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# 1 **Influence of organic matters on AsIII oxidation by the microflora of** 2 **polluted soils**

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## 14 **Abstract**

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

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

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

## 32 **Introduction**

33 High soil concentrations of the toxic element arsenic (As) may be linked to pollution originating from  
34 mining and industrial activities, long-term application of As-containing pesticides (Smith et al., 1998)  
35 or to the geochemical background. On polluted mining sites, characterized by high concentrations of  
36 metal(oid)s and poor fertility, organic amendments are often considered as a means of facilitating  
37 phyto-stabilization (Galende et al., 2014). In addition, agricultural soils affected by diffuse As  
38 pollution from pesticides can be fertilized with organic matter (Franchini, 2001; Chantigny et al.,  
39 2000; Chantigny et al., 2002). Many studies have described the geochemical interactions between  
40 arsenic and organic matter: change of As speciation (Redman et al., 2002), formation of soluble  
41 complexes (Saada et al., 2003; Redman et al., 2002), competition for sorption sites (Bauer and Blodau,  
42 2006). Conversely, organic matter is also a trophic resource for microorganisms but its influence on  
43 microbial activities linked to As is poorly documented, even though the soil microflora plays a major  
44 role in geochemical cycles and particularly in As speciation. Bacteria isolated from soils have been  
45 shown to oxidize AsIII and/or reduce AsV (Macur et al., 2004; Inskeep et al., 2007; Bachate et al.,  
46 2012), or to methylate this toxic metalloid (Huang et al., 2012). Filamentous fungi isolated from  
47 contaminated soils are able to reduce AsV and methylate As (Su et al., 2011). Microbial  
48 transformations of arsenic in soil have important implications because mobility, toxicity and  
49 bioavailability of the element are closely related to its speciation (Smedley and Kinniburgh, 2002).  
50 The global AsIII-oxidizing activity of the microflora should tend to reduce the risk of transfer of the  
51 toxic element from soil to surface water or groundwater.

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

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

## 87 **Material and methods**

### 88 Soil sampling and characterization

89  
90 Eight different soils were sampled on three highly polluted sites that could be candidates for the  
91 application of aided phyto-stabilization. All materials were surface, non-saturated soils (0–15 cm).  
92 Four different soils were sampled in and around the Cheni disused gold mine site (Limousin;  
93 45°32'59.90"N, 1°09'39.04"E): in a cultivated field, a meadow, a forest and on the site near the  
94 arsenic-containing waste dump. Two brownfield soil samples were taken from the Auzon disused  
95 industrial site (Haute-Loire; 45°23'13.67"N, 3°21'24.32"E) and two brownfield soil samples were  
96 collected on the Salsigne disused gold mine site (Aude; 43°31'89.25"N, 2°38'11.07"E and  
97 43°31'52.75"N, 2°38'73.83"E). The soils were sampled in sterile glass jars, sieved at 2 mm in sterile  
98 sieves then stored at 5°C in sterile glass jars. Their water content was determined by drying at 50°C  
99 for 24h. Mineral carbon and carbonates were determined by volumetric method per NF ISO 10693.  
100 Total carbon was analyzed per NF ISO 10694. After drying and hand grinding in a mortar, total

101 concentrations of As, Pb, Cd, Zn and Fe were determined in the soils using a portable NITON© X-ray  
102 fluorescence field analyzer (XLT999KWY, bulk mode, counting time 60s). Values for the elements  
103 Ag, U, Se, Hg, Cu, Ni, Cr and V were below the detection limit. Total As was also analyzed in all soils  
104 by Atomic Absorption Spectrophotometry after total digestion, per NF EN 13346. AsIII and AsV were  
105 analyzed by HPLC-ICP-MS after extraction with 10 mL of H<sub>3</sub>PO<sub>4</sub> 1M added to 0.4 g of sample and  
106 microwave heating (Vergara Gallardo et al., 2001) in a closed system at 120°C for 20 min. The  
107 remaining solution was diluted to 50 mL with ultrapure water and then analyzed with HPLC-ICP-MS,  
108 using quantification by standard additions to avoid matrix effects. Arsenic species separation was  
109 performed using an anion exchange column (Hamilton PRPX-100) and a mobile phase of ammonium  
110 hydrogen phosphate 15 mM at pH 8.5 (Thomas et al., 1997). The percentage of AsIII (%AsIII) was  
111 calculated as  $[\text{AsIII}] \times 100 / [\text{total As}]$ . Total nitrogen was analyzed by the Kjeldhal method per NF EN  
112 25663. Granulometry was determined per NF X 31.107, total phosphorus per NF X 31.161, K<sub>2</sub> per NF  
113 X 31.108 and pH per NF ISO 10390. The biodegradability of intrinsic organic matter was studied via  
114 CO<sub>2</sub> emission (soil respiration), per Rey et al., 2005.

115 Living bacteria were enumerated by microscopy using the Live/Dead® kit (BacLight™ Viability L-  
116 13152 Molecular Probes, Invitrogen) per Pascaud et al. (2009). The soils (stored at 5°C) were  
117 incubated at ambient temperature for 72 h and then suspended at 1% in sodium pyrophosphate and  
118 agitated reciprocally for 15 min, sonicated 2 x 20 s at 45 kHz. The suspension was settled for 1 min,  
119 1 mL of the supernatant was then sampled for filtration, application of coloration and observation with  
120 a Zeiss Axio Imager Z1 microscope equipped with UV HBO lamp. Living bacteria, exhibiting green  
121 fluorescence with the FITC filter, were enumerated on 10 independent fields (each of 5,800 μm<sup>2</sup>).  
122 Average cell concentrations were calculated from volume of sample used and known filter area.

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

#### 124 AsIII oxidizing activity tests

125 The basal culture medium was the CAsO1 medium (pH 6) described in Battaglia-Brunet et al. (2002).  
126 This was supplemented with 1 mM AsIII. The soils (stored at 5°C) were incubated at 25°C for 72 h  
127 before starting the tests which were performed in 250 mL Erlenmeyer flasks filled with 100 mL  
128 medium and plugged with cotton. Each flask was inoculated with a mass of soil equivalent to 0.2 g dry  
129 weight. Flasks were incubated at 25°C under reciprocal agitation (150 rpm). The flasks were sampled  
130 (3 mL) twice a day; samples were filtered at 45 μm and frozen at -20°C until AsIII/AsV separation  
131 was performed. The AsIII oxidation activities were analyzed in (i) simple basal medium, (ii) basal  
132 medium amended with 0.2 g/L or 1 g/L of yeast extract (Sigma), and (iii) basal medium amended with  
133 two concentrations (0.5 g/L or 2.5 g/L corresponding to equivalent carbon concentrations as 0.2 and 1  
134 g/L of yeast extract, i.e. 0.08 and 0.4 g/L of carbon) of a Synthetic Mixture of Organic Matter

135 (SMOM). The SMOM was designed to mimic the average composition of soil organic matter in terms  
136 of C/N ratio, functional groups, amino-acids and sugars, on the basis of data available from the  
137 International Humic Substances Society's website (IHSS – <http://www.humicsubstances.org>). It  
138 contained the following molecules: L-arginine (3.2% dry weight), L-aspartic acid monopotassium salt  
139 (2.5%), L-glutamic acid monosodium salt monohydrate (3.0%), glycine (1.2%), L-histidine (0.3%), L-  
140 isoleucine (0.7%), L-leucine (0.9%), methionine (0.3%), L-phenylalanine (0.6%), L-serine (1.3%), L-  
141 threonine (0.4%), L-tyrosine (0.4%), L-valine (1.5%), succinic acid disodium salt hexahydrate  
142 (19.1%), acetic acid (5.6%), propionic acid (2.8%), sodium tartrate dehydrate (8.3%), valeric acid  
143 (3.5%), calcium formate (3.7%), citric acid monohydrate dibasic (5.4%), butyric acid (2.1%), oxalic  
144 acid (0.8%), palmitic acid (6.7%), gallic acid (3.9%), vanillic acid (3.2%), 4-hydroxybenzoic acid  
145 (1.7%), (+)-catechin hydrate (2.9%), protocatechuic acid ethyl (1.2%), *trans*-ferulic acid (1.0%), *p*-  
146 coumaric acid (0.1%), rutin (5.0%), D-glucose (2.3%), D(+)-galactose (1.1%), D(+)-mannose (1.1%),  
147 D(+)-fucose (0.2%), L-rhamnose (0.8%), D(-)-arabinose (0.5%), D(-)-ribose (0.1%), D(+)-xylose  
148 (0.8%). The pH of the mixture was adjusted to that of the liquid medium (pH 6). The SMOM  
149 concentrations provided the same organic carbon concentration as the 0.2 and 1 g/L of yeast extract,  
150 with 1 g/L yeast extract corresponding to 0.4 g/L of organic carbon (Holwerda et al., 2012). The  
151 conditions were therefore 0.08 and 0.4 g/L of added organic carbon for both yeast extract and SMOM.

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

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

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

$$161 \quad \text{Ln[AsV]} = kt + \text{Constant} \quad (1)$$

#### 162 Principal Component Analysis

163 Statistical analyses were carried out using the XLSTAT 2014 software (Addinsoft, version  
164 16.2.01.6189). Pearson correlations were calculated with all soils parameters, and eight independent  
165 soil parameters – selected as those correlating most strongly with AsIII oxidizing rate constants and  
166 their ratios (Electronic Supplementary material ESM1) – were subjected to a principal component  
167 analysis (PCA). Rate constants and their ratios were integrated as supplementary data.

168 Soil incubations without addition of AsIII

169 The four most polluted soils in terms of As concentration were selected for incubation: Auzon 1,  
170 Auzon 2, Cheni site and Cheni forest. Experiments were performed in 60 mL flasks with cotton  
171 stoppers (aerobic conditions). Slurries were prepared by mixing a mass of soil corresponding to 2.5 g  
172 (dry weight) and 25 mL of spring water (Montcalm, Ca 3 mg/L, Mg 0.7 mg/L, Na 2.2 mg/L, K  
173 0.6 mg/L, SO<sub>4</sub> 10 mg/L, HCO<sub>3</sub> 5.2 mg/L, NO<sub>3</sub> 0.7 mg/L, Cl 0.6 mg/L, pH 6.8). Control blanks were  
174 prepared with soils sterilized as described in part 2.2. Spring water was autoclaved (120°C, 20 min).  
175 Incubations were performed with and without addition of SMOM at 0.4 g/L of organic carbon. For  
176 each condition (blank and experiment, with and without SMOM), six flasks were prepared. One  
177 triplicate was sacrificed after 15 min of incubation and the remaining flasks were incubated for 7 days  
178 at 25°C, under reciprocal agitation (150 rpm). When the incubations were sacrificed, 10 mL of slurry  
179 were filtered at 0.45 µm; 2.5 mL of this filtrate were used immediately for AsIII/AsV separation on  
180 resin (Ficklin, 1983), the remainder was acidified with 50 µL HCl 37% for flame AAS analysis of  
181 total Fe. Separated AsIII and AsV were quantified by graphite furnace AAS (detection limit 20 µg/L).  
182 Samples of biotic soil slurries were taken after 15 min and 7 days of incubation and stored at -20°C.  
183 For biomolecular analyses, genomic DNA was extracted from the -20°C stored soil slurries using the  
184 FastDNA® Spin Kit for Soil (Bio101). The community structure and its evolution were monitored by  
185 two methods, (1) Capillary electrophoresis–terminal restriction fragment length polymorphism (CE–T-  
186 RFLP) diversity analysis of the 16S rRNA gene, as described by Mercier et al. (2013) and (2)  
187 Capillary Electrophoresis Single-Strand Conformation Polymorphism (CE-SSCP, Delbès et al., 2000).  
188 CE-SSCP analyses were performed with an ABI Prism 310 genetic analyzer using a 47 cm long  
189 capillary, a non-denaturing 5.6% CAP polymer (Applied Biosystems). CE-SSCP electrophoregrams  
190 were analyzed using the StatFingerprints Version 2 software (Michelland et al., 2009). For total  
191 bacteria enumeration, bacteria were separated from soil particles and from eukaryotes using the  
192 methods validated by Lindahl and Bakken (1995), Lindahl (1996) and Bertrand et al., (2005),  
193 including a separation of bacteria from soil particles using the Nycodenz gradient method. The final  
194 pellet was suspended in 1 mL of NaCl 0.8%, mixed with 1 mL of absolute ethanol and stored at -20°C.  
195 Details about the methods are given in the Electronic Supplementary Material ESM4. Bacteria were  
196 enumerated after fluorescent staining, as described in Kumar et al. (2013).

## 197 **Results and discussion**

198 AsIII-oxidizing activity measurements

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

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

212 Many bacteria isolated from soils have been shown to oxidize AsIII (Macur et al., 2004; Inskeep et al.,  
213 2007; Bachate et al., 2012) and AsIII oxidation by complete soil microflora was evidenced by  
214 Yamamura et al. (2009). The bacterial AsIII oxidation is linked to the expression of *aio* genes that  
215 were evidenced in soil bacteria (Huang et al, 2012; Poirel et al., 2013).

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

223 Evolution of the AsIII oxidation rate constants when organic matter was introduced in the medium  
224 was examined through calculation of the ratio of rate constants,  $C_0/C_{0.08}$  and  $C_{0.08}/C_{0.4}$ . The  
225 evolution of these ratios according to soil respiration enlightens the influence of nature and  
226 concentration of organic matter on AsIII oxidation rate constants (Fig. 3). Soil respiration is linked to  
227 the intrinsic biodegradable organic matter. The  $C_0/C_{0.08}$  ratio is very similar for yeast extract and  
228 SMOM (Fig. 3a), and lower than 1 for all soils with the exception of the Cheni forest soil which  
229 presents the highest respiration level. Thus, the addition of organic substances at 0.08 g/L of organic  
230 carbon exerted a positive effect on AsIII oxidation rate constants, except when the soil contained  
231 enough biodegradable organic matter. These results suggest that the AsIII oxidation rate was limited  
232 by the availability of organic matter in natural conditions when no organic substrate was added to the  
233 liquid medium, and this limitation was removed by supplying 0.08 g/L of available organic carbon,  
234 provided by either yeast extract or SMOM. Conversely, the  $C_{0.08}/C_{0.4}$  ratio was higher than 1 in all  
235 conditions except when yeast extract was added to media inoculated by the four soils with the lowest  
236 respiration levels (Fig. 3b). In all other conditions, increasing the organic substrate concentration from



237 0.08 to 0.4 g/L of organic carbon induced a decrease of the AsIII oxidation rate constant. But the  
238 influence of yeast extract on the AsIII oxidizing rate differed from that of SMOM. SMOM exerted a  
239 negative effect on AsIII oxidizing rate, more pronounced than that of yeast extract, in particular with  
240 the soils with lower respiration levels.

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

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

257 The only parameters that influenced significantly the AsIII-oxidation rates constants and their ratios  
258 were either linked to organic matter, i.e. N, organic C, respiration and C/N, or pH (Table 2). One of  
259 the main parameters influencing soil biogeochemistry and microbial activities is pH (Rousk et al.,  
260 2011; Whittinghill and Hobbie, 2012), thus it is not surprising to enlighten an influence of this  
261 parameter on microbial As-related activities. Concerning specifically the soil organic matter  
262 properties, their significant influence on the AsIII oxidizing rate without added organic substrate (C0)  
263 decreased in the following order: total N > org. C > respiration, and the C/N ratio significantly  
264 influenced the C0.4 SMOM rate constant. These results suggest that microbial As oxidation may be  
265 limited not only by organic C but also by nitrogen. Thus, nitrogen-rich fraction of soil organic matter  
266 might influence As-related microbial activities. The C/N ratio in yeast extract is 4.25, whereas it is  
267 higher in SMOM (15.51) and in intrinsic organic matter of the eight soils (10 to 23). If the global soil  
268 respiration exerted a significant influence on AsIII-oxidizing rate constants, the proportion of  
269 biodegradable organic substances in soil organic matter was not related to any AsIII-oxidation related  
270 parameter (Table 2).

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

277 The As<sup>III</sup>-oxidizing activity in the soils also depends on the diversity and density of the different types  
278 of microorganisms, parameters that themselves depend on the environmental conditions of soils. The  
279 present study was focused on a global activity test that integrates all these variables. Such global  
280 activity measurements were developed and applied for other types of soil microbial activities: PAH  
281 biodegradation rate (Kästner and Mahro, 1996), thiosulfate-oxidizing activity in paddy fields (Stubner  
282 et al., 1998), and soil respiration, that integrates CO<sub>2</sub> production and consumption (Rey et al., 2005)  
283 as we integrate here As<sup>III</sup> production and consumption.

284 To date, all As<sup>III</sup>-oxidizing bacteria isolated from soils have been either heterotrophs (Macur et al.,  
285 2004; Bachate et al., 2012; Bahar et al., 2013) or facultative autotrophs (Santini et al., 2002, Inskeep et  
286 al., 2007; Garcia-Dominguez et al., 2008; Dong et al., 2014). The availability of organic substrates  
287 should therefore favor increase of cell density of As<sup>III</sup>-oxidizing bacteria. This might almost partly  
288 explain the positive effect of organic matters on the As<sup>III</sup> oxidizing rate constants between 0 and  
289 0.08 g/L of carbon, and the positive correlation of the constant with intrinsic organic matter in the  
290 without-amendment condition.

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

296 Conversely, high concentrations of organic substrates may decrease the specific As<sup>III</sup>-oxidizing  
297 activity of bacteria (Challan-Belval, 2009; Bachate et al., 2012) and may also stimulate the aerobic  
298 As<sup>V</sup>-reducing activity of soil microorganisms (Yamamura et al., 2009). These latter phenomena may  
299 explain the decrease in global As<sup>III</sup>-oxidizing rate constants between 0.08 and 0.4 g/L of added  
300 organic carbon observed in most of the conditions. In a liquid medium containing 0.5 g/L yeast extract  
301 and 1.8 g/L lactate (i.e. 0.92 g/L organic C), Yamamura et al. (2009) observed rapid reduction of  
302 1 mM As<sup>V</sup> by unpolluted soil inocula, thus demonstrating the presence of active As<sup>V</sup>-reducing  
303 microorganisms that may influence As speciation simultaneously with As<sup>III</sup>-oxidizing ones.  
304 Moreover, most As<sup>III</sup>-oxidizing bacteria have both oxidizing and reducing systems. In these

305 experiments, different groups of soil microorganisms may have been favored in each condition  
306 (0, 0.08 and 0.4 g/L of added carbon): according to van Gestel et al. (1993), autochthonous soil  
307 microorganisms are adapted to survive in soils containing recalcitrant material, where no abundant  
308 supply of easily oxidizable substrate occurs. These organisms exhibit K-selected behavior, with  
309 moderate growth rate and moderate nutrient demands, and are able to use diverse, complex materials  
310 (Odum, 1969; Metting, 1993; Langer et al., 2004). In contrast, zymogenous organisms show rapid  
311 growth when high energy-containing nutrients are added to soils (Paul and Clark, 1996), exhibiting r-  
312 selected behavior, characterized by rapid growth rate and use of simple and readily available  
313 substrates. Individual organisms may exhibit both r- and K-selected behavior, however amendment  
314 with organic substances should induce a change of behavior in soil microflora. The difference of  
315 effects exerted by yeast extract and SMOM at 0.04 g/L carbon may be related to the distinct  
316 composition of the two substrates. The r-strategy should be more prevalent in the presence of yeast  
317 extract than with the SMOM, whose composition includes some molecules that are not easily  
318 biodegradable. Soil incubations without added As

319 The experiment aimed to evaluate the short term (7 days) influence of amendment with SMOM  
320 (0.4 g/L organic carbon) on the mobility of arsenic present in the four most polluted soils, in relation  
321 to microbial activity. The incubations remained aerobic throughout the experiment (positive redox  
322 potential, Electronic Supplementary Material ESM4). Similar tendencies were observed with the four  
323 soils. When microbes were alive and without added SMOM, no arsenic was mobilized in the liquid  
324 phase (Fig. 5a). Conversely, in the presence of SMOM, arsenic was solubilized, and this mobilization  
325 was significantly higher when the microbes were alive than in the abiotic controls. Regarding the  
326 speciation of As in the liquid phase after seven days of incubation (Fig. 5b), AsIII was never detected  
327 in the absence of SMOM when microbes were alive. Conversely, AsIII was always detected in biotic  
328 conditions with SMOM, in proportion equivalent to that of the abiotic incubations (but with a higher  
329 variability between replicates). Present results showed mobilization of arsenic from soils contaminated  
330 for decades in presence of active bacteria and added organic matter, in aerobic condition. Iron  
331 concentration was increased by addition of SMOM in the soil slurries (Fig. 5c), either in biotic and  
332 abiotic conditions. This phenomenon was probably linked to the increase of Fe solubility through  
333 complexation with dissolved organic molecules: as a fact, SMOM contains some organic acids, such  
334 as citric and oxalic acids, known to chelate iron (Zhang et al., 1985). Whereas Fe concentration  
335 increased during incubation in biotic conditions, iron concentration always remained lower in biotic  
336 than abiotic conditions, contrary to total arsenic whose release in solution, with SMOM, was clearly  
337 higher in biotic than abiotic conditions. Thus, biological reduction of iron does not seem to represent  
338 the major mechanism inducing As mobilization. The organic molecules present in the SMOM and  
339 their organic degradation products may have mobilized some As by chemical complexation: arsenite  
340 and arsenate form aqueous complexes with humic acid and natural organic matter in the presence of

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

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

355 The bacterial community structure and diversity of the soils amended or not with SMOM were  
356 assessed. The four soils without added organic matter had a constant bacterial diversity profile  
357 showing that our incubation procedure alone did not change these characteristics (Fig. ESM4-1 a).  
358 When the soils were amended with SMOM, bacterial diversity changed during incubation and  
359 converged at the end of incubation for all soils. (Fig. ESM4-1 b). SMOM did not affect the bacterial  
360 diversity in the same way for all soils: it was decreased for the two soils that had the highest initial  
361 diversity, and was maintained or slightly increased for the two soils with lower initial diversity. In  
362 terms of total bacterial concentration, the mixture induced an increase in the bacterial cell  
363 concentration with the Cheni Site soil, however, for the other soils, bacterial counts did not differ with  
364 and without addition of the mixture (Fig. ESM4-2). The convergence of the SSCP fingerprint profiles  
365 for all amended soils may be related to the selective development of bacteria able to metabolize the  
366 most easily biodegradable compounds of the SMOM, as previously observed by Goldfarb et al.  
367 (2011), or may have resulted from the buffering effect of metabolized SMOM on pH. Nevertheless,  
368 the amendment of soils with the mixture did not result in the emergence of a few dominant species; it  
369 allowed the persistence of a diverse bacterial community, and did not significantly modify the total  
370 bacterial concentration.

## 371 **Conclusions**

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

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

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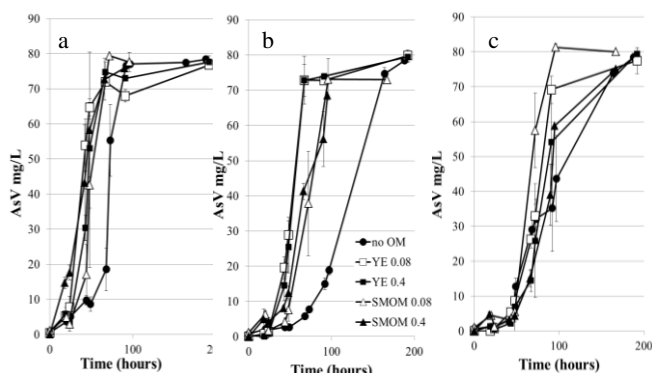
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544 **Figure Captions**

545

Figure 1

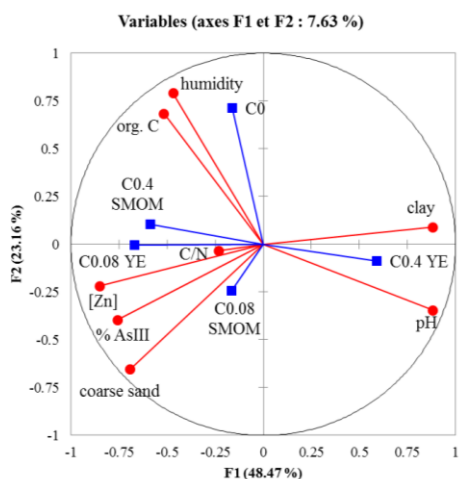


546

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

550

Figure 2



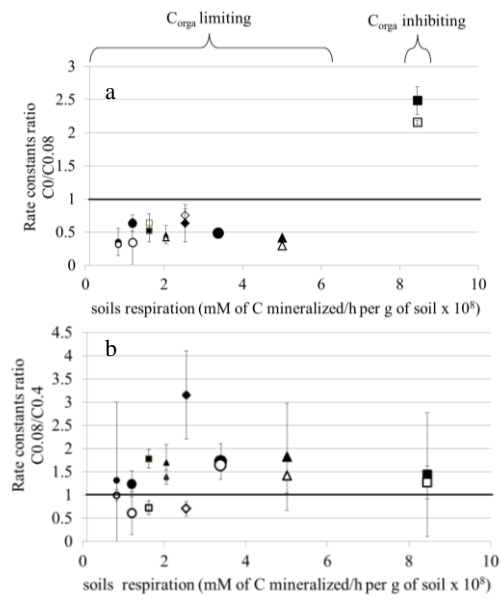
551

**Fig.2** Principal

552 Component Analysis of the soil characteristics (circles) with AsIII oxidation rate constants integrated  
 553 as supplementary data (squares)

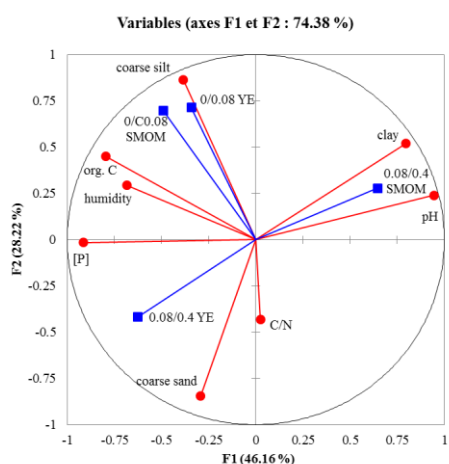
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Figure 3



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

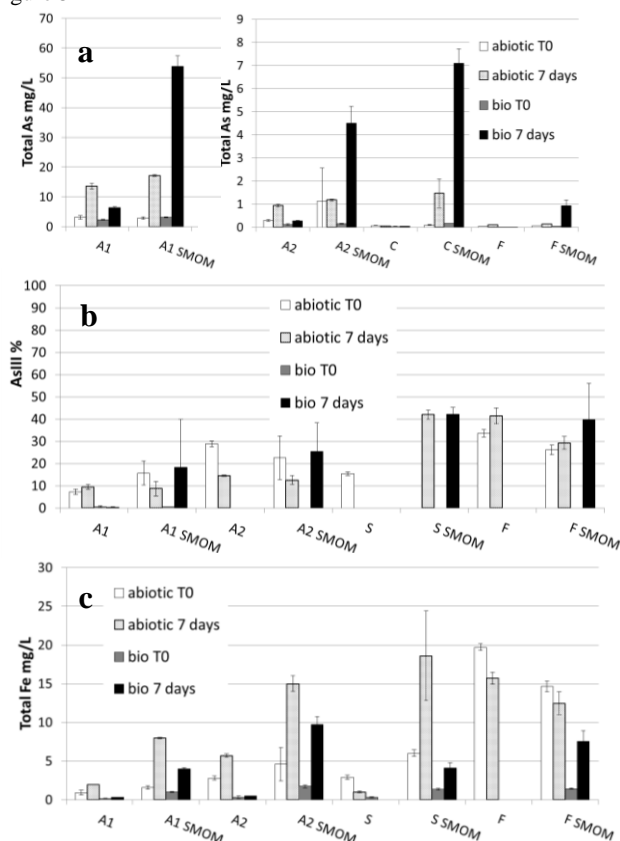
Figure 4



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 <sup>-8</sup>	respiration	3.38	8.45	8.27	1.20	2.,05	5.01	1.62	2.54
Bact/g x 10 <sup>8</sup>	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	<u>0.62</u>	-0.28	<u>0.54</u>	<u>0.67</u>	-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	<u>-0.52</u>	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	<u>0.60</u>
clay	0.15	<u>-0.62</u>	0.42	0.10	-0.32	0.26	<u>-0.68</u>	0.10	0.56
[CaCO <sub>3</sub> ]	-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	<u>0.57</u>	-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	<u>0.63</u>	0.53	0.38	0.61	-0.58
pH	-0.23	-0.56	<u>0.57</u>	0.09	-0.50	-0.13	<b>-0.74</b>	-0.27	<b>0.72</b>
[P]	0.30	0.32	-0.30	<u>-0.45</u>	0.07	0.22	0.41	0.39	<u>-0.64</u>
[N]	<b>0.88</b>	0.05	-0.45	-0.21	0.04	<b>0.84</b>	0.32	<b>0.90</b>	-0.31
org. C	<b>0.84</b>	0.17	-0.49	-0.14	0.14	<b>0.78</b>	0.40	<b>0.85</b>	-0.37
respiration	<b>0.77</b>	0.07	<u>-0.56</u>	-0.19	0.02	<b>0.74</b>	0.41	<b>0.79</b>	-0.31
C/N	-0.31	<u>0.61</u>	-0.05	<u>0.61</u>	<b>0.87</b>	-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 <sub>3</sub>	coarse silt	humidity	pH	[P]	[N]	org. C	resp.	C/N	IBCF h-1	living bact.
[As]	<b>1</b>	<b>0.991</b>	<b>0.899</b>	<b>0.798</b>	<b>0.979</b>	<b>0.832</b>	0.295	-0.523	-0.365	<b>0.807</b>	-0.260	-0.365	-0.099	-0.291	0.322	0.084	0.128	-0.033	0.059	-0.311	-0.195
[AsIII]	<b>0.991</b>	<b>1</b>	<b>0.874</b>	<b>0.839</b>	<b>0.991</b>	<b>0.779</b>	0.276	-0.480	-0.444	<b>0.824</b>	-0.209	-0.444	-0.207	-0.207	0.253	-0.023	0.015	-0.139	0.043	-0.312	-0.283
% AsIII	<b>0.899</b>	<b>0.874</b>	<b>1</b>	0.620	<b>0.808</b>	0.696	0.163	-0.534	-0.402	<b>0.733</b>	-0.226	-0.402	0.199	-0.427	0.217	0.007	0.074	-0.124	0.434	-0.471	-0.311
[Cd]	<b>0.798</b>	<b>0.839</b>	0.620	<b>1</b>	<b>0.851</b>	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]	<b>0.979</b>	<b>0.991</b>	<b>0.808</b>	<b>0.851</b>	<b>1</b>	<b>0.796</b>	0.301	-0.470	-0.415	<b>0.815</b>	-0.217	-0.415	-0.287	-0.167	0.280	-0.002	0.030	-0.103	-0.061	-0.245	-0.231
[Zn]	<b>0.832</b>	<b>0.779</b>	0.696	0.583	<b>0.796</b>	<b>1</b>	0.081	-0.703	-0.165	<b>0.711</b>	-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	<b>1</b>	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	<b>1</b>	0.057	-0.658	<b>0.774</b>	0.057	-0.268	<b>0.864</b>	<b>-0.793</b>	-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	<b>1</b>	-0.594	-0.441	<b>1.000</b>	0.441	-0.151	0.336	0.656	0.619	0.602	-0.439	-0.166	0.604
coarse sand	<b>0.807</b>	<b>0.824</b>	<b>0.733</b>	0.678	<b>0.815</b>	<b>0.711</b>	-0.086	-0.658	-0.594	<b>1</b>	-0.353	-0.594	-0.213	-0.415	0.303	-0.081	-0.039	-0.106	0.049	-0.055	-0.278
[CaCO <sub>3</sub> ]	-0.260	-0.209	-0.226	-0.296	-0.217	-0.368	-0.421	<b>0.774</b>	-0.441	-0.353	<b>1</b>	-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	<b>1.000</b>	-0.594	-0.441	<b>1</b>	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	<b>1</b>	-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	<b>0.864</b>	-0.151	-0.415	0.695	-0.151	-0.681	<b>1</b>	<b>-0.810</b>	-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	<b>-0.793</b>	0.336	0.303	-0.697	0.336	0.410	<b>-0.810</b>	<b>1</b>	0.677	<b>0.713</b>	0.652	-0.253	0.066	<b>0.720</b>
[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	<b>1</b>	<b>0.991</b>	<b>0.957</b>	-0.392	0.209	<b>0.842</b>
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	<b>0.713</b>	<b>0.991</b>	<b>1</b>	<b>0.963</b>	-0.298	0.209	<b>0.851</b>
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	<b>0.957</b>	<b>0.963</b>	<b>1</b>	-0.368	0.432	<b>0.909</b>
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	<b>1</b>	-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	<b>1</b>	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	<b>0.720</b>	<b>0.842</b>	<b>0.851</b>	<b>0.909</b>	-0.358	0.483	<b>1</b>