the soil properties (Table 2) was determined for all microbially active microcosms of all six soils without carbon amendment and with gasoline addition (Table EA2 of the Electronic Annex). The setup of soil AW without carbon amendment was excluded from these calculations due to its extreme decrease in MS in comparison to the other soils (Fig. 1). All tests and calculations were performed with the software package PASW statistics 17.0 from SPSS Inc. (Chicago, USA).

3. RESULTS

In order to determine the importance of microbial activity and geochemical conditions on the extent and temporal development of changes in soil MS in presence and absence of hydrocarbons, microcosms with six different soils and three different treatments (no carbon addition, addition of lactate/acetate or addition of gasoline) were set up (Table 1).

3.1. Microbial processes involved in changes of soil MS

3.1.1. Changes in MS of sterile vs. microbially active soil microcosms

The MS of microbially active soil microcosms with soils Hclean, Sbu, FB and AW without additional carbon or with gasoline amendment changed significantly during incubation with the exception of microcosms with soil FB amended with gasoline (Fig. 1, Table EA1 of the Electronic Annex). In contrast, for five out of eight sterile microcosms containing the same soils no significant changes in MS were observed (Fig. 1, Table EA1 of the Electronic Annex). The extent of MS change of the microbially active microcosms

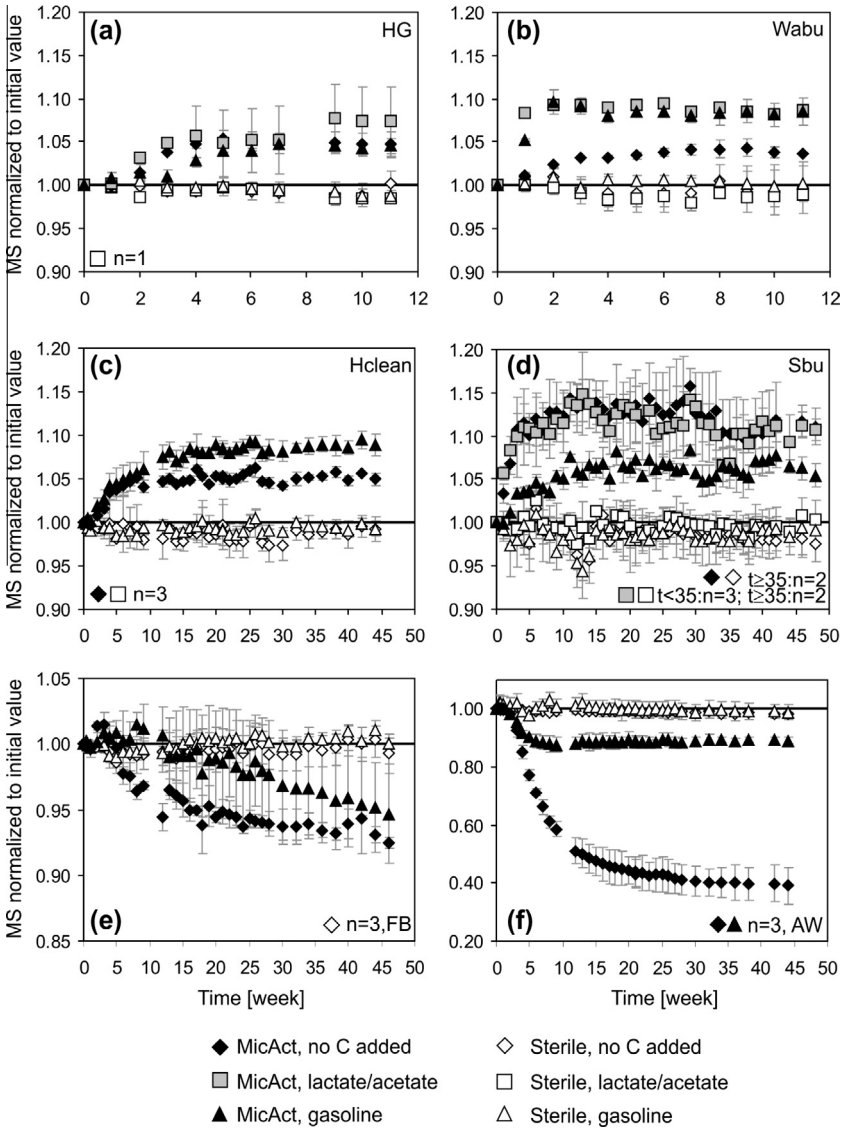

Fig. 1. Changes of magnetic susceptibility (MS) over time of microbially active (MicAct) and sterile soil microcosms either without carbon amendment (no C), amended with 15 mM lactate/acetate or amended with 3.6 μL gasoline/g field moist soil. Lactate/acetate was only added to soils HG, Wabu and Sbu. High-purity water was added to microcosms with soil Sbu. Microbial growth medium was added to all other microcosms. The MS values measured at each time point were normalized to the MS values measured directly after setting up the microcosms (time $t = 0$). Solid horizontal lines indicate MS without any change over time. Note the different scales of the axes. Results are means of two ($n = 2$, a and b) or four ($n = 4$, c–f) replicates, except those noted in the graphs. One replicate of sterile and microbially active setups of soil Sbu without carbon amendment and with lactate/acetate were harvested in week 35 for EXAFS measurements. Bars bracket the range of duplicates or indicate the standard deviation of three and four replicates.

varied between $4.0 \pm 0.4\%$ (soil Wabu, no carbon added) and $11.3 \pm 3.2\%$ (soil Sbu, no carbon added). In one particular microbially active setup (soil AW without any carbon amendment), MS decreased by $60.3 \pm 6.0\%$. On average (excluding the very high change of >60% observed with soil AW) the MS of all microbially active setups, including those with lactate/acetate amendment, changed by $7.3 \pm 2.4\%$. The change in MS of all sterile microcosms varied between $0.6 \pm 0.3\%$ (soil FB, gasoline) and $2.4 \pm 0.9\%$ (soil AW, gasoline) with an average change of $1.4 \pm 0.6\%$.

Whether MS of the non-sterile microcosms increased or decreased over time was dependent upon the soil used (but not upon the carbon source) (Fig. 1). Microcosms with soils HG, Wabu, Hclean and Sbu showed increases in MS, whereas soils FB and AW showed decreases in MS. Furthermore, the time needed until stable MS values were reached also mainly depended on the soil and only to a small extent on the added carbon source. Microcosms with soil Wabu and carbon amendment reached stable MS values within two weeks, whereas microcosms with soil FB

required around 30 weeks for stabilization. The reasons for the different time span until MS stabilizes are probably related to specific properties of the different soils such as soil composition, geochemistry, and microbial community structure.

3.1.2. Cell numbers of Fe-metabolizing microorganisms in soil Sbu

Differences of MS and Fe mineralogy (see Section 3.2.1.) observed between sterile and microbially active microcosms with soil Sbu suggest the activity of Fe(III)-reducing microorganisms. MPN quantification of Fe(III)-reducers in the original soil Sbu yielded $2.1 * 10^5$ cells/g dry soil (95% confidence interval: $8.3 * 10^4$–$5.5 * 10^5$ cells/g dry soil).

3.2. Geochemical parameters affecting changes in soil MS

3.2.1. Fe mineralogy changes in soil microcosms

In order to verify that MS changes in our microcosms were caused by changes in soil Fe mineralogy, we extracted three different Fe fractions from sterile and microbially active soils after incubation. Fig. 2 shows the Fe extraction data of microcosms with soil Sbu (Fig. 1d). The other soils were analyzed as well and showed comparable results (data not shown).

The amount of Fe_{tot} extractable with 1 M HCl at 70 °C (crystalline Fe minerals) was similar for the original soil Sbu and for soil Sbu incubated for approximately 1 year in sterile and microbially active microcosms (Fig. 2b), showing that the Fe_{tot} content was similar in all bottles. The total amount of Fe extractable with Na-acetate (adsorbed Fe and Fe carbonates) and 0.5 M HCl (poorly crystalline Fe minerals) was similar for the sterile and the original soil indicating no major changes in mineralogy during sterilization and incubation of the sterilized soil. In contrast, in the microbially active setups, especially those without carbon amendment and those with lactate/acetate addition, Fe_{tot} extracted by Na-acetate and 0.5 M HCl was much higher (Fig. 2a) indicating changes in the different Fe pools caused by microbial activity. Among all sterile setups as well as among all microbially active setups, the Fe(II):Fe_{tot} ratio of the 0.5 M HCl and 1 M HCl fraction, respectively, was very similar, independent of the carbon treatment. The percentage of total Fe present as Fe(II) as determined in the 0.5 M HCl extract was 14.3% in the original soil, 57.5 ± 2.7% on average in the sterile setups, and 97.4 ± 2.4% on average in the microbially active setups. In the 1 M HCl fraction, the percentage of total Fe present as Fe(II) in the sterile setups (69.2 ± 8.2%) was similar to the percentage in the original soil (69.7%), whereas in microbially active setups the Fe(II) content was approximately 20% higher (90.1 ± 2.4%).

The Fe extraction data indicate a difference in soil Fe mineralogy and Fe redox states in microbial active microcosms in comparison to the sterile microcosms (Fig. 2). In order to identify the Fe minerals that were (trans)formed in the soils during incubation, we performed Mössbauer and EXAFS spectroscopy analysis of the original soil Sbu and of soil Sbu from sterile and microbially active microcosms without carbon amendment, and with lactate/acetate addition after several months of incubation. Confirming the Fe extraction data, Mössbauer spectroscopy revealed that the original soil Sbu and soil Sbu from the two sterile setups contained more Fe(III) phases (82–92%, percentages refer to the relative absorption of γ-rays by Fe_{tot}) than the microbially active setups without carbon amendment (71%) or lactate/acetate addition (65%) (Table 3, Fig. 3). The main fraction of Fe(III) in the original soil and all four setups was a paramagnetic Fe(III) phase which might have been ferrihydrite, nano-goethite, lepidocrocite, Fe(III) in clays or Fe(III) associated with humic substances. Additionally, hematite (<10%) was identified in the original soil and in the microbially active setup with lactate/acetate (Fig. 3b). The amount of Fe(II) phases detected by Mössbauer spectroscopy varied between 29% and 35% in the microbially active setups, whereas it was between 12% and 19% in the sterile setups and only 8% in the original soil. In the microbially active setups, Fe(II) was present as siderite and an associated Fe(II) phase represented in the spectrum by an extra Fe(II) doublet occurring together with siderite (Fig. 3b). A similar spectral feature was also detected when siderite was chemically synthesized in our lab by mixing dissolved Fe^{2+} with an HCO_3^- solution at pH 8 and 25 °C (data not shown). Its identity is so far unknown, however,

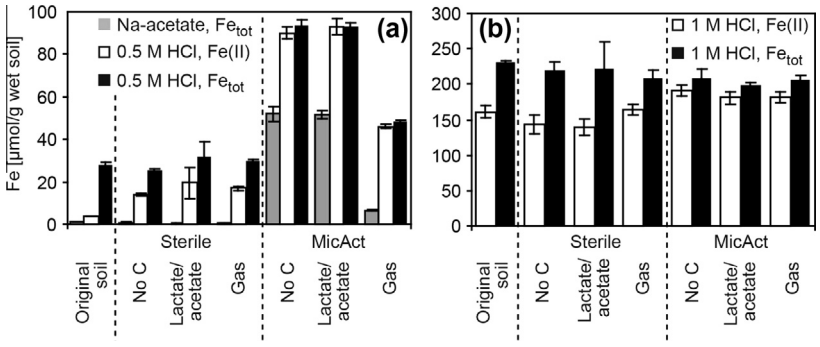

Fig. 2. (a) Fe_{tot} extracted with Na-acetate, Fe(II) and Fe_{tot} extracted with 0.5 M HCl from original soil Sbu and from sterile and microbially active (MicAct) microcosms with soil Sbu after 48 weeks of incubation without additional carbon (No C), with 15 mM lactate/acetate or with 3.6 µL gasoline/g field moist soil (Gas). (b) Fe(II) and Fe_{tot} extracted with 1 M HCl (70 °C) from the same soil samples as in (a). Results are means of triplicate measurements of one bottle per setup or of the original soil, except for the original soil which was extracted with 1 M HCl only in duplicates. Bars indicate the range of duplicates or the standard deviation of triplicates.

Table 3
Relative abundance and fitting data of different Fe phases determined by Mössbauer spectroscopy in the original soil Sbu and in soil Sbu from sterile and microbially active (MicAct) microcosms without additional carbon or with 15 mM lactate/acetate after 9-23 months of incubation. Fitting errors are given in parentheses.

Sample (incubation time [weeks])	Reduced χ^2 of fit	Fe phases	Abundance[f] [%]	CS[f] [mm/s]	QS[f] [mm/s]	H[f] [T]
Original soil	3.14	Paramagnetic Fe(III)[a]	83 (±0.3)	0.36 (<0.01)	0.64 (<0.01)	–
		Hematite	9 (±0.07)	0.37 *	−0.20 *	51.3*
		Fe(II) in silicates[b]	8 (±0.4)	1.08 (±0.01)	2.73 (±0.02)	–
Sterile microcosm, no carbon (99)	0.56	Paramagnetic Fe(III)[a]	82 (±2.1)	0.36 *	0.60 (±0.03)	–
		Fe(II) in silicates[b]	12 (±1.5)	1.14 *	2.67 *	–
		Fe(II) in silicates[b]	7 (±1.6)	1.10 *	1.94 *	–
Sterile microcosm, lactate/acetate (93)	0.88	Paramagnetic Fe(III)[a]	88 (±1.7)	0.35 *	0.65 *	–
		Unknown Fe(II) phase 1[c]	12 (±1.7)	1.31 (±0.07)	2.33 (±1.4)	–
MicAct microcosm, no carbon (62.5)	1.35	Paramagnetic Fe(III)[a]	71 (±1.2)	0.35 *	0.63 *	–
		Hematite	n.d.[e]	–	–	–
		Unknown Fe(II) phase 2[d]	13 (±0.9)	1.21 *	2.49 *	–
		Siderite	16 (±0.9)	1.20 *	1.80 *	–
MicAct microcosm, lactate/acetate (40)	1.29	Paramagnetic Fe(III)[a]	59 (±0.73)	0.31 (±0.01)	0.65 (±0.02)	–
		Hematite	6 (±0.12)	0.38 *	−0.20 *	51.2*
		Unknown Fe(II) phase 2[d]	18 (±0.64)	1.21 *	2.49 *	–
		Siderite	17(±0.73)	1.23 *	1.80 *	–

[a] Paramagnetic Fe(III) might be ferrihydrite, nano-goethite, lepidocrocite, Fe(III) in clays, Fe(III) associated with humic substances or a mix of these.
[b] Silicates are probably phyllosilicates such as clays and micas. Differences in the modeling parameters indicate Fe(II) present either in different silicates or in different sites of one silicate.
[c] Maybe a mixture of siderite and unknown Fe(II) phase 2.
[d] Unknown Fe(II) phase associated with siderite.
[e] Hematite could not be modeled, since the signal to noise ratio was too low.
[f] Abundance [%] = absorption of γ-ray [%]. CS = center shift, QS = quadrupol splitting, H = hyperfine field.
* Model parameter fixed during fitting.

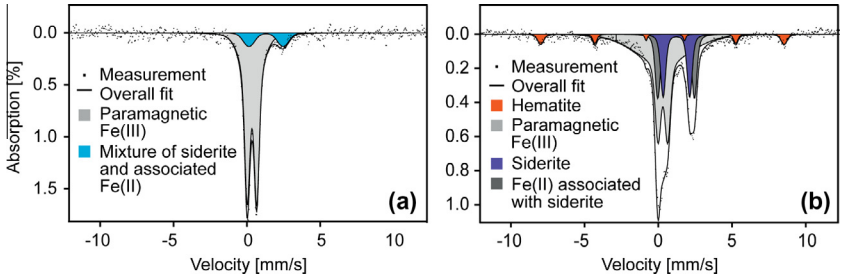

Fig. 3. Mössbauer spectra collected at room temperature of soil Sbu from (a) sterile and (b) microbially active microcosms with 15 mM lactate/acetate amendment after 93 and 40 weeks of incubation, respectively. Fitting parameters are given in Table 3.

based on the broadness of the signals, its crystallinity is similar to that of siderite. In the original soil and in sterile soil without carbon amendment, Fe(II) was present as ferrosilicates. The Fe(II) phase present in the sterile setup with lactate/acetate is unknown.

EXAFS analyses of soil Sbu from microcosms confirmed the results of Fe extraction and Mössbauer spectroscopy and showed that the Fe composition of the original soil was similar to the sterile setups regarding all Fe-containing phases identified by EXAFS (data not shown). Additionally, the EXAFS analyses showed that in microbially active setups without carbon addition or with lactate/acetate addition (Fig. EA1 of the Electronic Annex), the fraction of the Fe(III) minerals goethite and hematite decreased and the Fe(II)-bearing mineral siderite was formed (Table 4). The amount of Fe present as Fe(III) minerals in sterile setups decreased in microbially active setups by around 10 mol.%, which is less than the amount of Fe found as Fe(II) in siderite (14 mol.%) in the microbially active setups. However, the main fraction of Fe (71-74 mol.%) identified by EXAFS in sterile and microbially active setups was present in augite- and ferro-smectite-like minerals and likely in association with humic substances.

Table 4
Relative amounts of Fe phases (in % on a per mol basis) resulting from linear combination fits of k^3-weighted EXAFS spectra (the data were fit to $k = 11$[a] and the detection limit is approximately 5 mol.% Fe). The table gives the data for sterile and microbially active (MicAct) microcosms of soil Sbu without carbon amendment (no C) and with addition of 15 mM lactate/acetate (lac/ac). Dashes indicate Fe phases which were not detected due to their low concentration or absence. As an example, the linear combination fitting for the lactate/acetate-amended, microbially active sample is displayed in Fig. EA1 of the Electronic Annex.

Sample	Goethite [mol.% Fe]	Hematite [mol.% Fe]	Siderite [mol.% Fe]	Augite [mol.% Fe]	Ferro-Smectite [mol.% Fe]	Fe-HS[d] [mol.% Fe]	Reduced χ^2 of fit
Sterile, no C[b]	20	5	–	28	15	31	0.026
Sterile, lac/ac[b]	20	6	–	28	14	31	0.037
MicAct, no C[c]	16	–	14	19	6	46	0.031
MicAct, lac/ac[b]	15	–	14	38	-	33	0.021

[a] Data fit to $k = 14$ did not change the overall results.
[b] Measured at beamline 11-2 at the Stanford Synchrotron Radiation Lightsource.
[c] Measured at beamline 4-1 at the Stanford Synchrotron Radiation Lightsource 4 months later than samples labeled with (b).
[d] Fe associated with humic substances (HS).

3.2.2. Mobilization of organic carbon from soils

The MS of most microbially active setups without carbon amendment changed to a similar or even larger extent than that of setups with carbon amendment (Fig. 1). Since microcosms of soils AW, FB and Hclean amended with growth medium showed a similar change in MS compared to microcosms of the same soils amended only with water (Fig. EA2 of the Electronic Annex), we believe that neither carbon amendment nor the trace metals and vitamins in the medium were the key parameters causing MS changes. Instead, this suggests that the addition of liquid – independent of whether it was pure water or medium – mobilized organic carbon (natural organic matter) from the soil that drove metabolic processes and MS changes in all microbially active setups. In order to determine which dissolved carbon sources were available in the microcosms, the concentrations of organic acids (lactate, formate, acetate, and propionate) and dissolved inorganic (DIC) and organic (DOC) carbon in the liquid phase of microcosms with soil Sbu after 48 weeks of incubation were quantified (Fig. 4). The concentrations of the four different organic acids were similar in all sterile setups of soil Sbu, except in setups amended with lactate/acetate which resulted in higher lactate and acetate concentrations (Fig. 4a). In contrast, no organic acids besides small amounts of lactate (<2.5 mM) were detected in the microbially active setups of soil Sbu without carbon amendment, and in the setups with lactate/acetate addition. In the microbially active setups with gasoline, the lactate and formate concentrations were also low (<1.2 mM) but the acetate (37.4 mM) and propionate (8.2 mM) concentrations were higher than in all other sterile and microbially active setups.

The DIC in all sterile microcosms of soil Sbu was <0.1 g/L whereas in microbially active ones the DIC was higher (0.1–0.4 g/L) indicating microbial mineralization of organic carbon (Fig. 4b). The DOC in sterile setups without carbon addition and with gasoline was similar (~2 g/L) indicating the low solubility of the gasoline hydrocarbons, whereas in the sterile setup with lactate/acetate the DOC was 0.6 g/L higher than in setups without carbon addition. The DOC content in microbially active setups without carbon amendment and in setups with lactate/acetate addition was lower (both 0.3 g/L) than in setups amended with gasoline (1.9 g/L).

3.2.3. Influence of organic carbon and Fe bioavailability on changes in MS of soil microcosms

All microbially active soil microcosms with and without carbon addition reached stable MS values after a few weeks to several months (Fig. 1). This suggests that the soils reached a stable state and no further Fe mineral transformation occurred after that time. In order to determine whether the amount of bioavailable organic carbon was limiting the mineral transformation, we added lactate and acetate a second time to the respective setups of soils HG and Wabu (Table 1). Lactate and acetate were chosen, since the first addition of these compounds led to a similar or even higher change in MS than the addition of gasoline (Fig. 1). The carbon sources were added after 14.5 weeks of incubation at which time the MS values had stabilized (Fig. 1). In the following 10 weeks, the MS values of both the sterile and microbially active setups changed in all but one setup by less than 2.0% (data not shown), indicating that the amount of bioavailable carbon was probably not a primary limiting factor for ferro(i)magnetic mineral transformation.

In order to determine if bioavailable Fe(III) limitation in the soils led to cessation of changes in MS, ferrihydrite was added to microcosms with soil Hclean after 51 weeks of incubation when MS did not show further change (Table 1, Fig. 1). Ferrihydrite addition to soil Hclean led to an immediate increase in MS of 10.6–20.1% when measured directly after its addition (Fig. 5). In the following 73 weeks, MS in microbially active microcosms further increased by $5.4 \pm 3.4\%$ (no carbon addition) and $13.4 \pm 1.6\%$ (gasoline addition), respectively. From these MS values we can estimate that about 1% and 2% of the ferrihydrite has been converted into magnetite (calculation not shown). In the corresponding sterile setups the increase within the 73 weeks was only minor ($2.4 \pm 1.2\%$ and $0.8 \pm 0.0\%$).

3.2.4. Influences of soil properties on soil MS changes

In order to determine if soil properties had an influence on changes in MS, linear correlation analyses between the

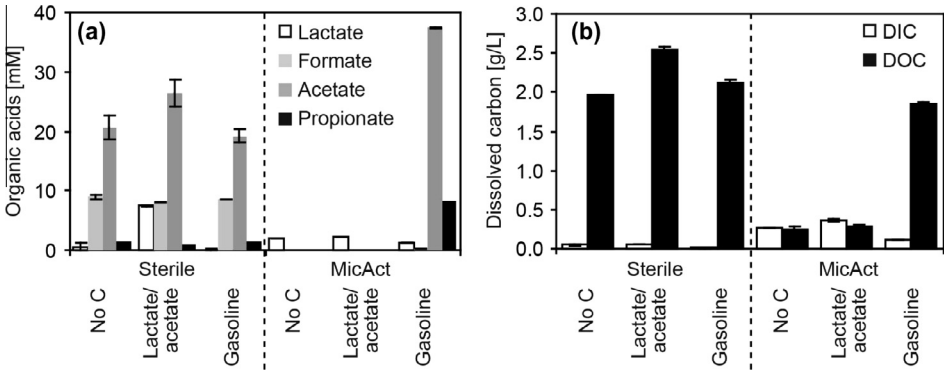

Fig. 4. (a) Concentration of short chain fatty acids lactate, formate, acetate and propionate and (b) dissolved inorganic (DIC) and dissolved organic carbon (DOC) in the water in soil Sbu microcosms after 48 weeks of incubation. Shown are data from sterile and microbially active (MicAct) microcosms without carbon addition (no C), with 15 mM lactate/acetate or with 3.6 μL gasoline/g field moist soil. Results are means of duplicate measurements of one bottle per setup. Propionate was measured only once. Bars bracket the range of duplicates.

MS dependent parameters of the microcosms (extent of MS change, absolute value of the extent of MS change, time required to reach stable MS values, Fig. 1) and the soil properties (Table 2) were performed. Since significant changes in MS occurred mainly in microbially active microcosms (Table EA1 of the Electronic Annex), only these setups were used for the analysis. Furthermore, only setups without carbon amendment and with gasoline addition were considered, since lactate/acetate was added to only three soils. As the MS of microbially active soil AW without carbon amendment decreased by >60% in contrast to the other microbially active microcosms (average MS change 7.3 ± 2.4%, Fig. 1), this setup was considered an exception and was also excluded from the calculations.

The MS dependent parameters of the microcosms were not linearly correlated with most of the soil parameters tested (Table EA2 of the Electronic Annex). There may be several reasons for the lack of a strong correlation. Either the correlation between the parameters was not linear, the relevant soil parameters were not tested, or the sample number (five to six) was too small to observe any correlation. However, our analysis revealed a significant positive correlation between the absolute value of the extent of MS change and the Fe_{tot} extractable with 0.5 M HCl for the microbially active microcosms without carbon amendment (Pearson correlation coefficient 0.959, 2-tailed significance 0.010) (Table EA2 of the Electronic Annex). The Fe_{tot} fraction extractable with 0.5 M HCl is considered to be the "bioavailable" Fe fraction. Hence, the results suggest that the higher the concentration of the bioavailable Fe fraction in a soil is, the higher the change in MS. For microbially active microcosms amended with gasoline, a linear correlation was neither observed between the absolute value of the extent of MS change and the Fe_{tot} extractable with 0.5 M HCl nor between the extent of MS change (including positive and negative MS values) and the Fe_{tot} extractable with 0.5 M HCl. However, a significant negative linear correlation between the extent of MS change and the initial mass specific MS of the soil was found (Pearson correlation coefficient −0.957, 2-tailed significance 0.003) (Table EA2 of the Electronic Annex). This suggests, that in soils with a low MS, ferro(i)magnetic minerals are formed, whereas in soils with a relatively high MS, ferro(i)magnetic minerals are transformed.

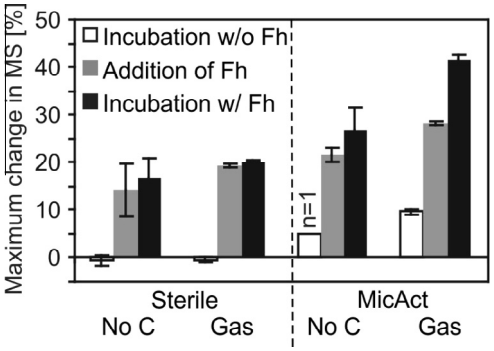

Fig. 5. Changes in magnetic susceptibility (MS) of sterile and microbially active (MicAct) microcosms with soil Hclean without carbon addition (No C) or with gasoline addition (Gas, 3.6 μL/g field moist soil) within 46 weeks of incubation (□, Fig. 1c). After 51 weeks 2 mL of a 0.5 M ferrihydrite suspension (Fh) was added to the microcosms leading to an immediate increase in MS (■) and to a slower MS increase in the following 73 weeks of incubation (■). All values are given in % relative to the MS values measured directly after setting up the microcosms. Results are means of duplicates, except those marked in the figure ($n = 1$). Bars bracket the range of duplicates.

4. DISCUSSION

Magnetic susceptibility measurements of microcosms with six different sterile and microbially active soils showed that during anoxic incubation of the soils microbially mediated processes were predominantly responsible for changes in the MS. Furthermore, the results indicated that besides microbial activity the bioavailable Fe and organic carbon content as well as the initial mass specific MS were the main factors controlling the changes in MS of the soil microcosms. In the following sections, we therefore first discuss how microorganisms potentially have been involved in the

(trans)formation of magnetite, a ferro(i)magnetic mineral, and of non-ferro(i)magnetic Fe-containing minerals in the microcosms. Secondly, we describe how the mobilization of bioavailable soil organic carbon or the addition of bioavailable carbon (e.g. gasoline) may have influenced the changes of soil MS in the microcosms indirectly by influencing the Fe(III)-reducing microorganisms.

4.1. Microbial (trans)formation of Fe-containing soil minerals

4.1.1. (Trans)formation of ferro(i)magnetic minerals

The increase and decrease in MS of the soil microcosms (Fig. 1) indicated the formation and transformation of ferro(i)magnetic minerals. Statistical analysis suggested that in microcosms without additional carbon amendment the extent of the increase or decrease in MS depended on the amount of bioavailable Fe in the soil (Table EA2 of the Electronic Annex). This hypothesis is supported by the experiment in which ferrihydrite was added as an easily reducible and thus as bioavailable Fe(III) source to microcosms after they had reached stable MS values (Fig. 5). The ferrihydrite addition to the microbially active microcosms led to further changes in the MS over time, indicating that either the ferrihydrite served as precipitation site for magnetite by a reaction of the ferrihydrite with Fe^{2+} present in the soil and/or that the ferrihydrite itself was reduced. For microbially active microcosms amended with gasoline, the statistical analysis (Table EA2 of the Electronic Annex) suggested, that in soils with a low mass specific MS, ferro(i)magnetic minerals are formed, whereas in soils with a relatively high mass specific MS, ferro(i)magnetic minerals are transformed.

The most important ferro(i)magnetic minerals in soils are the mixed-valent Fe mineral magnetite (Fe_3O_4) and the Fe(III) oxide maghemite (γ-Fe_2O_3) (Mullins, 1977). In sulfur-rich environments also the ferro(i)magnetic minerals greigite (Fe_3S_4) and pyrrhotite (Fe_7S_8) may form (Farina et al., 1990; Stanjek et al., 1994). Since the total sulfur content of the soils was low (<1100 ppm in comparison to 6000–38,000 ppm Fe_{tot}, Table 2) and since both EXAFS and Mössbauer spectroscopy did not provide evidence for the presence of Fe-sulfide minerals, (trans)formation of greigite or pyrrhotite did probably not contribute considerably to the change of MS of the soil microcosms.

Microbially controlled formation of magnetite and greigite inside cells is known from magnetotactic bacteria (Blakemore, 1975). Since other studies showed that the cell numbers of magnetotactic bacteria in soils were too low to contribute significantly to the soil magnetic properties (Fassbinder et al., 1990; Dearing et al., 1996), we assume that their contribution to the changes in MS of our microcosms was also minor.

In contrast, ferro(i)magnetic Fe minerals can be produced in large amounts extracellularly by secondary mineral formation during microbial Fe(III) reduction under anoxic conditions. Magnetite formation was observed, for example, during microbial reduction of ferrihydrite (Lovley et al., 1987) and hematite (Behrends and Van Cappellen, 2007). Ferrihydrite, hematite and goethite are common Fe minerals in soils (Cornell and Schwertmann, 2003) and we identified these Fe(III) phases by Mössbauer spectroscopy and/or EXAFS measurements in the original soil Sbu (no amendments, stored at 4 °C) that also contained Fe(III)-reducing bacteria (see Section 3.1.2.). However, although MS measurements of microbially active soil Sbu indicated an increase in ferro(i)magnetic minerals (e.g. magnetite, Fig. 1d), such minerals were neither detected by Mössbauer spectroscopy nor by EXAFS, probably due to their low content in the soil. For identification and quantification of a certain Fe mineral in soils by Mössbauer spectroscopy or EXAFS, the Fe present in this respective Fe mineral must represent at least 5–10 wt.% of the total Fe content in the sample. In contrast, MS measurements are more sensitive. An increase in the initial soil Fe content of soil FB by addition of magnetite by less than 3 wt.% (corresponding to less than 0.1 wt.% magnetite in the soil) led to an increase in the soil MS by more than 100% (Fig. EA3 of the Electronic Annex).

In contrast to setups with soils HG, Wabu, Hclean and Sbu where MS increased over time, a decline of the MS over time was observed in setups with soils FB and AW indicating a microbially mediated decrease in the ferro(i)magnetic mineral content, e.g. magnetite, in the soils. Magnetite can serve also as electron acceptor for Fe(III)-reducing microorganisms (Kostka and Nealson, 1995; Brown et al., 1997) and microbial Fe(III) reduction could potentially have led to the transformation of ferro(i)magnetic minerals in our anoxic microcosms.

4.1.2. (Trans)formation of non-ferro(i)magnetic Fe-containing minerals

Besides magnetite, non-ferro(i)magnetic Fe minerals can form during microbial Fe(III) reduction. Depending on geochemical conditions such as the presence of anions, mineral nucleation sites and humic substances and depending on the Fe(III) reduction rates, the formation of dissolved Fe^{2+}, goethite, green rust, vivianite, or siderite has been reported (for reviews see Konhauser, 1998; Fortin and Langley, 2005). Mössbauer and EXAFS spectroscopy revealed that microbially active soil Sbu contained more siderite than the sterile and the original soil Sbu. The carbonate necessary for the siderite formation might originate from the soil Sbu itself (it showed the highest carbonate content of all soils, Table 2) or from microbial degradation of organic matter. Microbial CO_2 production was indicated by a higher DIC content in microbially active soil Sbu in comparison to the sterile soil (Fig. 4b).

Both Mössbauer and EXAFS spectroscopy of soil Sbu microcosms revealed that part of the Fe was also present in silicates, augite-like and ferro-smectite-like phases and in association with humic substances (Tables 3 and 4). These Fe phases are typical soil constituents (Cornell and Schwertmann, 2003) and are also non-ferro(i)magnetic. Fe-containing minerals which are non-ferro(i)magnetic have only a small positive MS. However, when the concentration of ferro(i)magnetic minerals in a soil is low, these minerals can contribute considerably to the soil MS (Mullins, 1977).

4.2. Influences of organic carbon sources on microbially induced soil MS changes

4.2.1. Mobilized soil organic carbon as substrate for microorganisms

Analysis of the water and microbial growth medium added to soil Sbu revealed that organic compounds including organic acids were mobilized from the soil matrix (Fig. 4). DOC represents an important source of bioavailable carbon in soils (Marschner and Kalbitz, 2003). The mobilized natural organic matter was partly consumed in microbially active microcosms leading to a change in soil MS comparable to setups amended with a carbon source (Fig. 1). The fact that the microbial (trans)formation of ferro(i)magnetic minerals was not limited by the availability of organic carbon was supported by the experiment in which a second amendment of lactate/acetate to microcosms with soils HG and Wabu did not lead to a further change in MS (data not shown).

4.2.2. Gasoline as organic carbon source

In all microbially active microcosms, the addition of gasoline led to changes in MS within the first five weeks (Fig. 1), indicating that gasoline was not toxic for all soil microorganisms and that at least some of them remained active and caused changes in MS. Previous studies showed that microbial communities in uncontaminated soils could adapt to added hydrocarbons such as crude oil and gasoline and were able to partly degrade them (Bundy et al., 2002; Hamamura et al., 2006). The microbial degradation of hydrocarbons can be directly coupled to the reduction of Fe(III) (Lovley et al., 1989; Lovley and Anderson, 2000). Additionally, nitrate-reducers, sulfate-reducers and methanogens are able to degrade hydrocarbons under anoxic conditions (Grbić-Galić and Vogel, 1987; Kuhn et al., 1988; Rueter et al., 1994) and the metabolic products (e.g. sulfide or nitrite) can react abiotically with Fe(III) and Fe(II) and thus influence the soil Fe mineralogy (Moraghan and Buresh, 1977; dos Santos Afonso and Stumm, 1992; Klueglein and Kappler, 2013).

The changes in MS and the formation of acetate and propionate in the microbially active setup of soil Sbu amended with gasoline suggest that the microorganisms were indeed active and have degraded at least part of the added gasoline (Cozzarelli et al., 1994). However, the smaller changes in MS (Fig. 1) and the lower content of 0.5 M extractable Fe(II) (Fig. 2a) compared to microbially active setups of soil Sbu without carbon addition indicate that the transformation of Fe minerals in soil Sbu was less pronounced in the presence of gasoline than without gasoline. This suggests that gasoline had an inhibiting effect on the microbial (trans)formation of ferro(i)magnetic minerals in this soil. Nevertheless, in some soils the change in MS was similar (HG, FB) or even larger (Wabu, Hclean) in setups with gasoline than without carbon amendment (Fig. 1). These results suggest that the microorganisms were well adapted to the presence of hydrocarbons.

5. CONCLUSIONS

The motivation for our study was the idea that soil magnetic susceptibility measurements as a fast screening of soil magnetic properties can be used to localize hydrocarbon contamination in the field. However, this study suggests that hydrocarbon addition is not the only factor driving changes in soil MS. The major findings of the present soil microcosm study were that (i) changes in soil MS were mainly microbially mediated, (ii) the MS of the soils started to change within the first few weeks of incubation, (iii) the extent of the MS change depended on the bioavailable Fe content of the soils, (iv) the MS value of the original soils determined whether the MS increased or decreased during incubation, and (v) mobilization of natural organic matter from the soil by addition of water also led to a change in MS similar to setups with hydrocarbon contamination. Although the changes in MS were only around 4–11% for most soil microcosms, the high sensitivity of MS analysis allowed their detection with high accuracy. In contrast, Mössbauer and EXAFS spectroscopy analysis are less sensitive to small changes in soil ferro(i)magnetic mineral content and are more time and labor intensive.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2013.12.001.

REFERENCES

Aldana M., Costanzo-Alvarez V., Gomez L., Gonzalez C., Diaz M., Silva P. and Rada M. (2011) Identification of magnetic minerals related to hydrocarbon authigenesis in venezuelan oil fields using an alternative decomposition of isothermal remanence curves. *Stud. Geophys. Geod.* **55**, 343–358.

Amstaetter K., Borch T. and Kappler A. (2012) Influence of humic acid imposed changes of ferrihydrite aggregation on microbial Fe(III) reduction. *Geochim. Cosmochim. Acta* **85**, 326–341.

Behrends T. and Van Cappellen P. (2007) Transformation of hematite into magnetite during dissimilatory iron reduction – conditions and mechanisms. *Geomicrobiol. J.* **24**, 403–416.

Blakemore R. (1975) Magnetotactic bacteria. *Science* **190**, 377–379.

Blume H.-P., Deller B., Leschber R., Paetz A. and Wilke B.-M. (2000) *Handbuch der Bodenuntersuchung: Terminologie, Verfahrensvorschriften und Datenblätter – Physikalische, chemische, biologische Untersuchungsverfahren – Gesetzliche Regelwerke*. Wiley-VCH, Weinheim.

Borch T., Masue Y., Kukkadapu R. K. and Fendorf S. (2007) Phosphate imposed limitations on biological reduction and alteration of ferrihydrite. *Environ. Sci. Technol.* **41**, 166–172.

Borch T., Kretzschmar R., Kappler A., Cappellen P. V., Ginder-Vogel M., Voegelin A. and Campbell K. (2010) Biogeochemical redox processes and their impact on contaminant dynamics. *Environ. Sci. Technol.* **44**, 15–23.

Brown D. A., Sherriff B. L. and Sawicki J. A. (1997) Microbial transformation of magnetite to hematite. *Geochim. Cosmochim. Acta* **61**, 3341–3348.

Bundy J. G., Paton G. I. and Campbell C. D. (2002) Microbial communities in different soil types do not converge after diesel contamination. *J. Appl. Microbiol.* **92**, 276–288.

Chaudhuri S. K., Lack J. G. and Coates J. D. (2001) Biogenic magnetite formation through anaerobic biooxidation of Fe(II). *Appl. Environ. Microbiol.* **67**, 2844–2848.

Cornell R. M. and Schwertmann U. (2003) *The Iron Oxides, Structure, Properties, Reactions, Occurences and Uses*. Wiley-VCH, Weinheim.

Cozzarelli I. M., Baedecker M. J., Eganhouse R. P. and Goerlitz D. F. (1994) The geochemical evolution of low-molecular-weight organic acids derived from the degradation of petroleum contaminants in groundwater. *Geochim. Cosmochim. Acta* **58**, 863–877.

Dearing J. A., Hay K. L., Baban S. M. J., Huddleston A. S., Wellington E. M. H. and Loveland P. J. (1996) Magnetic susceptibility of soil: an evaluation of conflicting theories using a national data set. *Geophys. J. Int.* **127**, 728–734.

Dippon U., Pantke C., Porsch K., Larese-Casanova P. and Kappler A. (2012) Potential function of added minerals as nucleation sites and effect of humic substances on mineral formation by the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp BoFeN1. *Environ. Sci. Technol.* **46**, 6556–6565.

dos Santos Afonso. M. and Stumm W. (1992) Reductive dissolution of iron(III) (hydr)oxides by hydrogen sulfide. *Langmuir* **8**, 1671–1675.

Dunlop D. J. and Özdemir Ö. (1997) *Rock Magnetism: Fundamentals and Frontiers*. Cambridge University Press, Cambridge.

Emmerich M., Bhansali A., Losekann-Behrens T., Schroder C., Kappler A. and Behrens S. (2012) Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of lake Kasin, Southern Russia. *Appl. Environ. Microbiol.* **78**, 4386–4399.

Farina M., Esquivel D. M. S. and Debarros H. (1990) Magnetic iron-sulphur crystals from a magnetotactic microorganism. *Nature* **343**, 256–258.

Fassbinder J. W. E., Stanjek H. and Vali H. (1990) Occurence of magnetic bacteria in soil. *Nature* **343**, 161–163.

Fortin D. and Langley S. (2005) Formation and occurence of biogenic iron-rich minerals. *Earth Sci. Rev.* **72**, 1–19.

Fredrickson J. K., Zachara J. M., Kennedy D. W., Dong H., Onstott T. C., Hinman N. W. and Li S.-M. (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta* **62**, 3239–3257.

Grbić-Galić D. and Vogel T. M. (1987) Transformation of toluene and benzene by mixed methanogenic cultures. *Appl. Environ. Microbiol.* **53**, 254–260.

Guzman O., Costanzo-Alvarez V., Aldana M. and Diaz M. (2011) Study of magnetic contrasts applied to hydrocarbon exploration in the Maturin Sub-Basin (Eastern Venezuela). *Stud. Geophys. Geod.* **55**, 359–376.

Hamamura N., Olson S. H., Ward D. M. and Inskeep W. P. (2006) Microbial population dynamics associated with crude-oil biodegradation in diverse soils. *Appl. Environ. Microbiol.* **72**, 6316–6324.

Hansel C. M., Benner S. G. and Fendorf S. (2005) Competing Fe(II)-induced mineralization pathways of ferrihydrite. *Environ. Sci. Technol.* **39**, 7147–7153.

Hegler F., Posth N. R., Jiang J. and Kappler A. (2008) Physiology of phototrophic iron(II)-oxidizing bacteria: implications for modern and ancient environments. *FEMS Microbiol. Ecol.* **66**, 250–260.

Hohmann C., Winkler E., Morin G. and Kappler A. (2010) Anaerobic Fe(II)-oxidizing bacteria show As resistance and immobilize As during Fe(III) mineral precipitation. *Environ. Sci. Technol.* **44**, 94–101.

Ivakhnenko O. P. and Potter D. K. (2004) Magnetic susceptibility of petroleum reservoir fluids. *Phys. Chem. Earth* **29**, 899–907.

Jiao Y. Y. Q., Kappler A., Croal L. R. and Newman D. K. (2005) Isolation and characterization of a genetically tractable photo autotrophic Fe(II)-oxidizing bacterium, *Rhodopseudomonas palustris* strain TIE-1. *Appl. Environ. Microbiol.* **71**, 4487–4496.

Kappler A. and Straub K. L. (2005) Geomicrobiological cycling of iron. *Rev. Mineral. Geochem.* **59**, 85–108.

Klueglein N. and Kappler A. (2013) Abiotic oxidation of Fe(II) by reactive nitrogen species in cultures of the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp. BoFeN1 – questioning the existence of enzymatic Fe(II) oxidation. *Geobiology* **11**, 180–190.

Konhauser K. O., Kappler A. and Roden E. E. (2011) Iron in microbial metabolisms. *Elements* **7**, 89–93.

Konhauser K. O. (1998) Diversity of bacterial iron mineralization. *Earth Sci. Rev.* **43**, 91–121.

Kostka J. E. and Nealson K. H. (1995) Dissolution and reduction of magnetite by bacteria. *Environ. Sci. Technol.* **29**, 2535–2540.

Kuhn E. P., Zeyer J., Eicher P. and Schwarzenbach R. P. (1988) Anaerobic degradation of alkylated benzenes in denitrifying laboratory aquifer columns. *Appl. Environ. Microbiol.* **54**, 490–496.

Lovley D. R. and Anderson R. T. (2000) Influence of dissimilatory metal reduction on fate of organic and metal contaminants in the subsurface. *Hydrogeol. J.* **8**, 77–88.

Lovley D. R., Stolz, Jr., J. F., Nord G. L. and Phillips E. J. P. (1987) Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature* **330**, 252–254.

Lovley D. R., Baedecker M. J., Lonergan D. J., Cozzarelli I. M., Phillips E. J. P. and Siegel D. I. (1989) Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature* **339**, 297–300.

Marschner B. and Kalbitz K. (2003) Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma* **113**, 211–235.

Masak J., Machackova J., Siglova M., Cejkova A. and Jirku V. (2003) Capacity of the bioremediation technology for clean-up of soil and groundwater contaminated with petroleum hydrocarbons. *J. Environ. Sci. Health A Toxic Hazard. Subst. Environ. Eng.* **A38**, 2447–2452.

McCabe C., Sassen R. and Saffer B. (1987) Occurence of secondary magnetite within biodegraded oil. *Geology* **15**, 7–10.

Mendelssohn I. A., Andersen G. L., Baltz D. M., Caffey R. H., Carman K. R., Fleeger J. W., Joye S. B., Lin Q. X., Maltby E., Overton E. B. and Rozas L. P. (2012) Oil impacts on coastal wetlands: Implications for the Mississippi river delta ecosystem after the deepwater horizon oil spill. *Bioscience* **62**, 562–574.

Moeslund L., Thamdrup B. and Jørgensen B. B. (1994) Sulfur and iron cycling in a coastal sediment: radiotracer studies and seasonal dynamics. *Biogeochemistry* **27**, 129–152.

Moraghan J. T. and Buresh R. J. (1977) Chemical reduction of nitrite and nitrous-oxide by ferrous iron. *Soil Sci. Soc. Am. J.* **41**, 47–50.

Mullins C. E. (1977) Magnetic susceptibility of soil and its significance in soil science – a review. *J. Soil Sci.* **28**, 223–246.

Pantke C., Obst M., Benzerara K., Morin G., Ona-Nguema G., Dippon U. and Kappler A. (2012) Green rust formation during Fe(II) oxidation by the nitrate-reducing *Acidovorax* sp strain BoFeN1. *Environ. Sci. Technol.* **46**, 1439–1446.

Petrovský E. and Ellwood B. B. (1999) Magnetic monitoring of air-, land- and water-pollution. In *Quarternary Climates, Environments and Magnetism* (eds. B. A. Maher and R. Thompson). Cambridge University Press, Cambridge, pp. 279–322.

Piepenbrock A., Dippon U., Porsch K., Appel E. and Kappler A. (2011) Dependence of microbial magnetite formation on humic substance and ferrihydrite concentrations. *Geochim. Cosmochim. Acta* **75**, 6844–6858.

Porsch K. and Kappler A. (2011) Fe^{II} oxidation by molecular O_2 during HCl extraction. *Environ. Chem.* **8**, 190–197.

Porsch K., Dippon U., Rijal M. L., Appel E. and Kappler A. (2010) In-situ magnetic susceptibility measurements as a tool to follow geomicrobiological transformation of Fe minerals. *Environ. Sci. Technol.* **44**, 3846–3852.

Raven K. P., Jain A. and Loeppert R. H. (1998) Arsenite and arsenate adsorption on ferrihydrite: kinetics, equilibrium, and adsorption envelopes. *Environ. Sci. Technol.* **32**, 344–349.

Readman J. W., Fowler S. W., Villeneuve J. P., Cattini C., Oregioni B. and Mee L. D. (1992) Oil and combustion-product contamination of the Gulf marine environment following the war. *Nature* **358**, 662–665.

Rijal M. L., Appel E., Petrovsky E. and Blaha U. (2010) Change of magnetic properties due to fluctuations of hydrocarbon contaminated groundwater in unconsolidated sediments. *Environ. Pollut.* **158**, 1756–1762.

Rijal M. L., Porsch K., Kappler A. and Appel E. (2012) Magnetic signatures of hydrocarbon-contaminated soils and sediments at the former oil-field Hänigsen, Germany. *Stud. Geophys. Geod.* **56**, 889–908.

Roden E. E. and Zachara J. M. (1996) Microbial reduction of crystalline iron(III) oxides: influence of oxide surface area and potential for cell growth. *Environ. Sci. Technol.* **30**, 1618–1628.

Rueter P., Rabus R., Wilkes H., Aeckersberg F., Rainey F. A., Jannasch H. W. and Widdel F. (1994) Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. *Nature* **372**, 455–458.

Schumacher D. (1996) Hydrocarbon-induced alteration of soils and sediments. In *Hydrocarbon Migration and its Near-Surface Expression* (eds. D. Schumacher and M. A. Abrams). AAPG, pp. 71–89.

Sikkema J., Debont J. A. M. and Poolman B. (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* **59**, 201–222.

Stanjek H., Fassbinder J. W. E., Vali H., Wägele H. and Graf W. (1994) Evidence of biogenic greigite (ferrimagnetic Fe_3S_4) in soil. *Eur. J. Soil Sci.* **45**, 97–103.

Stookey L. L. (1970) Ferrozine – a new spectrophotometric reagent for iron. *Anal. Chem.* **42**, 779–781.

Straub K. L., Kappler A. and Schink B. (2005) Enrichment and isolation of ferric-iron- and humic-acid-reducing bacteria. *Methods Enzymol.* **397**, 58–77.

Webb S. M. (2005) SIXPack a graphical user interface for XAS analysis using IFEFFIT. *Phys. Scr.* **T115**, 1011–1014.

Weber K. A., Achenbach L. A. and Coates J. D. (2006) Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat. Rev. Microbiol.* **4**, 752–764.

Zachara J. M., Kukkadapu R. K., Gassman P. L., Dohnalkova A., Fredrickson J. K. and Anderson T. (2004) Biogeochemical transformation of Fe minerals in a petroleum-contaminated aquifer. *Geochim. Cosmochim. Acta* **68**, 1791–1805.

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