Influence of organic matters on AsIII oxidation by the microflora of polluted soils

Tiffanie Lescure, J. Moreau, C. Charles, T. Ben Ali Saanda, Hugues Thouin, N. Pillas, P. Bauda, I. Lamy, Fabienne Battaglia-Brunet

Manuscript submitted to « Environmental Geochemistry and Health »

Influence of organic matters on AsIII oxidation by the microflora of polluted soils

Lescure T.[1,2,3,4], Moreau J.[1], Charles C.[1], Ben Ali Saanda T.[1], Thouin H.[1,2], Pillas N.[1], Bauda P.[3], Lamy I.[5], Battaglia-Brunet F.[1,2*]

[1]BRGM, 3 avenue Claude Guillemin, 45060 Orléans, France

[2]BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France

[3]LIEC UMR 7360 CNRS-Université de Lorraine, Campus Bridoux, rue du Général Delestraint, 57070 Metz, France

[4]Agence de l'Environnement et de la Maîtrise de l'Energie, 20 avenue du Grésillé, BP 90406, 49004, Angers Cedex 01, France

[5]INRA Centre de Versailles-Grignon, RD 10, 78026 Versailles Cedex, France

*Author for correspondence (tel.: +33 238 643 930; fax: +33 238 643 680; e-mail: f.battaglia@brgm.fr)

Abstract

The global AsIII oxidizing activity of microorganisms in eight surface soils from polluted sites were quantified with and without addition of organic substrates. The organic substances provided differed by their nature: either yeast extract, commonly used in microbiological culture media, or a synthetic mixture of defined organic matters (SMOM) presenting some common features with natural soil organic matter. Correlations were sought between soil characteristics and both the AsIII oxidizing rate constants and their evolution in accordance with inputs of organic substrates.

In the absence of added substrate, the global AsIII oxidation rate constant correlated positively with concentration of intrinsic organic matter in the soil, suggesting that AsIII oxidizing activity was limited by organic substrate availability in nutrient-poor soils. This limitation was, however, removed by 0.08 g/L of added organic carbon. In most conditions, the AsIII oxidation rate constant decreased as organic carbon input increased from 0.08 to 0.4 g/L. Incubations of polluted soils in aerobic conditions, amended or not with SMOM, resulted in short term As mobilization in the presence of SMOM and active microorganisms. In contrast, microbial AsIII oxidation seemed to stabilize As when no organic substrate was added. Results suggest that microbial speciation of arsenic driven by nature and concentration of organic matter exerts a major influence on the fate of this toxic element in surface soils.

Key words: arsenic, polluted soils, organic matter, microorganisms, AsIII oxidation

Introduction

High soil concentrations of the toxic element arsenic (As) may be linked to pollution originating from mining and industrial activities, long-term application of As-containing pesticides (Smith et al., 1998) or to the geochemical background. On polluted mining sites, characterized by high concentrations of metal(oid)s and poor fertility, organic amendments are often considered as a means of facilitating phyto-stabilization (Galende et al., 2014). In addition, agricultural soils affected by diffuse As pollution from pesticides can be fertilized with organic matter (Franchini, 2001; Chantigny et al., 2000; Chantigny et al., 2002). Many studies have described the geochemical interactions between arsenic and organic matter: change of As speciation (Redman et al., 2002), formation of soluble complexes (Saada et al., 2003; Redman et al., 2002), competition for sorption sites (Bauer and Blodau, 2006). Conversely, organic matter is also a trophic resource for microorganisms but its influence on microbial activities linked to As is poorly documented, even though the soil microflora plays a major role in geochemical cycles and particularly in As speciation. Bacteria isolated from soils have been shown to oxidize AsIII and/or reduce AsV (Macur et al., 2004; Inskeep et al., 2007; Bachate et al., 2012), or to methylate this toxic metalloid (Huang et al., 2012). Filamentous fungi isolated from contaminated soils are able to reduce AsV and methylate As (Su et al., 2011). Microbial transformations of arsenic in soil have important implications because mobility, toxicity and bioavailability of the element are closely related to its speciation (Smedley and Kinniburgh, 2002). The global AsIII-oxidizing activity of the microflora should tend to reduce the risk of transfer of the toxic element from soil to surface water or groundwater.

Important bacterial mechanisms involved in AsIII/AsV transformations are the *ars* system, whose primary function is detoxification with AsV being reduced by an arsenate reductase ArsC, the *aio* oxidation system through arsenite oxidase, and the *arr* system of AsV dissimilatory reduction in anaerobic conditions (Inskeep et al., 2007). Huang et al. (2012) examined the effect of vegetal organic amendments on arsenic speciation and volatilization in flooded paddy fields. They observed that concentration and type of organic matter had a significant effect on AsIII oxidizing activities and AsV reduction via bacterial *arsC* genes expression. The organic matter amendments stimulated the methylation and volatilization of As. Yamamura et al. (2009) showed that uncontaminated surface soil microflora exhibit both AsIII oxidizing and AsV reducing activities. However, the influence of organic matter on the behavior of As-transforming bacteria in unsaturated soils was not described. As several studies have suggested that organic substances could lower the efficiency of bacterial arsenic oxidation in the presence of oxygen (Challan-Belval et al., 2009; Bachate et al., 2012), there is a need to acquire information about the influence of the nature and concentration of organic matter on As oxidizing activities in polluted soils.

In this context, the present study aimed to determine the influence of organic matters on the AsIII oxidizing activity of complete microflora of polluted soils in aerobic conditions. Activity measurements made in specific liquid media to assess the global level of target microbial metabolisms are usual: this type of measurement has been proposed to quantify PAH biodegradation rate in soils (Kästner and Mahro, 1996), thiosulfate-oxidizing activity in paddy fields (Stubner et al., 1998), denitrification (Buys et al., 2000), anaerobic oxidation of ammonium (Dapena-Mora, 2007), and FeII oxidizing activity (Senko et al., 2008). For this study, AsIII oxidizing activities were monitored in a previously optimized mineral liquid culture medium (Battaglia-Brunet et al., 2002) to which two organic substrates differing in their composition, i.e. yeast extract or a synthetic mixture of defined molecules (SMOM) were added at two different concentrations. The composition of the yeast extract is not defined exactly but is close to that of living cells; it is also a source of vitamins that might stimulate the growth of some microorganisms. SMOM is a well-defined complex mixture of molecules displaying some of the main characteristics of natural soil organic matter such as C/N ratio and supply of phenolic and carboxylic groups that may interact with inorganic soil components. Thus, a substrate usually used in microbiology (yeast extract) and an organic amendment designed to present common features with natural organic matter (SMOM) were compared in terms of influence on AsIII biooxidation in conditions of equivalent organic carbon concentrations. The AsIII oxidizing rate constants were correlated with the presence of organic substrates and characteristics of eight polluted soils (physico-chemical, structural and biological features). A study was then made of the combined influence of microbial activities and amendment with complex organic substrates on the speciation and mobilization of intrinsic arsenic in the four most polluted soils.

Material and methods

Soil sampling and characterization

Eight different soils were sampled on three highly polluted sites that could be candidates for the application of aided phyto-stabilization. All materials were surface, non-saturated soils (0–15 cm). Four different soils were sampled in and around the Cheni disused gold mine site (Limousin; 45°32'59.90"N, 1°09'39.04"E): in a cultivated field, a meadow, a forest and on the site near the arsenic-containing waste dump. Two brownfield soil samples were taken from the Auzon disused industrial site (Haute-Loire; 45°23'13.67"N, 3°21'24.32"E) and two brownfield soil samples were collected on the Salsigne disused gold mine site (Aude; 43°31’89.25"N, 2°38’11.07"E and 43°31’52.75"N, 2°38’73.83"E). The soils were sampled in sterile glass jars, sieved at 2 mm in sterile sieves then stored at 5°C in sterile glass jars. Their water content was determined by drying at 50°C for 24h. Mineral carbon and carbonates were determined by volumetric method per NF ISO 10693. Total carbon was analyzed per NF ISO 10694. After drying and hand grinding in a mortar, total

concentrations of As, Pb, Cd, Zn and Fe were determined in the soils using a portable NITON© X-ray fluorescence field analyzer (XLT999KWY, bulk mode, counting time 60s). Values for the elements Ag, U, Se, Hg, Cu, Ni, Cr and V were below the detection limit. Total As was also analyzed in all soils by Atomic Absorption Spectrophotometry after total digestion, per NF EN 13346. AsIII and AsV were analyzed by HPLC-ICP-MS after extraction with 10 mL of H_3PO_4 1M added to 0.4 g of sample and microwave heating (Vergara Gallardo et al., 2001) in a closed system at 120°C for 20 min. The remaining solution was diluted to 50 mL with ultrapure water and then analyzed with HPLC-ICP-MS, using quantification by standard additions to avoid matrix effects. Arsenic species separation was performed using an anion exchange column (Hamilton PRPX-100) and a mobile phase of ammonium hydrogen phosphate 15 mM at pH 8.5 (Thomas et al., 1997). The percentage of AsIII (%AsIII) was calculated as [AsIII]x100/[total As]. Total nitrogen was analyzed by the Kjeldhal method per NF EN 25663. Granulometry was determined per NF X 31.107, total phosphorus per NF X 31.161, K_2 per NF X 31.108 and pH per NF ISO 10390. The biodegradability of intrinsic organic matter was studied via CO_2 emission (soil respiration), per Rey et al., 2005.

Living bacteria were enumerated by microscopy using the Live/Dead® kit (BacLight™ Viability L-13152 Molecular Probes, Invitrogen) per Pascaud et al. (2009). The soils (stored at 5°C) were incubated at ambient temperature for 72 h and then suspended at 1% in sodium pyrophosphate and agitated reciprocally for 15 min, sonicated 2 x 20 s at 45 kHz. The suspension was settled for 1 min, 1 mL of the supernatant was then sampled for filtration, application of coloration and observation with a Zeiss Axio Imager Z1 microscope equipped with UV HBO lamp. Living bacteria, exhibiting green fluorescence with the FITC filter, were enumerated on 10 independent fields (each of 5,800 μm^2). Average cell concentrations were calculated from volume of sample used and known filter area.

The characteristics determined for the eight soils samples are detailed in Table 1.

AsIII oxidizing activity tests

The basal culture medium was the CAsO1 medium (pH 6) described in Battaglia-Brunet et al. (2002). This was supplemented with 1 mM AsIII. The soils (stored at 5°C) were incubated at 25°C for 72 h before starting the tests which were performed in 250 mL Erlenmeyer flasks filled with 100 mL medium and plugged with cotton. Each flask was inoculated with a mass of soil equivalent to 0.2 g dry weight. Flasks were incubated at 25°C under reciprocal agitation (150 rpm). The flasks were sampled (3 mL) twice a day; samples were filtered at 45 µm and frozen at -20°C until AsIII/AsV separation was performed. The AsIII oxidation activities were analyzed in (i) simple basal medium, (ii) basal medium amended with 0.2 g/L or 1 g/L of yeast extract (Sigma), and (iii) basal medium amended with two concentrations (0.5 g/L or 2.5 g/L corresponding to equivalent carbon concentrations as 0.2 and 1 g/L of yeast extract, i.e. 0.08 and 0.4 g/L of carbon) of a Synthetic Mixture of Organic Matter

(SMOM). The SMOM was designed to mimic the average composition of soil organic matter in terms of C/N ratio, functional groups, amino-acids and sugars, on the basis of data available from the International Humic Substances Society's website (IHSS – http://www.humicsubstances.org). It contained the following molecules: L-arginine (3.2% dry weight), L-aspartic acid monopotassium salt (2.5%), L-glutamic acid monosodium salt monohydrate (3.0%), glycine (1.2%), L-histidine (0.3%), L-isoleucine (0.7%), L-leucine (0.9%), methionine (0.3%), L-phenylalanine (0.6%), L-serine (1.3%), L-threonine (0.4%), L-tyrosine (0.4%), L-valine (1.5%), succinic acid disodium salt hexahydrate (19.1%), acetic acid (5.6%), propionic acid (2.8%), sodium tartrate dehydrate (8.3%), valeric acid (3.5%), calcium formate (3.7%), citric acid monohydrate dibasic (5.4%), butyric acid (2.1%), oxalic acid (0.8%), palmitic acid (6.7%), gallic acid (3.9%), vanillic acid (3.2%), 4-hydroxybenzoic acid (1.7%), (+)-catechin hydrate (2.9%), protocatechuic acid ethyl (1.2%), *trans*-ferulic acid (1.0%), *p*-coumaric acid (0.1%), rutin (5.0%), D-glucose (2.3%), D(+)-galactose (1.1%), D(+)-mannose (1.1%), D(+)-fucose (0.2%), L-rhamnose (0.8%), D(-)-arabinose (0.5%), D(-)-ribose (0.1%), D(+)-xylose (0.8%). The pH of the mixture was adjusted to that of the liquid medium (pH 6). The SMOM concentrations provided the same organic carbon concentration as the 0.2 and 1 g/L of yeast extract, with 1 g/L yeast extract corresponding to 0.4 g/L of organic carbon (Holwerda et al., 2012). The conditions were therefore 0.08 and 0.4 g/L of added organic carbon for both yeast extract and SMOM.

Incubations were performed in triplicate. The following controls were performed: as all experiments could not be carried simultaneously, the absence of effect of soil storage at 5°C was verified by repeating the test at the beginning and at the end of the study with one of the polluted soils. Abiotic AsIII oxidation controls were performed in the absence of soil inoculation and with sterile soils. For these latter controls, soils were sterilized by autoclaving four times at 120°C, 1h, at 24 h intervals.

AsV was quantified by flame atomic absorption spectrophotometry, after AsV/AsIII separation with the PDC/MIBK method (Battaglia-Brunet, 2002).

First order AsIII oxidizing rate constants were determined by linear least squares regression fitting of the ln[AsV] versus time line, using the following equation:

$$\mathrm{Ln[AsV]} = kt + \mathrm{Constant} \quad (1)$$

Principal Component Analysis

Statistical analyses were carried out using the XLSTAT 2014 software (Addinsoft, version 16.2.01.6189). Pearson correlations were calculated with all soils parameters, and eight independent soil parameters – selected as those correlating most strongly with AsIII oxidizing rate constants and their ratios (Electronic Supplementary material ESM1) – were subjected to a principal component analysis (PCA). Rate constants and their ratios were integrated as supplementary data.

Soil incubations without addition of AsIII

The four most polluted soils in terms of As concentration were selected for incubation: Auzon 1, Auzon 2, Cheni site and Cheni forest. Experiments were performed in 60 mL flasks with cotton stoppers (aerobic conditions). Slurries were prepared by mixing a mass of soil corresponding to 2.5 g (dry weight) and 25 mL of spring water (Montcalm, Ca 3 mg/L, Mg 0.7 mg/L, Na 2.2 mg/L, K 0.6 mg/L, SO_4 10 mg/L, HCO_3 5.2 mg/L, NO_3 0.7 mg/L, Cl 0.6 mg/L, pH 6.8). Control blanks were prepared with soils sterilized as described in part 2.2. Spring water was autoclaved (120°C, 20 min). Incubations were performed with and without addition of SMOM at 0.4 g/L of organic carbon. For each condition (blank and experiment, with and without SMOM), six flasks were prepared. One triplicate was sacrificed after 15 min of incubation and the remaining flasks were incubated for 7 days at 25°C, under reciprocal agitation (150 rpm). When the incubations were sacrificed, 10 mL of slurry were filtered at 0.45 µm; 2.5 mL of this filtrate were used immediately for AsIII/AsV separation on resin (Ficklin, 1983), the remainder was acidified with 50 µL HCl 37% for flame AAS analysis of total Fe. Separated AsIII and AsV were quantified by graphite furnace AAS (detection limit 20 µg/L). Samples of biotic soil slurries were taken after 15 min and 7 days of incubation and stored at -20°C. For biomolecular analyses, genomic DNA was extracted from the -20°C stored soil slurries using the FastDNA® Spin Kit for Soil (Bio101). The community structure and its evolution were monitored by two methods, (1) Capillary electrophoresis–terminal restriction fragment length polymorphism (CE–T-RFLP) diversity analysis of the 16S rRNA gene, as described by Mercier et al. (2013) and (2) Capillary Electrophoresis Single-Strand Conformation Polymorphism (CE-SSCP, Delbès et al., 2000). CE-SSCP analyses were performed with an ABI Prism 310 genetic analyzer using a 47 cm long capillary, a non-denaturating 5.6% CAP polymer (Applied Biosystems). CE-SSCP electrophoregrams were analyzed using the StatFingerprints Version 2 software (Michelland et al., 2009). For total bacteria enumeration, bacteria were separated from soil particles and from eukaryotes using the methods validated by Lindahl and Bakken (1995), Lindahl (1996) and Bertrand et al., (2005), including a separation of bacteria from soil particles using the Nycodenz gradient method. The final pellet was suspended in 1 mL of NaCl 0.8%, mixed with 1 mL of absolute ethanol and stored at -20°C. Details about the methods are given in the Electronic Supplementary Material ESM4. Bacteria were enumerated after fluorescent staining, as described in Kumar et al. (2013).

Results and discussion

AsIII-oxidizing activity measurements

Experiments were performed in order to evaluate the influence of some soil characteristics on the AsIII oxidizing activity of microbes, with and without added organic matter. Controls showed no abiotic oxidation in sterile media nor with autoclaved soils (data not shown). Storage at 5°C did not

significantly influence the AsIII oxidation rate (Electronic Supplementary material ESM2). The kinetics of AsIII oxidation differed between soils. Contrasting kinetics according to nature and concentration of added substrates obtained with three soils are shown in Fig.1. With the Cheni forest soil, high AsIII oxidation rate was obtained without added organic matter (Fig. 1a). With the Cheni site soil (Fig. 1b), results were clearly grouped according to the type of organic matter added and the kinetic was slower without added organic matter. With Cheni meadow soil (Fig. 1c), the higher AsIII oxidizing rates were obtained at 0.08 g/L of added organic matter, the highest being observed with SMOM. The other kinetics are shown in Electronic Supplementary Material ESM3. The time preceding AsIII oxidation varied between 0 and 50 hours, and AsIII was entirely oxidized within 200 hours of incubation.

Many bacteria isolated from soils have been shown to oxidize AsIII (Macur et al., 2004; Inskeep et al., 2007; Bachate et al., 2012) and AsIII oxidation by complete soil microflora was evidenced by Yamamura et al. (2009). The bacterial AsIII oxidation is linked to the expression of *aio* genes that were evidenced in soil bacteria (Huang et al, 2012; Poirel et al., 2013).

When no organic substance was added to the medium (C0) and according to the PCA with rate constants as supplementary variables (Fig. 2), the AsIII oxidation rate constant correlated positively with organic carbon and humidity. The highest significant Pearson correlation coefficients were obtained between C0 and the intrinsic soil organic matter parameters (C, N and respiration) (Table 2). When the culture medium was enriched with organic substrates, the rate constants no longer correlated with intrinsic soil organic matter parameters (Fig. 2 and Table 2), with the exception of a positive significant correlation between C0.4 SMOM and the C/N ratio (Table 2)..

Evolution of the AsIII oxidation rate constants when organic matter was introduced in the medium was examined through calculation of the ratio of rate constants, C0/C0.08 and C0.08/C0.4. The evolution of these ratios according to soil respiration enlightens the influence of nature and concentration of organic matter on AsIII oxidation rate constants (Fig. 3). Soil respiration is linked to the intrinsic biodegradable organic matter. The C0/C0.08 ratio is very similar for yeast extract and SMOM (Fig. 3a), and lower than 1 for all soils with the exception of the Cheni forest soil which presents the highest respiration level. Thus, the addition of organic substances at 0.08 g/L of organic carbon exerted a positive effect on AsIII oxidation rate constants, except when the soil contained enough biodegradable organic matter. These results suggest that the AsIII oxidation rate was limited by the availability of organic matter in natural conditions when no organic substrate was added to the liquid medium, and this limitation was removed by supplying 0.08 g/L of available organic carbon, provided by either yeast extract or SMOM. Conversely, the C0.08/C0.4 ratio was higher than 1 in all conditions except when yeast extract was added to media inoculated by the four soils with the lowest respiration levels (Fig. 3b). In all other conditions, increasing the organic substrate concentration from

0.08 to 0.4 g/L of organic carbon induced a decrease of the AsIII oxidation rate constant. But the influence of yeast extract on the AsIII oxidizing rate differed from that of SMOM. SMOM exerted a negative effect on AsIII oxidizing rate, more pronounced than that of yeast extract, in particular with the soils with lower respiration levels.

The PCA with ratios as supplementary variables (Fig. 4) showed that the C0/C0.08 ratios obtained with yeast extract and SMOM are located very close to one another on the graph. The positive correlation between C0/C0.08 ratio and intrinsic organic matter concentrations in soils, for both types of added organic substrates, is confirmed by the significant values of the correlation coefficients (Table 2). Conversely, the C0.08/C0.4 ratio no longer correlated with intrinsic soil organic matter, and behaved differently for yeast extract and SMOM (Fig. 4). This ratio correlated negatively with pH for yeast extract and positively with this parameter for SMOM (Tables 2 andFig. 4). However, these correlations, although significant, were weaker than the positive link between C0/C0.08 and the intrinsic organic matter.

Significant correlations between soils parameters are detailed in Table 3. Living bacteria concentration correlated significantly with respiration, C, N and P, indicating that living bacteria concentration was positively correlated with the intrinsic organic matter of the soils. However, the correlation between living bacteria and AsIII-oxidizing rate constants parameters did not reach the significance level (Table 2), even if results have the same pattern as that between intrinsic organic matter and AsIII-oxidizing rate constants parameters. This weakness of significance may be linked to a variable proportion of living As-transforming bacteria between soils.

The only parameters that influenced significantly the AsIII-oxidation rates constants and their ratios were either linked to organic matter, i.e. N, organic C, respiration and C/N, or pH (Table 2). One of the main parameters influencing soil biogeochemistry and microbial activities is pH (Rousk et al., 2011; Whittinghill and Hobbie, 2012), thus it is not surprising to enlighten an influence of this parameter on microbial As-related activities. Concerning specifically the soil organic matter properties, their significant influence on the AsIII oxidizing rate without added organic substrate (C0) decreased in the following order: total N > org. C > respiration, and the C/N ratio significantly influenced the C0.4 SMOM rate constant. These results suggest that microbial As oxidation may be limited not only by organic C but also by nitrogen. Thus, nitrogen-rich fraction of soil organic matter might influence As-related microbial activities. The C/N ratio in yeast extract is 4.25, whereas it is higher in SMOM (15.51) and in intrinsic organic matter of the eight soils (10 to 23). If the global soil respiration exerted a significant influence on AsIII-oxidizing rate constants, the proportion of biodegradable organic substances in soil organic matter was not related to any AsIII-oxidation related parameter (Table 2).

The soil microbial communities include organisms able to oxidize AsIII and/or reduce AsV (Macur et al., 2004), even in unpolluted environments (Yamamura et al., 2009).The AsIII oxidation rate constant is thus linked to the global activity of all microorganisms involved in As speciation. The global rate constant value should be linked to the following parameters, whose relative contributions have not been quantified: the cell density of As-transforming organisms, their physiological state and growth rate, and the kinetic parameters of the reactions (maximum rate and Km) for each organism.

The AsIII-oxidizing activity in the soils also depends on the diversity and density of the different types of microorganisms, parameters that themselves depend on the environmental conditions of soils. The present study was focused on a global activity test that integrates all these variables. Such global activity measurements were developed and applied for other types of soil microbial activities: PAH biodegradation rate (Kästner and Mahro, 1996), thiosulfate-oxidizing activity in paddy fields (Stubner et al., 1998), and soil respiration, that integrates CO_2 production and consumption (Rey et al., 2005) as we integrate here AsIII production and consumption.

To date, all AsIII-oxidizing bacteria isolated from soils have been either heterotrophs (Macur et al., 2004; Bachate et al., 2012; Bahar et al., 2013) or facultative autotrophs (Santini et al., 2002, Inskeep et al., 2007; Garcia-Dominguez et al., 2008; Dong et al., 2014). The availability of organic substrates should therefore favor increase of cell density of AsIII-oxidizing bacteria. This might almost partly explain the positive effect of organic matters on the AsIII oxidizing rate constants between 0 and 0.08 g/L of carbon, and the positive correlation of the constant with intrinsic organic matter in the without-amendment condition.

The growth with yeast extract or SMOM of two AsIII-oxidizing bacterial strains of contrasting AsIII metabolisms was verified in the conditions of the AsIII-oxidizing tests (data not shown). Both strains grew with both substrates, however they displayed different behaviors with the SMOM: the mixotrophic strain using AsIII as energy source presented a higher growth yield at 0.08 g/L of added C, whereas the heterotrophic organism showed a higher growth yield at 0.4 g/L of added carbon.

Conversely, high concentrations of organic substrates may decrease the specific AsIII-oxidizing activity of bacteria (Challan-Belval, 2009; Bachate et al., 2012) and may also stimulate the aerobic AsV-reducing activity of soil microorganisms (Yamamura et al., 2009). These latter phenomena may explain the decrease in global AsIII-oxidizing rate constants between 0.08 and 0.4 g/L of added organic carbon observed in most of the conditions. In a liquid medium containing 0.5 g/L yeast extract and 1.8 g/L lactate (i.e. 0.92 g/L organic C), Yamamura et al. (2009) observed rapid reduction of 1 mM AsV by unpolluted soil inocula, thus demonstrating the presence of active AsV-reducing microorganisms that may influence As speciation simultaneously with AsIII-oxidizing ones. Moreover, most AsIII-oxidizing bacteria have both oxidizing and reducing systems. In these

experiments, different groups of soil microorganisms may have been favored in each condition (0, 0.08 and 0.4 g/L of added carbon): according to van Gestel et al. (1993), autochthonous soil microorganisms are adapted to survive in soils containing recalcitrant material, where no abundant supply of easily oxidizable substrate occurs. These organisms exhibit K-selected behavior, with moderate growth rate and moderate nutrient demands, and are able to use diverse, complex materials (Odum, 1969; Metting, 1993; Langer et al., 2004). In contrast, zymogenous organisms show rapid growth when high energy-containing nutrients are added to soils (Paul and Clark, 1996), exhibiting r-selected behavior, characterized by rapid growth rate and use of simple and readily available substrates. Individual organisms may exhibit both r- and K-selected behavior, however amendment with organic substances should induce a change of behavior in soil microflora. The difference of effects exerted by yeast extract and SMOM at 0.04 g/L carbon may be related to the distinct composition of the two substrates. The r-strategy should be more prevalent in the presence of yeast extract than with the SMOM, whose composition includes some molecules that are not easily biodegradable. Soil incubations without added As

The experiment aimed to evaluate the short term (7 days) influence of amendment with SMOM (0.4 g/L organic carbon) on the mobility of arsenic present in the four most polluted soils, in relation to microbial activity. The incubations remained aerobic throughout the experiment (positive redox potential, Electronic Supplementary Material ESM4). Similar tendencies were observed with the four soils. When microbes were alive and without added SMOM, no arsenic was mobilized in the liquid phase (Fig. 5a). Conversely, in the presence of SMOM, arsenic was solubilized, and this mobilization was significantly higher when the microbes were alive than in the abiotic controls. Regarding the speciation of As in the liquid phase after seven days of incubation (Fig. 5b), AsIII was never detected in the absence of SMOM when microbes were alive. Conversely, AsIII was always detected in biotic conditions with SMOM, in proportion equivalent to that of the abiotic incubations (but with a higher variability between replicates).Present results showed mobilization of arsenic from soils contaminated for decades in presence of active bacteria and added organic matter, in aerobic condition. Iron concentration was increased by addition of SMOM in the soil slurries (Fig. 5c), either in biotic and abiotic conditions. This phenomenon was probably linked to the increase of Fe solubility through complexation with dissolved organic molecules: as a fact, SMOM contains some organic acids, such as citric and oxalic acids, known to chelate iron (Zhang et al., 1985). Whereas Fe concentration increased during incubation in biotic conditions, iron concentration always remained lower in biotic than abiotic conditions, contrary to total arsenic whose release in solution, with SMOM, was clearly higher in biotic than abiotic conditions. Thus, biological reduction of iron does not seem to represent the major mechanism inducing As mobilization. The organic molecules present in the SMOM and their organic degradation products may have mobilized some As by chemical complexation: arsenite and arsenate form aqueous complexes with humic acid and natural organic matter in the presence of

bridging metals (Redman et al., 2002; Ko et al., 2004; Kim et al., 2015). In addition, SMOM probably influenced the microbial speciation of As, either decreasing AsIII-oxidizing activity and/or stimulating the AsV-reducing heterotrophs as observed by Yamamura et al. (2009) when they incubated uncontaminated soils spiked with AsV and glucose. Incubating sediments sampled from a disused mine in aerobic conditions, Lee et al. (2005) observed bio-stimulation of As mobility by addition of acetate and lactate, but not by glucose, and linked the behavior of As to evolution of pH associated to the metabolism of organic substrates. Here, pH tended to increase in biotic conditions with SMOM (Table ESM4-1), which may have caused desorption of AsV According to Dixit and Hering (2003), sorption of AsV onto iron oxides decreases when pH increases, contrary to AsIII, whose sorption should be favored by pH increase within the pH range of present experiments (pH 4.5 to 7.5). Thus, increase of pH may explain the increase of total As concentration but not that of AsIII.

In addition to influencing microbial speciation of As, presence of SMOM might also have exerted a priming effect (Hamer and Marschner, 2002), stimulating the biodegradation of the intrinsic organic matter of the soils and thus mobilizing some arsenic associated with natural organic matter.

The bacterial community structure and diversity of the soils amended or not with SMOM were assessed. The four soils without added organic matter had a constant bacterial diversity profile showing that our incubation procedure alone did not change these characteristics (Fig. ESM4-1 a). When the soils were amended with SMOM, bacterial diversity changed during incubation and converged at the end of incubation for all soils. (Fig. ESM4-1 b). SMOM did not affect the bacterial diversity in the same way for all soils: it was decreased for the two soils that had the highest initial diversity, and was maintained or slightly increased for the two soils with lower initial diversity. In terms of total bacterial concentration, the mixture induced an increase in the bacterial cell concentration with the Cheni Site soil, however, for the other soils, bacterial counts did not differ with and without addition of the mixture (Fig. ESM4-2). The convergence of the SSCP fingerprint profiles for all amended soils may be related to the selective development of bacteria able to metabolize the most easily biodegradable compounds of the SMOM, as previously observed by Goldfarb et al. (2011), or may have resulted from the buffering effect of metabolized SMOM on pH. Nevertheless, the amendment of soils with the mixture did not result in the emergence of a few dominant species; it allowed the persistence of a diverse bacterial community, and did not significantly modify the total bacterial concentration.

Conclusions

Results suggest that the global AsIII-oxidizing activity of microorganisms in polluted soils is linked to availability of biodegradable organic substrates. AsIII oxidation can be stimulated by some input of organic matter when the natural organic content of the soil is low; the limitation being already removed in the presence of 0.08 g/L organic carbon. Higher intakes of organic substances no longer

stimulate AsIII oxidation and, conversely, may induce short term As mobilization through decrease of bacterial AsIII oxidation, stimulation of AsV-reducing organisms and formation of soluble As complexes with organic molecules. In most natural conditions, AsV has lower mobility than AsIII and the speciation of As in soils therefore influences its mobility. These phenomena should be given careful consideration when designing efficient management strategies for highly polluted sites and agricultural lands affected by diffuse As contamination.

Acknowledgements

This work was supported by the French Environment and Energy Management Agency (ADEME), BRGM (convention TEZ 11-16), and the Labex Voltaire (ANR-10-LABX-100-01). The authors gratefully acknowledge Catherine Joulian for expert judgment concerning SSCP profiles and technical support from Dominique Breeze and Pascal Auger.

References cited

Bachate, S. P., Khapare, R. M., Kodam, K. M. (2012). Oxidation of arsenite by two β-proteobacteria isolated from soils. Applied Microbiology and Biotechnology, 93, 2135-2145.

Bahar, M. M., Megharaj, M., Naidu, R. (2013). Kinetics of arsenite oxidation by *Variovorax* sp. MM-1 isolated from a soil and identification of arsenite oxidase gene. Journal of Hazardous Materials, 262, 997-1003.

Battaglia-Brunet, F., Dictor, M. C., Garrido, F., Crouzet, C., Morin, D., Dekeyser, K., Clarens, M., Baranger, P. (2002). An arsenic(III)-oxidizing bacterial population: selection, characterisation, and performance in reactors. Journal of Applied Microbiology, 93, 656-667.

Bauer, M., Blodau, C. (2006). Mobilization of arsenic by dissolved organic matter from iron oxides, soils and sediments. Science of the Total Environment, 354, 179-190.

Bertrand, H., Poly, F., Van, V. T., Lombard, N., Nalin, R., Vogel, T. M., Simonet, P. (2005) High molecular weight DNA recovery from soils prerequisite for biotechnological metagenomic library construction. Journal of Microbiological Methods, 62, 1-11.

Buys, B. R., Mosquera-Corral, A., Sanchez, M., Mendez, R. (2000). Development and application of a denitrification test based on gas production. Water Science and Technology, 41, 113-120.

Challan-Belval, S., Garnier, F., Michel, C., Chautard, S., Breeze, D., Garrido, F. (2009). Enhancing pozzolana colonization by As(III)-oxidizing bacteria for bioremediation purposes. Applied Microbiology and Biotechnology, 84, 565-573.

Chantigny, M. H., Angers, D. A., Beauchamps, C. J. (2000). Decomposition of de-inking paper sludge in agricultural soils as characterized by carbohydrate analysis. Soil Biology and Biochemistry, 31, 543-550.

Chantigny, M. H., Angers, D. A., Rochette, P. (2002). Fate of carbon and nitrogen from animal manure and crop residues in wet and cold soils. Soil Biology and Biochemistry, 34, 509-517.

Dapena-Mora, A., Fernandez, I., Campos, J. L., Mosquera-Corral, A., Mendez, R., Jetten, M. S. M. (2007). Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. Enzyme and Microbial Technology, 40, 859-865.

Delbès, C., Moletta, R., Godon, J. J. (2000) Monitoring of Activity Dynamics of an Anaerobic Digester Bacterial Community Using 16S rRNA Polymerase Chain Reaction-Single-strand Conformation Polymorphism Analysis. Environmental Microbiology, 2, 506-515.

Dixit, S., Hering, J. G., (2003). Comparison of Arsenic(V) and Arsenic(III) sorption onto iron oxide minerals: implications for arsenic mobility. Environmental Science & Technology, 37, 82-4189.

Dong, D., Ohtsuka, T., Tao Dong, D., Mamachi, S. (2014). Arsenite oxidation by a facultative chemolithoautotrophic *Sinorhizobium* sp. KGO-5 isolated from arsenic-contaminated soil. Bioscience, Biotechnology, and Biochemistry, 78, 1963-1970.

Ficklin, W. H. (1983). Separation of arsenic(III) and arsenic(V) in ground waters by ion-exchange. Talanta, 30, 371-373.

Franchini, J. C., Gonzalez-Vila, F. J., Cabrera, F., Miyazawa, M., Pavan, M. A. (2001). Rapid transformations of plant water-soluble organic compounds in relation to cation mobilization in an acid oxisol. Plant and Soil, 231, 55-63.

Galende, M. A., Becerril, J. M., Barrutia, O., Artetxe, U., Garbisu, C., Hernandez, A. (2014). Field assessment of the effectiveness of organic amendments for aided phytostabilization of a Pb-Zn contaminated mine soil. Journal of Geochemical Exploration, 145, 181-189.

Garcia-Dominguez, E., Mumford, A., Rhine, E., Paschal, A., Young, L. Y. (2008). Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. FEMS Microbiology Ecology, 66, 401-410.

van Gestel, M., Merckx, R., Vlassak, K. (1993). Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing microorganisms in soils from different climates. Soil Biology and Biochemistry, 25, 109-123.

437 Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K.,
438 Wallenstein, M. D., Brodie, E. L. (2011). Differential Growth Response of Soil Bacterial Taxa to
439 Carbon Substrates of Varying Chemical Recalcitrance. Frontiers in Microbiology, Vol. 2, Article 94.
440 doi 10.3389/fmicb.2011/00094.

441 Hamer, U., Marschner, B. (2002). Priming effects of sugars, amino acids, organic acids and catechol
442 on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science, 165, 261-268.

443 Holwerda, E. K., Hirst, K. D., Lynd, L. R. (2012). A defined growth medium with very low
444 background carbon for culturing *Clostridium thermocellum*. Journal of Industrial Microbiology &
445 Biotechnology, 39, 943-947.Huang, H., Jia, Y., Sun, G. X., Zhu, Y. G. (2012). Arsenic speciation and
446 volatilization from flooded paddy soils amended with different organic matters. Environmental
447 Science & Technology, 46, 2163-2168.

448 Inskeep, W. P., Macur, R. E., Hamamura, N. H., Warelow, T. P., Ward, S. A., Santini, J. M. (2007).
449 Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. Environmental
450 Microbiology, 9, 934-943.

451 Kästner, M., Mahro, B. (1996). Microbial degradation of polycyclic aromatic hydrocarbons in soils
452 affected by the organic matrix of compost. Applied Microbiology and Biotechnology, 44, 668-675.

453 Kim, E. J., Hwang, B.-R., Baek, K. (2015). Effects of natural organic matter on the coprecipitation of
454 arsenic with iron. Environmental Geochemistry and Health, DOI 10.1007/s10653-015-9692-1.

455 Ko, I., Kim, J.-Y., Kim, K.-W. (2004). Arsenic speciation and sorption kinetics in the As–hematite–
456 humic acid system. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 234, 43–50.

457 Kumar, N., Millot, R., Battaglia-Brunet, F., Négrel, P., Diels, L., Rose, J., Bastiaens, L. (2013) Sulfur
458 and Oxygen Isotope Tracing in Zero Valent Iron Based In situ Remediation System for Metal
459 Contaminants. Chemosphere 90, 1366-1371.

460 Langer, U., Böhme, L., Böme, F. (2004). Classification of soil microorganisms based on growth
461 properties: a critical view of some commonly used terms. Journal of Plant Nutrition and Soil Science,
462 167, 267-269.

463 Lee, J. U., Lee, S. W., Kim, K. W., Yoon, C. H. (2005). The effects of different carbon sources on
464 microbial mediation of arsenic in arsenic-contaminated sediment. Environmental Geochemistry and
465 Health, 27, 159-168.

466 Lindahl, V. (1996) Improved soil dispersion procedures for total bacterial counts, extraction of
467 indigenous bacteria and cell survival. Journal of Microbiological Methods, 25, 279-286.

468 Lindahl, V., Bakken, L. R. (1995) Evaluation of Methods for Extraction of Bacteria from Soil. FEMS
469 Microbiology Ecology, 16, 135–142.

470

471 Macur, R. E., Jackson, C. R., Botero, L. M., McDermott, T., Inskeep, W. P. (2004). Bacterial
472 populations associated with the oxidation and reduction of arsenic in an unsaturated soil.
473 Environmental Science & Technology, 38, 104-111.

474 Mercier, A., Wille, G., Michel, C., Harris-Hellal, J., Amalric, L., Morlay, C., Battaglia-Brunet, F.
475 (2013) Biofilm formation vs PCB adsorption on granular activated carbon in a PCB contaminated
476 aquatic sediment. Journal of Soils and Sediments, 13, 793-800.

477 Metting, F. B. Jr. (1993). Soil microbial ecology. Marcel Dekker Inc., New York, p. 11-17.

478 Michelland, R. J., Dejean, S., Combes, S., Fortun-Lamothe, L., Cauquil, L. (2009) StatFingerprints: A
479 Friendly Graphical Interface Program for Processing and Analysis of Microbial Fingerprint Profiles.
480 Molecular Ecology Resources, 9, 1359–1363.

481 Odum, E. P. (1969). The strategy of ecosystem development. Science, 164, 262-270.

482 Pascaud, A., Amellal, S., Soulas, M.-L., Soulas, G. (2009). A fluorescence-based assay for measuring
483 the viable cell concentration of mixed microbial communities in soil. Journal of Microbiological
484 Methods, 76, 81-87.

485 Paul, E., Clark, F. (1996). Soil microbiology and biochemistry. Academic Press, San Diego, p. 69-74.

486 Poirel, J., Joulian, C., Leyval, C., Billard, P. (2013) Arsenite-induced changes in abundance and
487 expression of arsenite transporter and arsenite oxidase genes of a soil microbial community. Research
488 in Microbiology, 164, 457-465.Redman, A. D., Macalady, D. L., Ahmann, D. (2002). Natural organic
489 matter affects arsenic speciation and sorption onto hematite. Environmental Science & Technology,
490 36, 2889-96.

491 Rey, A., Petsikos, C., Jarvis, P.G., Grace, J. (2005). Effect of temperature and moisture on rates of
492 carbon mineralization in a Mediterranean oak forest soil under controlled and field conditions.
493 European Journal of Soil Science, 56, 589–599.

494

495 Rousk, J., Brookes, P. C., Bååth, E. (2011). Fungal and bacterial growth response to N fertilization and
496 pH in the 150-year 'Park Grass' UK grassland experiment. FEMS Microbiology Ecology, 76, 89-99.

497 Saada, A., Breeze, D., Crouzet, C., Cornu, S., Baranger, P. (2003). Adsorption of arsenic(V) on
498 kaolinite and on kaolinite-humic acid complexes. Role of humic acid nitrogen groups. Chemosphere,
499 51, 757-763.

500 Santini, J. M., Wen, L. I. S. A., Comrie, D., de Wulf-Durand, P., Macy, J. M. (2002). New arsenite-
501 oxidizing bacteria isolated from Australian gold mining environments - phylogenetic relationships.
502 Geomicrobiology Journal, 19, 67-76.

503 Senko, J. M., Wanjugi, P., Lucas, M., Bruns, M. A., Burgos, W. D. (2008). Characterization of Fe(II)
504 oxidizing bacterial activities and communities at two acidic Appalachian coalmine drainage-impacted
505 sites. The ISME Journal, 2, 1134-1145.

506 Smedley, P. L., Kinniburg, D. G. (2002). A review of the source, behavior and distribution of arsenic
507 in natural waters. Applied Geochemistry, 17, 517-568.

508 Smith, E., Naidu, R., Alston, M. M., (1998). Arsenic in the soil environment: a review. Advances in
509 Agronomy, 64, 149-195.

510 Su, S., Zeng, X., Bai, L., Li, L., Duan, R. (2011). Arsenic biotransformation by arsenic-resistant fungi
511 *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxyporum* CZ-
512 8F1. Science of the Total Environment, 409, 5057-5062.

513 Stubner, S., Wind, T., Conrad, R. (1998). Sulfur oxidation in rice field soil: activity, enumeration,
514 isolation and characterization of thiosulfate-oxidizing bacteria. Systematic and Applied Microbiology,
515 21, 569-578.

516 Thomas, P., Finnie, J. K., Williams, J. G. (1997). Feasibility of identification and monitoring of
517 arsenic species in soil and sediment samples by coupled high-performance liquid chromatography -
518 inductively coupled plasma mass spectrometry. Journal of Analytical Atomic spectrometry, 12, 1367-
519 1372.

520 Vergara Gallardo, M., Bohari, Y., Astruc, A., Potin-Gautier M., Astruc, M. (2001). Speciation analysis
521 of arsenic in environmental solids reference materials by high-performance liquid-chromatography-
522 hydride generation-atomic fluorescence spectrometry following orthophosphoric acid extraction.
523 Analytica Chimica Acta, 441, 257-268.

524 Whittinghill, K. A., Hobbie, S. E. (2012) Effects of pH and calcium on soil organic matter dynamics in
525 Alaskan toundra. Biogeochemistry, 111, 569-581.

526 Yamamura, S., Watanabe, M., Yamamoto, N., Sei, K., Ike, M. (2009). Potential for microbially
527 mediated redox transformations and mobilization of arsenic in uncontaminated soils. Chemosphere 77,
528 169-174.

529 Zhang, Y., Kallay, N., Matijevic, E. (1985) Interaction of metal hydrous oxides with chelating agents.
530 7. hematite-oxalic acid and –citric acid systems. Langmuir, 1, 201-206.

531

532

533

534

535

536

537

538

539

540

541

542

543

544 **Figure Captions**

545

Figure 1

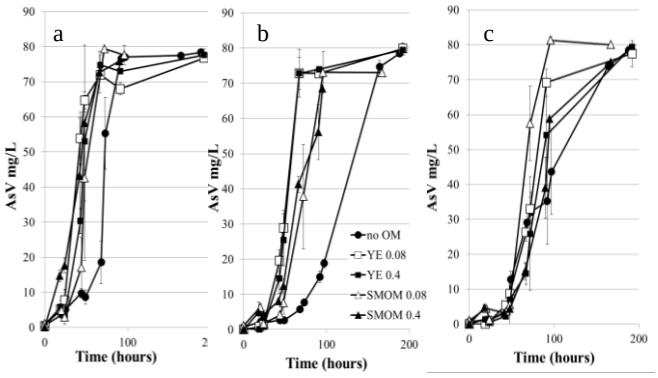


Fig.1 Examples of evolution of AsV concentration in the AsIII oxidizing tests performed with three soils: (a) Cheni forest soil, (b) Cheni site soil, (c) Cheni meadow soil. Graphs for the other soils are given in ESM3. Error bars represent the standard deviation of the mean of three replicates

Figure 2

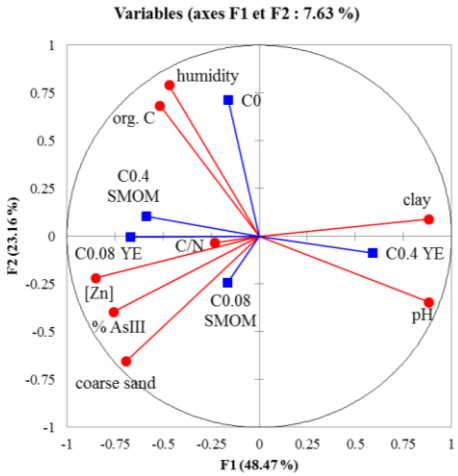
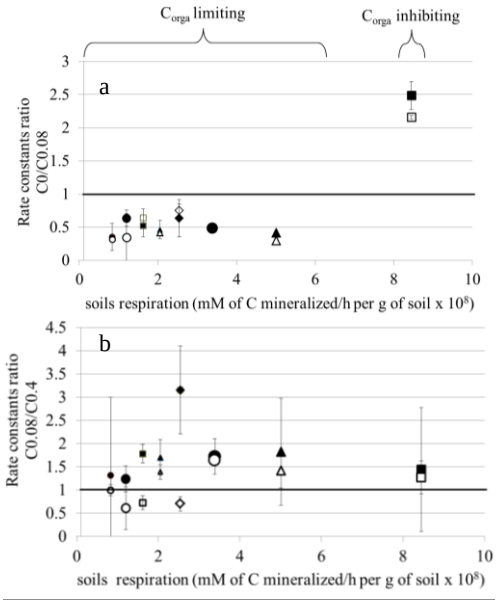


Fig.2 Principal Component Analysis of the soil characteristics (circles) with AsIII oxidation rate constants integrated as supplementary data (squares)

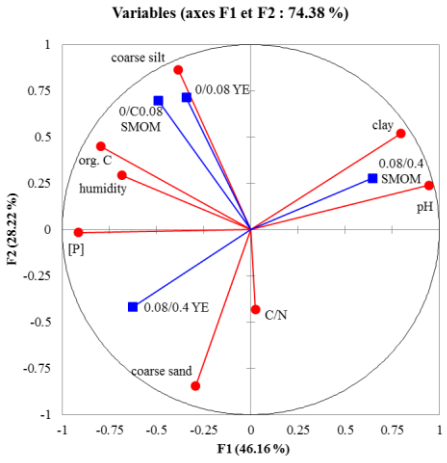
554

Figure 3



555 **Fig.3** Evolution of
556 AsIII oxidation rate constant ratio with soil respiration rate. a: ratio C0/C0.08; b: ratio C0.08/C0.4.
557 Open symbols: yeast extract. Closed symbols: SMOM. Diamonds: Salsigne ZE2. Small squares:
558 Salsigne ZE1. Small circles: Cheni site. Average size circles: Cheni field. Big circles: Cheni meadow.
559 Small triangles: Auzon 1. Big triangles: Auzon 2. Big squares: Cheni forest. Error bars represent the
560 standard deviation of the ratios calculated with the Taylor expansion method, based on three replicates

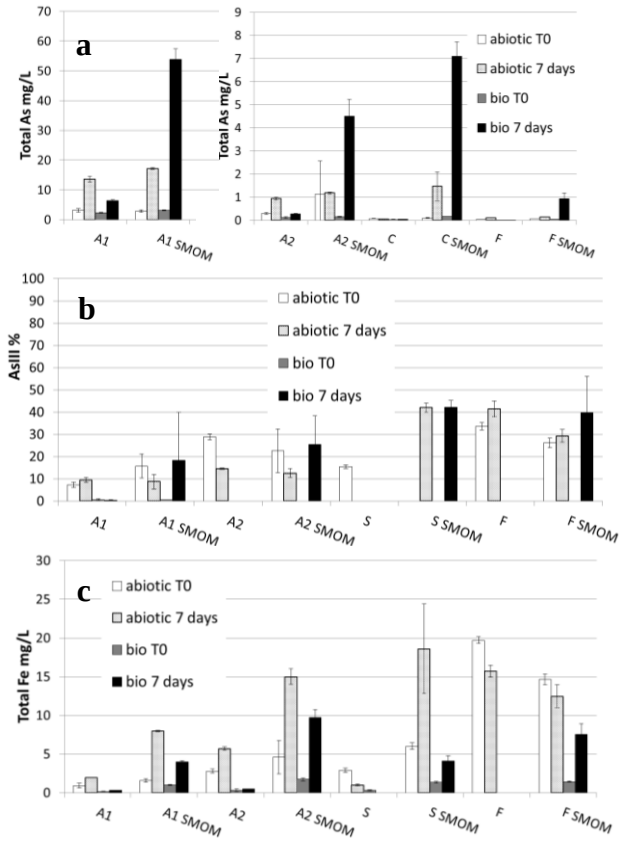
Figure 4



561

562 **Fig.4** Principal Component Analysis of the soil characteristics (circles) with the ratios of AsIII
563 oxidation rate constants integrated as supplementary data (squares)
564

Figure 5



565

566 **Fig.5** Incubations of soils with and without SMOM. (a) Concentrations in total As; (b) percentage of
567 AsIII in the aqueous phase. (c) Concentration in total Fe. A1: Auzon 1. A2: Auzon 2. S: Cheni site. F:
568 Cheni forest. White bars: abiotic T0. Light gray: abiotic 7 days. Dark gray: biotic T0. Black: biotic 7
569 days. Error bars represent the standard deviation of the mean of three replicates
570

571

Table 1. Characteristics of the polluted soils.

		Cheni meadow	Cheni forest	Cheni site	Cheni field	Auzon 1	Auzon 2	Salsigne ZE1	Salsigne ZE2
ppm	[As]*	182	617	785	80	3680	358	89	119
mg/kg	[AsIII]**	0.6	4.32	23	0	180	0	0	0
%	AsIII***	0.39	1.22	3.76	0.00	5.75	0.00	0.00	0.00
mg/kg	[Cd]	0	0	0	47.99	86.65	0	0	0
mg/kg	[Pb]	36	48	67	31	2041	139	36	39
mg/kg	[Zn]	110	145	104	76	240	159	83	33
g/kg	[Fe]	18	30	24	26	32	29	18	39
%	[K]	1.7	1.1	1.5	1.7	1.,9	1.7	0.7	0.9
%	[CaCO3]	0.1	0.1	2.9	0.1	0.1	0.1	14.2	2.9
%	humidity	14	37	34	17	10	16	10	8
	pH	5.33	4.8	4.91	6.02	5.65	5.7	8.84	9.02
%	clay	11.3	17.1	11.8	12.3	5	9.3	33.3	29.9
%	coarse sand	48.4	26.3	35	29.6	62.,4	29.2	25.8	24
%	coarse silt	11.1	2.4	12.2	15.8	9.,2	18.6	8.9	18.6
%	org. C	1.69	5.76	1.16	1.16	2.03	2.79	0.76	0.35
g/kg	[N]	1.7	6.6	0.6	1.2	1.9	2.6	0.6	0.4
mg/kg	[P]	1079	1722	798	1532	1471	1573	288	139
mM/h/mol/g $x10^{-8}$	respiration	3.38	8.45	8.27	1.20	2.,05	5.01	1.62	2.54
Bact/g x 10^{8}	living bacteria	6.31	11.6	4.32	7.28	4.,98	10.1	6.17	3.24

(*) Total As analyzed by NITON; (**) AsIII analyzed by HPLC_ICP-MS; (***)AsIII/(AsIII + AsV) analyzed by HPLC_ICP-MS

Table 2. Pearson correlation coefficients between soil characteristics and the AsIII oxidation rate constants and their ratios. Values in bold are significant for a level of significance alpha = 0.05, and the three highest coefficients, for each variable are underlined. IBCF: Indicator of biodegradable carbon fraction (ratio respiration/organic carbon concentration).

Variables	C0	C0.08 YE	C0.4 YE	C0.08 SMOM	C0.4 SMOM	C0/C0.08 YE	C0.08/C0.4 YE	C0/C0.08 SMOM	C0.08/C0.4 SMOM
[As]	-0.02	0.44	-0.25	0.35	0.34	-0.10	0.35	-0.08	-0.14
[AsIII]	-0.11	0.37	-0.18	0.32	0.27	-0.18	0.28	-0.17	-0.10
% AsIII	-0.02	0.62	-0.28	0.54	0.67	-0.12	0.47	-0.10	-0.28
[Cd]	-0.24	0.11	0.18	-0.19	-0.04	-0.27	-0.07	-0.19	-0.23
[Pb]	-0.12	0.33	-0.17	0.26	0.17	-0.18	0.25	-0.17	-0.05
[Zn]	0.13	0.55	-0.52	0.19	0.36	0.03	0.61	0.09	-0.39
[Fe]	0.24	0.17	0.31	0.14	-0.30	0.20	-0.20	0.19	0.60
clay	0.15	-0.62	0.42	0.10	-0.32	0.26	-0.68	0.10	0.56
[$CaCO_3$]	-0.13	-0.35	0.28	0.27	0.08	-0.06	-0.46	-0.19	0.13
coarse sand	-0.28	0.29	-0.51	0.19	0.25	-0.33	0.57	-0.31	-0.22
coarse silt	0.66	-0.02	0.10	-0.29	-0.30	0.65	-0.11	0.70	0.17
humidity	0.61	0.40	-0.29	0.09	0.63	0.53	0.38	0.61	-0.58
pH	-0.23	-0.56	0.57	0.09	-0.50	-0.13	**-0.74**	-0.27	**0.72**
[P]	0.30	0.32	-0.30	-0.45	0.07	0.22	0.41	0.39	-0.64
[N]	**0.88**	0.05	-0.45	-0.21	0.04	**0.84**	0.32	**0.90**	-0.31
org. C	**0.84**	0.17	-0.49	-0.14	0.14	**0.78**	0.40	**0.85**	-0.37
respiration	**0.77**	0.07	-0.56	-0.19	0.02	**0.74**	0.41	**0.79**	-0.31
C/N	-0.31	0.61	-0.05	0.61	**0.87**	-0.38	0.29	-0.39	-0.37
IBCF	0.04	-0.33	-0.42	-0.21	-0.19	0.09	0.17	0.06	-0.17
living bacteria	0.59	0.03	-0.27	-0.44	-0.09	0.56	0.19	0.66	-0.44

Table 3. Pearson correlation coefficients between textural, physical, chemical and biogeochemical soil characteristics. Values in bold are significant for a level of significance alpha=0.05. IBCF: Indicator of biodegradable carbon fraction (ratio respiration/organic carbon concentration).

Variables	[As]	[AsIII]	% AsIII	[Cd]	[Pb]	[Zn]	[Fe]	clay	coarse silt	coarse sand	CaCO 3	coarse silt	humi-dity	pH	[P]	[N]	org. C	resp.	C/N	IBCF h-1	living bact.
[As]	**1**	**0.991**	**0.899**	**0.798**	**0.979**	**0.832**	0.295	-0.523	-0.365	**0.807**	-0.260	-0.365	-0.099	-0.291	0.322	0.084	0.128	-0.033	0.059	-0.311	-0.195
[AsIII]	**0.991**	**1**	**0.874**	**0.839**	**0.991**	**0.779**	0.276	-0.480	-0.444	**0.824**	-0.209	-0.444	-0.207	-0.207	0.253	-0.023	0.015	-0.139	0.043	-0.312	-0.283
% AsIII	**0.899**	**0.874**	**1**	0.620	**0.808**	0.696	0.163	-0.534	-0.402	**0.733**	-0.226	-0.402	0.199	-0.427	0.217	0.007	0.074	-0.124	0.434	-0.471	-0.311
[Cd]	**0.798**	**0.839**	0.620	**1**	**0.851**	0.583	0.239	-0.502	-0.356	0.678	-0.296	-0.356	-0.293	-0.175	0.401	-0.087	-0.082	-0.237	-0.161	-0.354	-0.195
[Pb]	**0.979**	**0.991**	**0.808**	**0.851**	**1**	**0.796**	0.301	-0.470	-0.415	**0.815**	-0.217	-0.415	-0.287	-0.167	0.280	-0.002	0.030	-0.103	-0.061	-0.245	-0.231
[Zn]	**0.832**	**0.779**	0.696	0.583	**0.796**	**1**	0.081	-0.703	-0.165	**0.711**	-0.368	-0.165	0.102	-0.575	0.664	0.425	0.495	0.417	-0.016	0.085	0.310
[Fe]	0.295	0.276	0.163	0.239	0.301	0.081	**1**	0.003	0.545	-0.086	-0.421	0.545	-0.112	0.171	0.034	0.143	0.114	-0.006	-0.373	-0.651	-0.103
clay	-0.523	-0.480	-0.534	-0.502	-0.470	-0.703	0.003	**1**	0.057	-0.658	**0.774**	0.057	-0.268	**0.864**	**-0.793**	-0.237	-0.313	-0.233	-0.111	0.165	-0.229
coarse silt	-0.365	-0.444	-0.402	-0.356	-0.415	-0.165	0.545	0.057	**1**	-0.594	-0.441	**1.000**	0.441	-0.151	0.336	0.656	0.619	0.602	-0.439	-0.166	0.604
coarse sand	**0.807**	**0.824**	**0.733**	0.678	**0.815**	**0.711**	-0.086	-0.658	-0.594	**1**	-0.353	-0.594	-0.213	-0.415	0.303	-0.081	-0.039	-0.106	0.049	-0.055	-0.278
[CaCO3]	-0.260	-0.209	-0.226	-0.296	-0.217	-0.368	-0.421	**0.774**	-0.441	-0.353	**1**	-0.441	-0.273	0.695	-0.697	-0.393	-0.407	-0.326	0.281	0.379	-0.255
coarse silt	-0.365	-0.444	-0.402	-0.356	-0.415	-0.165	0.545	0.057	**1.000**	-0.594	-0.441	**1**	0.441	-0.151	0.336	0.656	0.619	0.602	-0.439	-0.166	0.604
humidity	-0.099	-0.207	0.199	-0.293	-0.287	0.102	-0.112	-0.268	0.441	-0.213	-0.273	0.441	**1**	-0.681	0.410	0.599	0.640	0.529	0.398	-0.185	0.463
pH	-0.291	-0.207	-0.427	-0.175	-0.167	-0.575	0.171	**0.864**	-0.151	-0.415	0.695	-0.151	-0.681	**1**	**-0.810**	-0.530	-0.599	-0.517	-0.208	0.064	-0.461
[P]	0.322	0.253	0.217	0.401	0.280	0.664	0.034	**-0.793**	0.336	0.303	-0.697	0.336	0.410	**-0.810**	**1**	0.677	**0.713**	0.652	-0.253	0.066	**0.720**
[N]	0.084	-0.023	0.007	-0.087	-0.002	0.425	0.143	-0.237	0.656	-0.081	-0.393	0.656	0.599	-0.530	0.677	**1**	**0.991**	**0.957**	-0.392	0.209	**0.842**
org. C	0.128	0.015	0.074	-0.082	0.030	0.495	0.114	-0.313	0.619	-0.039	-0.407	0.619	0.640	-0.599	**0.713**	**0.991**	**1**	**0.963**	-0.298	0.209	**0.851**
respiration	-0.033	-0.139	-0.124	-0.237	-0.103	0.417	-0.006	-0.233	0.602	-0.106	-0.326	0.602	0.529	-0.517	0.652	**0.957**	**0.963**	**1**	-0.368	0.432	**0.909**
C/N	0.059	0.043	0.434	-0.161	-0.061	-0.016	-0.373	-0.111	-0.439	0.049	0.281	-0.439	0.398	-0.208	-0.253	-0.392	-0.298	-0.368	**1**	-0.236	-0.358
IBCF h-1	-0.311	-0.312	-0.471	-0.354	-0.245	0.085	-0.651	0.165	-0.166	-0.055	0.379	-0.166	-0.185	0.064	0.066	0.209	0.209	0.432	-0.236	**1**	0.483
living bact.	-0.195	-0.283	-0.311	-0.195	-0.231	0.310	-0.103	-0.229	0.604	-0.278	-0.255	0.604	0.463	-0.461	**0.720**	**0.842**	**0.851**	**0.909**	-0.358	0.483	**1**