

## Supplementary material

### Role of microbial reducing activity in antimony and arsenic release from an unpolluted wetland soil: a lab scale study using sodium azide as a microbial inhibiting agent

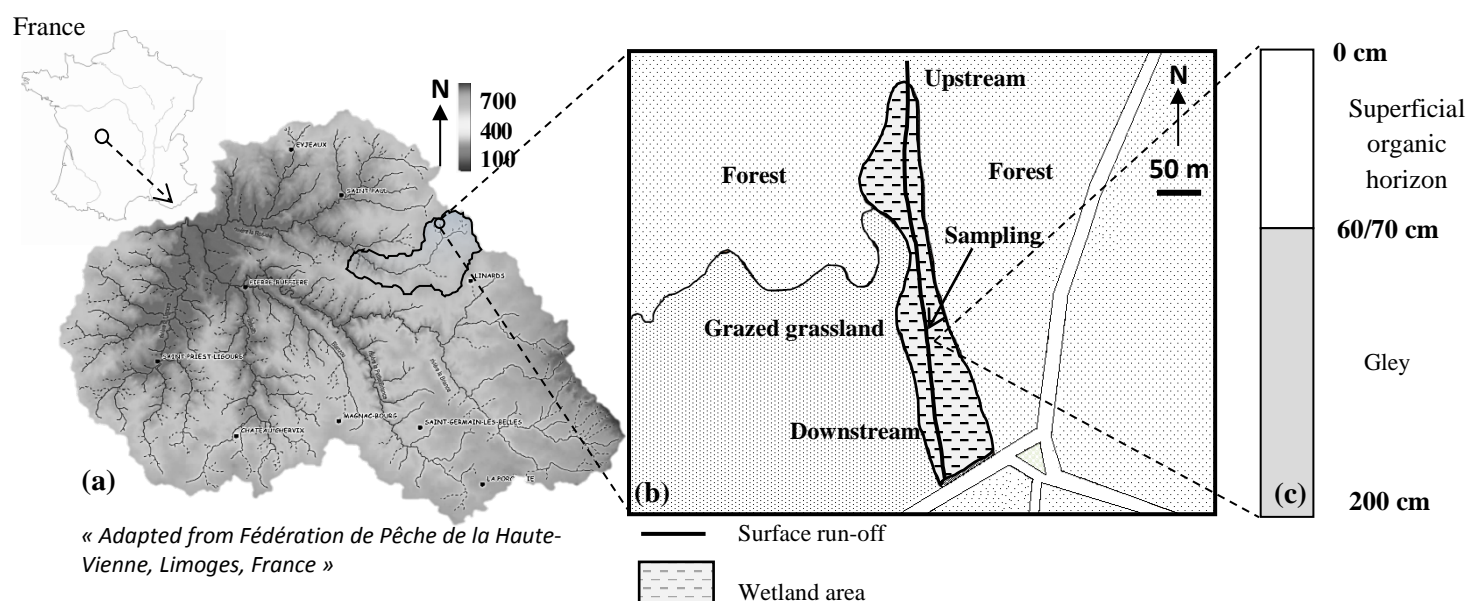
Asmaa Rouwane,<sup>A,B</sup> Marion Rabiet,<sup>A,B</sup> Isabelle Bourven,<sup>A</sup> Malgorzata Grybos,<sup>A</sup> Lucie Mallet<sup>A</sup> and Gilles Guibaud<sup>A</sup>

<sup>A</sup>Groupement de Recherche Eau Sol Environnement (GRESE), University of Limoges, 123 Av. Albert Thomas, 87060 Limoges cedex, France.

<sup>B</sup>Corresponding authors. Email: asmaa.rouwane@etu.unilim.fr; marion.rabiet@unilim.fr

#### 1. Site description

Soil was sampled from the uppermost organic horizon of a wetland (1 ha) located on an agricultural sub-catchment (Fig S1), with a substratum of relatively impermeable crystalline material (granites and gneisses). The site is classified as an oligotrophic wet meadow according to the “Conservatoire botanique national du Massif Central”<sup>[1]</sup>. The climate is continental oceanic with mean annual rainfall varying between 800 and 1700 mm and the mean annual ground temperature is of about 10.5 °C<sup>[2]</sup>. The wetland is crossed by a surface run-off feeding “La Roselle” River. The wetland soils are classified as gleys and pseudogleys<sup>[3]</sup>.



**Fig. S1.** Sketch map showing (a) location of the “La Roselle” sub-catchment in Limousin, France (b) seasonally flooded wetland and the soil sampling site and (c) the soil profile according to World Reference Base (WRB) classification.

#### 2. Soil sample analysis

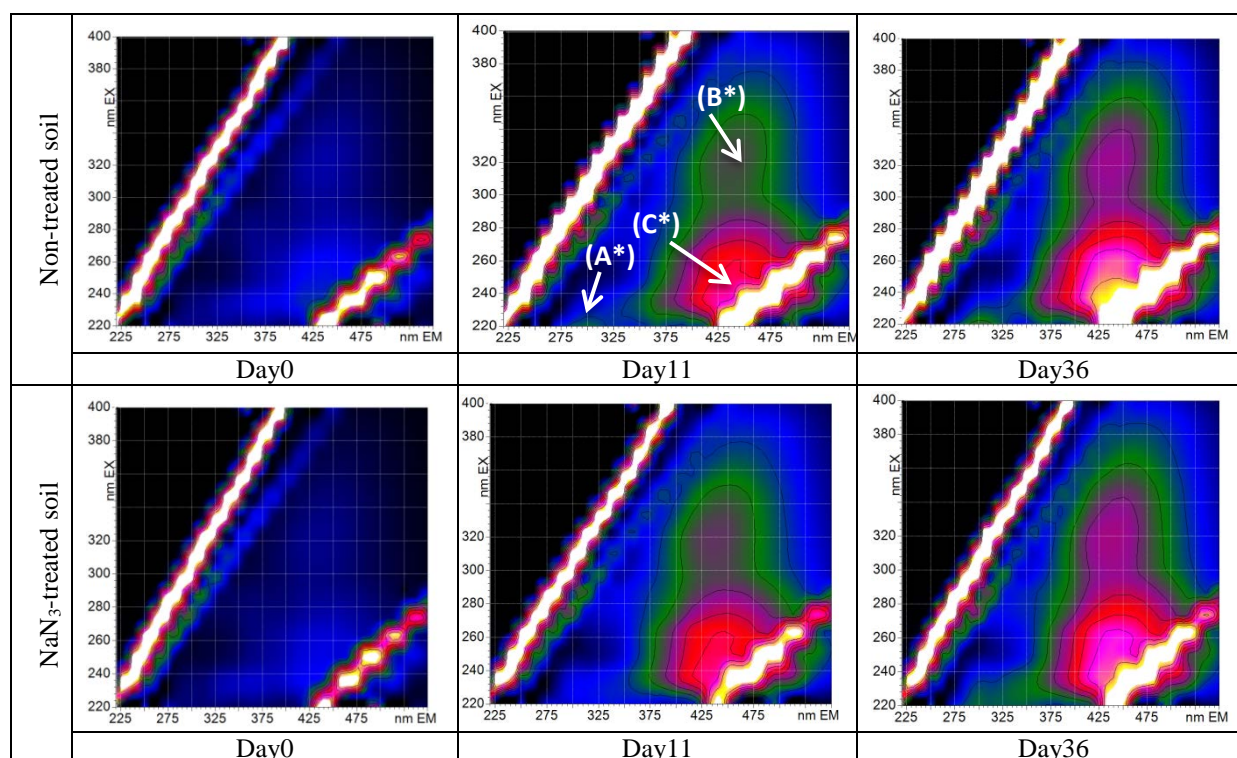
Soil sample was homogenized, dried at room temperature and sieved (< 2 mm).

**Table S1. Soil analysis methods**

Characteristic	Analysis method
Total organic carbon (TOC)	AFNOR NF ISO 10694
Total nitrogen	AFNOR NF ISO 13878
Organic phosphorus	Calcination method (INRA Arras laboratory, France)
Total content of As, Sb, Fe and Mn	Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) after aqua regia digestion (Acme Analytical Laboratories, Canada)
pH <sub>water</sub> and pH <sub>KCl</sub>	AFNOR NF ISO 10390
Cation exchange capacity (CEC)	AFNOR NF–X 31–130
Soil porosity	Determined by the weight difference between water saturated and a dry soil sample.

### 3. Three dimensional excitation emission fluorescence matrix (3D EEM) of soil solution DOM

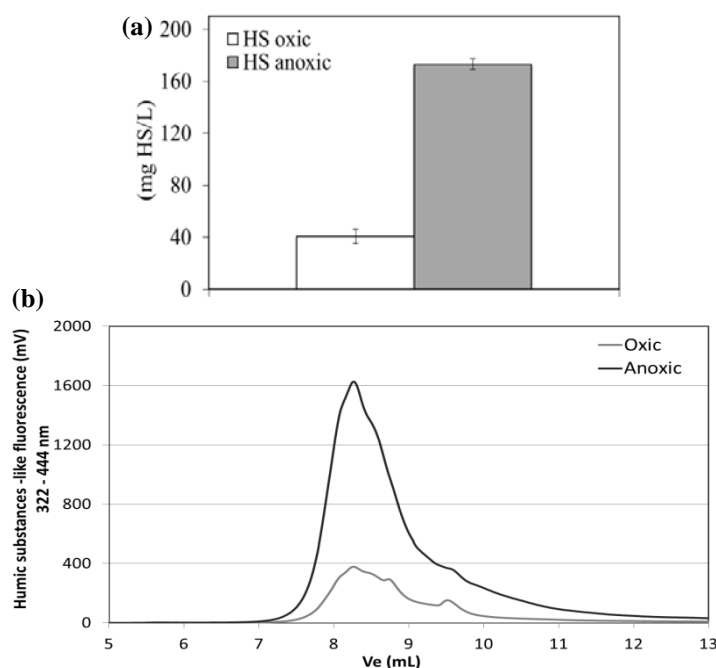
Three-dimensional excitation emission matrix (3-D EEM) were collected on a Shimadzu RF-5301 PCspectrofluorophotometer with a 150-W Xenon lamp at the excitation source using a 1.0 cm quartz cell. Scans were collected for excitation wavelengths between 220 and 400 nm at 12 nm intervals and emission wavelengths between 225 and 550 nm at 5 nm intervals. Fluorescence data was processed using the Panorama Fluorescence 3.1 software (LabCognition, Japan). Before analysis, soil solution was filtered with a 0.22 µm cellulose acetate membrane (SPARTAN 13/0.2 RC Whatman) then samples were diluted in Tris, HCl and NaCl buffer at pH 7 in order to keep the fluorophores at similar ionization level and to avoid fluorescence signal saturation. Analyzed samples were diluted in Tris, HCl buffer at the pH7, samples were kept at room temperature (22+/-2°C) for about 20 min before analysis.



\*A: protein–tyrosine-like compounds; B and C: humic substances-like compounds

**Fig. S2.** Three dimensional excitation emission fluorescence matrix (3D EEM) of non-treated and  $\text{NaN}_3$ -treated soil solutions on day0, day11 and day36.

#### 4. Dissolved humic substances on day36 under oxic and anoxic conditions



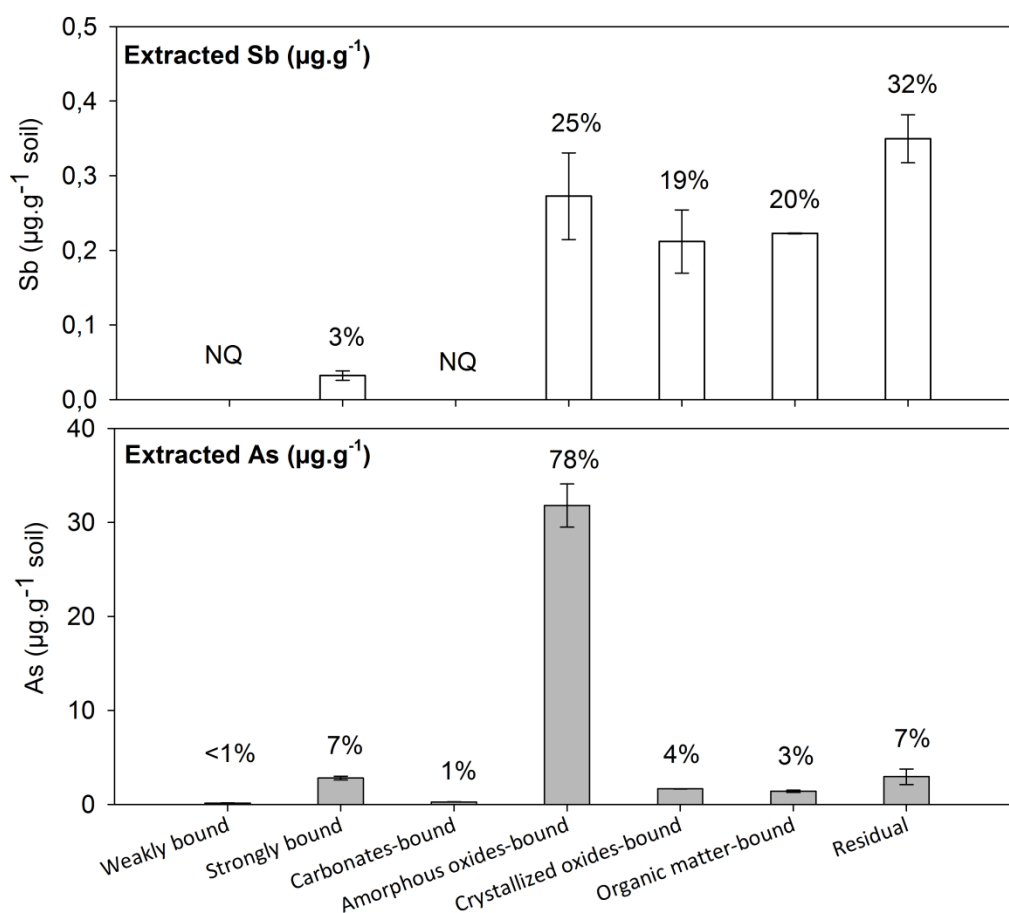
**Fig. S3.** (a) Amount of released humic substances (HS) and (b) HPSEC fingerprints of HS-like compounds (detection at Ex – Em of 322 nm – 444 nm; mobile phase 50 mM phosphate buffer and 100mM NaCl at pH7) on day36 of oxic and anoxic soil incubations.

#### 5. Chemical fractionation of Sb and As on soil

A modified sequential extraction procedure of 7 steps based on Javed et al.<sup>[4]</sup> was applied on soil sample (n=3) on a S:L ratio of 1:40. Table S1 summarizes the sequential extraction procedure. The pH of all extractants was adjusted with NaOH and  $\text{HNO}_3$ . Separation of solid and liquid phases was made by centrifugation at 6000g for 15min. The supernatants were filtered through a 0.2 $\mu\text{m}$  acetate filters (Minisart) then acidified with 50 $\mu\text{L}$  of  $\text{HNO}_3$  65% (50 $\mu\text{L}$ ) to prevent precipitation before analysis. Dissolved Sb and As in the supernatant were measured by ICP-MS. Between each extraction step, soil sample was washed with ultra-pure water for 30 min.

**Table S2. Sequential extraction procedure for As- and Sb-bearing solid phases**

Step	Target phase	Extractant	Procedure/duration	Reference
F1	Loosly adsorbed As and Sb	1M sodium acetate pH 8.2	2h shaking	[5]
F2	Strongly adsorbed As and Sb	0.05M sodium phosphates pH 5	16h shaking	[6]
F3	Carbonates bound As and Sb	1M sodium acetate pH 5	5h shaking	[5]
F4	As and Sb coprecipitated with amorphous Fe, Al and Mn (oxy)hydroxides	Tamm's reagent (0.2M ammonium oxalate /oxalic acid pH 3	2h shaking in the dark (performed twice)	[6]
F5	As and Sb coprecipitated with crystalline Fe, Al and Mn (oxy)hydroxides	0.2M ammonium oxalate /oxalic acid + ascorbic acid (0.1M) pH 3.25	30 minutes in the dark at 96°C (performed twice)	[7]
F6	As and Sb associated with OM and secondary sulfides	30% H <sub>2</sub> O <sub>2</sub> + 3.2 M ammonium acetate pH 2	1) 2 h 6ml of 0.02 M HNO <sub>3</sub> and 10 ml of 30% H <sub>2</sub> O <sub>2</sub> (pH 2) at 85°C for 2h, 2) 6ml of 30% H <sub>2</sub> O <sub>2</sub> (pH 2) at 85°C for 3 h, 3) 10 ml of 3.2M NH <sub>4</sub> OAc in 20% (v/v) HNO <sub>3</sub> for 30 min	[5]
F7		HCL+HNO <sub>3</sub> digestion		



**Fig. S4.** Chemical distribution of Sb and As on soil host phases (percentage (%) correspond to the amount of Sb or As in each fraction out of the sum of all fractions). N.Q refers to non-quantifiable.

## References

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- [2] A. Logeais, *Atlas agronomique du Limousin*. Chambre Régionale de l'Agriculture du Limousin, Limoges, **1993**. [In French]
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- [4] M.B. Javed, G. Kachanoski, T. Siddique, A modified sequential extraction method for arsenic fractionation in sediments. *Analytica. Chimica. Acta*. **2013**, 787, 102. doi:10.1016/j.aca.2013.05.050
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