

Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s

Questa è la versione Post print del seguente articolo:

Original

Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s / Garau, Giovanni; Silveti, Margherita; Vasileiadis, S.; Donner, E.; Diquattro, Stefania; Deiana, Salvatore Andrea; Lombi, Enzo; Castaldi, Paola. - In: SOIL BIOLOGY & BIOCHEMISTRY. - ISSN 0038-0717. - 111:(2017), pp. 25-35. [10.1016/j.soilbio.2017.03.014]

Availability:

This version is available at: 11388/175317 since: 2022-05-23T13:25:09Z

Publisher:

Published

DOI:10.1016/j.soilbio.2017.03.014

Terms of use:

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

Publisher copyright

note finali coverpage

(Article begins on next page)

Manuscript Details

Manuscript number	SBB_2016_378
Title	Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s
Article type	Research Paper

Abstract

Iron-rich water treatment residues (Fe-WTRs) and municipal solid waste compost (MSWC) were added together at two different total rates (i.e. 0.5% Fe-WTRs+0.5% MSWC and 1% Fe-WTRs+1% MSWC) to a degraded sub-alkaline soil (pH 8.0) contaminated with Sb (~110 mg·kg⁻¹ soil), Pb (~1,200 mg·kg⁻¹), Cd (~23 mg·kg⁻¹), and Zn (~5,400 mg·kg⁻¹). A large number of chemical and biological endpoints were evaluated to assess the efficacy of the treatments after five months of incubation. Both treatments significantly reduced the labile fractions of the metal(loid)s in soil, especially Sb, while increasing the abundance of culturable heterotrophic bacteria, actinomycetes and fungi (i.e. up to 6.3-, 1.6- and 4.1-fold higher than control respectively). Soil enzyme activities, i.e. dehydrogenase, β -glucosidase and urease, were also significantly enhanced in the treated soils (i.e. up to ~12-, 3- and 2-fold higher than control respectively). The amendment addition affected the structure of the soil microbial community as highlighted by the higher metabolic potential and catabolic versatility of treated soils (Biolog CLPP) and by the significantly higher α -diversity values based on high throughput partial 16S rRNA gene sequencing. Moreover, analysis of the dominant operational taxonomic units (OTUs) showed differences in the microbial communities of untreated and treated soils. Plant growth (*Helichrysum italicum*) in the treated soils was greatly stimulated while metal(loid)s uptake was significantly reduced. Overall, the results indicated that the applied treatment could be ideal for the chemical and (micro)biological recovery of sub-alkaline soils contaminated with Sb and co-occurring metals, and *H. italicum* appears to be a promising plant species for aided phytostabilisation of such soils.

Keywords	municipal solid waste compost; water treatment residues; antimony; soil microbial community; enzyme activity; community level physiological profile.
Taxonomy	Soil Microbial Community, Soil Rehabilitation, Soil Enzyme Activity, Contaminated Soils, Soil Microbial Populations, Soil Inorganic Pollutant
Manuscript region of origin	Europe
Corresponding Author	Giovanni Garau
Corresponding Author's Institution	University of Sassari
Order of Authors	Giovanni Garau, Margherita Silvetti, Sotirios Vasileiadis, Erica Donner, Stefania Diquattro, Salvatore Deiana, Enzo Lombi, Paola Castaldi
Suggested reviewers	Gudny Okkenhaug, yuan Wei, Esther Álvarez Ayuso, Daniele Daffonchio

Submission Files Included in this PDF

File Name [File Type]

Cover letter.docx [Cover Letter]

Response to reviewers.docx [Response to Reviewers]

Manuscript revised_R2.docx [Manuscript File]

Figure 1.pptx [Figure]

Figure 2.pptx [Figure]

Figure 3.pptx [Figure]

Figure 4.pptx [Figure]

Figure 5.pptx [Figure]

Highlights.docx [Highlights]

SI Tables S1_3.docx [Supporting File]

Figure S1.pptx [Supporting File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Dear Professor Kate Scow
Chief Editor
Soil Biology and Biochemistry,

attached to this mail you will find our revised version (R2) of the manuscript titled:

“Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s”

authored by

Giovanni Garau^{1*}, Margherita Silveti¹, Sotirios Vasileiadis², Erica Donner², Stefania Diquattro¹, Salvatore Deiana¹, Enzo Lombi², Paola Castaldi^{1*}

Please, note that in this revised version (R2), the original title was modified (as above) according to the reviewers suggestion. This was the only revision requested by the reviewers, and no other changes have been applied to the revised manuscript.

Best regards
Giovanni Garau

Authors reply to comments from the editors and reviewers.

We wish to thank the anonymous reviewers and the journal editor for the time devoted to improve our manuscript.

The specific point raised by the editor and reviewers was addressed by the authors and the manuscript revised accordingly.

Editor and Reviewers suggestions.

The only comment is to shorten your title to one which is shorter and more general. The current title makes the work seem very specific and provincial, not of interest to a broader audience. I suggest "Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metals".

Authors

We agree with the suggestion. The title in this revised version was changed to "Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s".

Given the co-occurrence in soil of both metalloids and metals, we used the word "metal(loid)s" instead of "metals".

Best regards
Giovanni Garau

1 Use of municipal solid wastes for chemical and microbiological recovery of soils
2 contaminated with metal(loid)s

3

4

5 Giovanni Garau^{1*}, Margherita Silveti¹, Sotirios Vasileiadis², Erica Donner², Stefania Diquattro¹,

6 Salvatore Deiana¹, Enzo Lombi², Paola Castaldi^{1*}

7

8

9 **1** Dipartimento di Agraria, University of Sassari, Viale Italia 39, 07100 Sassari, Italy, **2** Future

10 Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

11

12 *ggarau@uniss.it (GG); castaldi@uniss.it (PC)

13

14

15 **Abstract**

16 Iron-rich water treatment residues (Fe-WTRs) and municipal solid waste compost (MSWC) were
17 added together at two different total rates (i.e. 0.5% Fe-WTRs+0.5% MSWC and 1% Fe-WTRs+1%
18 MSWC) to a degraded sub-alkaline soil (pH 8.0) contaminated with Sb (~110 mg·kg⁻¹ soil), Pb
19 (~1,200 mg·kg⁻¹), Cd (~23 mg·kg⁻¹), and Zn (~5,400 mg·kg⁻¹). A large number of chemical and
20 biological endpoints were evaluated to assess the efficacy of the treatments after five months of
21 incubation. Both treatments significantly reduced the labile fractions of the metal(loid)s in soil,
22 especially Sb, while increasing the abundance of culturable heterotrophic bacteria, actinomycetes
23 and fungi (i.e. up to 6.3-, 1.6- and 4.1-fold higher than control respectively). Soil enzyme activities,
24 i.e. dehydrogenase, β-glucosidase and urease, were also significantly enhanced in the treated soils
25 (i.e. up to ~12-, 3- and 2-fold higher than control respectively). The amendment addition affected
26 the structure of the soil microbial community as highlighted by the higher metabolic potential and
27 catabolic versatility of treated soils (Biolog CLPP) and by the significantly higher α-diversity values
28 based on high throughput partial 16S rRNA gene sequencing. Moreover, analysis of the dominant
29 operational taxonomic units (OTUs) showed differences in the microbial communities of untreated
30 and treated soils. Plant growth (*Helichrysum italicum*) in the treated soils was greatly stimulated
31 while metal(loid)s uptake was significantly reduced. Overall, the results indicated that the applied
32 treatment could be ideal for the chemical and (micro)biological recovery of sub-alkaline soils
33 contaminated with Sb and co-occurring metals, and *H. italicum* appears to be a promising plant
34 species for aided phytostabilisation of such soils.

35

36 Key words: Municipal solid waste compost; water treatment residues; antimony; soil microbial
37 community; enzyme activity; community level physiological profile.

38

39 **1. Introduction**

40 Antimony (Sb) is a non-essential plant element which occurs naturally in soil, with its main source
41 being the weathering of soil parent materials containing minerals such as stibnite (Sb_2S_3) and
42 valentinite (Sb_2O_3) (Kabata-Pendias, 2011). While natural background concentrations of Sb in soils
43 range from 0.25 to 1.4 $\text{mg}\cdot\text{kg}^{-1}$ (Kabata-Pendias, 2011), much higher concentrations can be found
44 in the proximity of mining sites, mineral processing facilities and/or shooting ranges (due to
45 human activities). In recent years, heavily Sb-polluted sites have been identified all around Europe,
46 e.g. in Italy ($\sim 15,000 \text{ mg}\cdot\text{kg}^{-1}$ soil), Germany ($\sim 500 \text{ mg}\cdot\text{kg}^{-1}$ soil), Switzerland ($\sim 17,000 \text{ mg}\cdot\text{kg}^{-1}$ soil),
47 France ($\sim 5,700 \text{ mg}\cdot\text{kg}^{-1}$ soil) (Tschan et al., 2009) as well as worldwide (Wang et al., 2010 and
48 Sanderson et al., 2014). These sites, often characterised by critical concentrations of co-occurring
49 metal(loid)s (Wang et al., 2010, Wang et al., 2011, Okkenhaug et al., 2013 and Sanderson et al.,
50 2014), can be of particular environmental concern since they represent hazardous multi-element
51 contamination sources for neighbouring soils and water bodies and constitute a threat for soil
52 functionality and fertility.

53 Bioavailable Sb and co-occurring metal(loid)s can severely compromise soil functionality by
54 affecting the size, composition and activity of the resident microbial communities (Garau et al.,
55 2011, Wang et al., 2011, Garau et al., 2014 and Wei et al., 2015) as well as plant growth (Castaldi
56 et al., 2009, Kabata-Pendias, 2011 and Pan et al., 2011). For instance, previous studies have
57 reported reduced growth and biomass of both grasses and legumes under Sb and/or heavy
58 metal(loid)s stress (Castaldi et al., 2009, Pan et al., 2011 and Garau et al., 2014) as well as
59 decreased abundance of culturable soil microbial populations and reduced soil enzyme activities
60 (Garau et al., 2007, Wang et al., 2011 and Garau et al., 2014). Moreover, it was recently shown
61 that elevated Sb reduced the diversity of arbuscular mycorrhizal fungi (Wei et al., 2015) and

62 increased the distribution, diversity and abundance in soil bacterial populations of selected genes
63 involved in Sb detoxification (Luo et al., 2014).

64 Although the co-occurrence of critical concentrations of Sb and additional metal(loid)s represents
65 a substantial threat for soil and ecosystem functioning, effective and reliable approaches for the
66 remediation of these polluted sites are currently lacking. In particular, the different speciation,
67 mobility and bioavailability of Sb with respect to selected co-occurring metal(loid)s (e.g. trace
68 metal cations) makes the identification of suitable amendments a very challenging task.

69 Antimony is commonly present in the environment in the trivalent (III) and pentavalent (V) states
70 with the inorganic antimonite $[\text{Sb}(\text{OH})_3]$ and antimonate $[\text{Sb}(\text{OH})_6^-]$ being the dominant species in
71 aqueous systems across a wide pH range (i.e. pH 4-10) (Okkenhaug et al., 2013). In aerated soils,
72 the anionic species $\text{Sb}(\text{OH})_6^-$ is prevalent (if not exclusive) (Johnson et al., 2005 and Filella et al.,
73 2009) and displays a high affinity to amorphous and crystalline Fe-(hydr)oxides with which it can
74 form stable bidentate inner-sphere complexes (Guo et al., 2014). In the pH range of the majority
75 of soils (i.e. pH 5-9), such interactions are particularly favoured by the high point of zero charge of
76 amorphous and crystalline Fe-(hydr)oxides (e.g. pH_{PZC} 7.5-9.0 for goethite, 8.5 for hematite, 7.0-
77 9.0 for ferrihydrite, 9.5-10 for akaganeite (Strawn et al., 2015)). However, in the same pH range
78 (i.e. pH 5-9), heavy metals such as Pb, Cd and Zn behave quite differently, being commonly
79 present in the soil solution as divalent and/or monovalent (hydroxylated) cations at acidic and
80 circumneutral pH or as soluble SOM-metal(II) complexes at higher pH values (Kabata-Pendias,
81 2011). Moreover, at neutral and/or alkaline pH substantial amounts of heavy metals are
82 immobilised as Me-hydroxides, -carbonates and/or -hydroxycarbonates. Due to their cationic
83 nature, and in contrast to Sb(V), soluble heavy metals show a limited affinity for positively charged
84 soil surfaces but interact more strongly with negatively charged components (e.g. soil organic
85 matter and clay minerals).

86 In the last decades, a variety of potential sorbent materials for the in-situ remediation of
87 metal(loid) polluted soils have been proposed and tested (Castaldi et al., 2009, Garau et al., 2011
88 and Garau et al., 2014), however Sb-contaminated (or co-contaminated) sites have been largely
89 overlooked and neglected until recently. To date, only a few amendments, mostly based on Fe-
90 and Al-containing materials/minerals, have been tested with variable success as Sb-immobilising
91 agents. For instance, adding olivine and hematite to a contaminated soil was found to have no
92 effect on porewater Sb concentration (Okkenhaug et al., 2012), while red mud (alumina industry
93 residues high in Fe-oxides) addition reduced porewater Sb only in some cases (Sanderson et al.,
94 2015). The addition of organic amendments (e.g. mussel shell, cow bone, chicken manure, sodium
95 humate) have mostly been deemed ineffective at fixing Sb (Conesa et al., 2010, Ahmad et al., 2014
96 and Shtangeeva et al., 2014), although mussel shell and cow bone, and also red muds, reduced the
97 labile co-occurring Pb from the soil solution (Ahmad et al., 2014 and Sanderson et al., 2015). Other
98 research has shown that a 2% commercial Fe-oxyhydroxide, and very high rates of amorphous Fe-
99 and Al-oxyhydroxides, could effectively stabilise the antimony in different polluted soils (Alvarez-
100 Ayuso et al., 2013, Okkenhaug et al., 2013 and Okkenhaug et al., 2016). However, the mobilisation
101 of co-occurring metals (e.g. Pb, Cu, and Zn) was detected after the addition of Fe-based materials
102 to Sb-contaminated soils (Okkenhaug et al., 2013). These findings, together with the limited
103 number of studies addressing the remediation of Sb-contaminated sites, highlight the need to
104 deepen our knowledge on the physico-chemical factors governing the mobility of Sb (and co-
105 occurring metal(loid)s) in soil and to further select for sorbents with ideal Sb-immobilizing
106 capabilities. Such sorbents, other than reducing the mobility and phytoavailability of Sb and co-
107 occurring metal(loid)s, should also ideally improve soil fertility as well as soil microbial abundance,
108 diversity and functionality.

109 In this context, the aim of this work was to evaluate the suitability of the combined application of
110 two low-cost and sustainable amendments for the chemical and biological recovery of a degraded
111 alkaline soil contaminated with Sb, Pb, Cd and Zn. Iron-rich drinking-water treatment residuals (Fe-
112 WTRs) and municipal solid waste compost (MSWC) were the selected amendments.
113 The presence in Fe-WTRs of an abundant mineral component rich in Fe-(hydr)oxides makes these
114 residues potentially effective for the immobilisation of Sb in our alkaline soil (Guo et al., 2014),
115 while earlier studies also suggest their suitability as metal-fixing agents (Castaldi et al., 2015). On
116 the other hand, the application of MSWC together with Fe-WTRs was expected to contribute to
117 soil recovery mainly by improving its nutritional and functional status.
118 The effectiveness of the combined application of Fe-WTRs and MSWC in a 1:1 ratio, at two
119 different total rates (i.e. 1 and 2%), was assessed in this study using a comprehensive approach
120 that spanned chemical investigation of metal(loid)-mobility, plant growth and pollutant
121 phytoavailability, soil microbial abundance and bacterial diversity, community level physiological
122 profiling, and soil enzyme activity.

123

124

125 **2. Materials and Methods**

126

127 *2.1. Soil and amendment origins, characteristics and microcosm set up*

128

129 Sixty topsoil samples (0-20 cm depth; ~ 2 kg each) were collected from an area of approximately 1
130 ha located in the abandoned mining site of Argentiera (N40°44'11'', E8°8'54'') in north-western
131 Sardinia (Italy), where Pb, Ag and Zn were extracted for about one century (1867-1963) from
132 silver-rich galena [(Pb, Ag)S] and sphalerite (ZnS). The site is characterized by the presence of Sb-

133 rich minerals such as boulangerite ($\text{Pb}_5\text{Sb}_4\text{S}_{11}$), tetrahedrite [$(\text{Cu,Fe})_{12}\text{Sb}_4\text{S}_{13}$], freibergite
134 [$(\text{Ag,Cu,Fe})_{12}(\text{Sb,As})_4\text{S}_{13}$], bournonite (PbCuSbS_3) and pyrargyrite (Ag_3SbS_3) (Pirri, 1996). According
135 to particle-size analysis, carried out using the pipette method (Day, 1965), the soil in the sampled
136 area was a sandy-loam (USDA classification) with 68% coarse sand, 15% fine sand and 17% silt.
137 The collected soil samples were bulked together, air dried and sieved to <2 mm before being
138 employed for the set-up of different soil microcosms, each consisting of approx. 10 kg soil.
139 Triplicate microcosms were amended with 0.5% (w/w) Fe-WTRs + 0.5% (w/w) MSWC (1% total
140 amendment rate) or 1% (w/w) Fe-WTRs + 1% (w/w) MSWC (2% total amendment rate) or left
141 untreated as controls. These rates were selected based on the specific metal(loid)s immobilising
142 capabilities of Fe-WTRs and MSWC highlighted in previous studies (Manzano et al., 2016 and
143 Garau et al., 2014). Before addition to the microcosms, Fe-WTRs (provided by the Bidighinzu plant
144 of the Abbanoa industry, Sassari, Italy) and MSWC (provided by the Secit S.p.A. facility plant of the
145 Consorzio ZIR, Chilivani-Ozieri, Sassari, Italy) were oven dried at 60°C for 48 h and sieved to <2
146 mm.
147 After amendment, treated (and untreated) soils were carefully mixed, brought to 60% of their
148 water holding capacity and equilibrated for 5 months at 20°C. During this period, soils were mixed
149 twice a week and maintained at 60-70% relative humidity.

150

151

152 *2.2. Amendments and soil chemical analyses*

153

154 Selected physico-chemical characteristics of the treated and untreated soils after equilibration,
155 and of the amendments used, are reported in Tables 1-2. The pH and electric conductivity (EC)
156 were determined using a 1:2.5 soil/amendment:deionised water ratio according to national

157 standard guidelines (Gazzetta Ufficiale, 1999 and 2002). The total organic matter and N were
158 determined according to the Walkley-Black and to the Kjeldhal method respectively (Gazzetta
159 Ufficiale, 1999 and 2002) while total P was determined spectrophotometrically (ascorbic acid
160 method) (Gazzetta Ufficiale, 1999 and 2002). The dissolved organic carbon (DOC) was estimated as
161 described by Brandstetter et al. (1996) while the point of zero charge (pH_{pzc}) has been determined
162 by potentiometric titration as described in Appel et al. (2003). The method reported by Ciavatta et
163 al. (1990) was used to determine the content of humic substances (i.e. humic and fulvic acids)
164 while the specific surface area was determined by applying the BET model to the N_2 adsorption
165 results obtained from a Carlo Erba Sorptomatic instrument (Milan, Italy) (Castaldi et al., 2010).
166 Total and active carbonates were determined by acid dissolution (volumetric calcimeter method)
167 and by reaction of soil with 0.1 M ammonium oxalate respectively (Gazzetta Ufficiale, 1999 and
168 2002). Soil cation exchange capacity (CEC) was determined using the $BaCl_2$ and triethanolamine
169 method (Gazzetta Ufficiale, 1999).

170 Total concentration of metal(loid)s in soils and amendments was determined after digestion with
171 an HNO_3/HCl mixture (1:3 v/v ratio) using a flame atomic absorption spectrometer equipped with
172 a HGA-600 graphite furnace (Perkin Elmer Analyst 600). The NIST-SRM 2710 was used as standard
173 reference material for quality assurance and quality control.

174 The Sb mobility in soil was assessed through the sequential extraction procedures of Wenzel et al.
175 (Wenzel et al., 2001) (originally designed for soil As fractionation) while the protocol of Basta and
176 Gradwohl (Basta and Gradwohl, 2000) was used to assess the mobility of Cd, Zn and Pb. Briefly,
177 labile and surface-bound Sb in soil was assessed after extraction with 0.05 M $(NH_4)_2SO_4$ (Step 1)
178 and 0.05 M $(NH_4)H_2PO_4$ (Step 2) respectively, while the Sb associated to amorphous and crystalline
179 Al- and Fe-oxides was determined after extraction with 0.2 M NH_4 -oxalate (Step 3) and 0.2 M NH_4 -
180 oxalate solution + 0.1 M ascorbic acid (Step 4) respectively (Wenzel et al., 2001). Exchangeable

181 and readily soluble Cd, Zn and Pb in soil were determined after extraction with 0.5 M $\text{Ca}(\text{NO}_3)_2$
182 while acid-soluble metals and weak surface complexes were determined after extraction with 1 M
183 NaOAc (pH 5) (Basta and Gradwohl, 2000). Surface complexes and metal precipitates were
184 determined after 0.1 M Na_2EDTA extraction. After each step of the respective extraction
185 procedures, the samples were centrifuged at 8000 rpm for 10 min, filtered to completely separate
186 the liquid and solid phases and metal(loid)s in the supernatant determined using a flame atomic
187 absorption spectrometer equipped with a HGA-600 graphite furnace (Perkin Elmer Analyst 600).
188 The residual Sb in soil as well as the very insoluble and/or occluded metals were determined after
189 microwave digestion with an HNO_3/HCl mixture as previously mentioned.

190

191

192 *2.3. Heterotrophic microorganisms, Community Level Physiological Profiles and soil enzyme* 193 *activities*

194

195 Fast-growing culturable bacteria, fungi and actinomycetes were enumerated in soil microcosms
196 (after equilibration) using the conventional ten-fold serial dilution and spread plate method.
197 Solidified (15 g L^{-1} agar) 1:10 strength TSA (Tryptic Soy Agar, Microbiol, Cagliari, Italy), GYEP at pH
198 4.5 (Glucose Yeast Extract Peptone medium) and AIA (Actinomycete Isolation Agar, Difco, Milan,
199 Italy) were used as growth media for the enumeration of culturable bacteria, actinomycetes and
200 fungi respectively (Pinna et al., 2012). Microbial counts were conducted after incubation of the
201 plates for 72h at 25°C.

202 The Community Level Physiological Profile (CLPP) of microbial communities was determined using
203 Biolog Ecoplates (Biolog Inc., Hayward, CA). Microbial communities were extracted from the
204 respective soils and separately inoculated in each of the 96 microtiter wells of a Biolog Ecoplate as

205 previously described (Pinna et al., 2012). The Biolog Ecoplate contains a triplicate set of 31 carbon
206 sources of soil/environmental relevance, each present in a separate well, and three control wells
207 with no carbon. A tetrazolium dye incorporated in the plate wells reveals oxidative catabolism as
208 purple colour formation. This latter was recorded for each well every 15 min for 96 h using a
209 Biolog Omnilog unit (i.e. a charge-coupled-device camera; Biolog, Hayward, CA) to obtain a colour
210 vs. time curve. For each well/substrate, the area under this curve was control-subtracted and the
211 potential catabolic activity of each microbial community was calculated as the average well area
212 over the 31 carbon sources (AWCD; Garau et al., 2007 and Garau et al., 2014). Richness values (i.e.
213 the number of carbon sources metabolised by each microbial community) were determined as the
214 number of wells with (control-subtracted) areas higher than 1,000 (Biondi et al., 2009).
215 Selected enzyme activities, i.e. dehydrogenase (DHG), β -glucosidase (GLU) and urease (URE), were
216 determined (colorimetrically) in duplicate soil samples collected from each microcosm as
217 previously described (Garau et al., 2007). Briefly, DHG activity was quantified by determining the
218 concentration of triphenyl formazan produced after incubation of soil samples (5 g each) with
219 triphenyltetrazolium chloride for 24 h at 30°C. GLU activity was determined as p-nitrophenol
220 released after incubation of soil samples (1 g each) with p-nitrophenyl glucoside for 2 h at 37°C.
221 URE activity was determined as ammonia released after incubation of soil samples (5 g each) with
222 urea for 2 h at 37°C.

223 224 225 *2.4. Molecular analysis of soil bacterial communities and bioinformatics*

226
227 Total DNA was extracted from soil samples (~500 mg) of each microcosm using the FastDNA™
228 SPIN Kit and FastPrep® Instrument (MP Biomedicals, Santa Ana, CA) according to the

229 manufacturer's instructions with the homogenization step performed 3 times at maximum speed
230 and time ($3 \times 6.5 \text{ m sec}^{-1} \times 60 \text{ sec}$). DNA extracts were quantified using the Qubit® dsDNA HS Assay
231 kit (Invitrogen, Waltham, Massachusetts, USA) with the Qubit® v2.0 Fluorometer (Invitrogen,
232 ThermoFisher-Scientific, Waltham, Massachusetts, USA) and stored at -20°C prior to downstream
233 analyses. The V3-4 hypervariable region of the bacterial small ribosomal subunit coding gene (SSU)
234 was amplified via polymerase chain reaction (PCR) performed on 0.2 ng of the DNA extracts using
235 the 343SF forward primer (5'-TACGGGAGGCAGCAG-3'; modified version of the S-D-Bact-0343-a-S-
236 15 (Vasileiadis et al., 2012)) and a modified version of the 802R reverse primer (5'-
237 TACNVGGGTWTCTAATCC-3'; version of S-D-Bact-0785-b-A-18 (Claesson et al., 2009)) in 20 μl
238 reactions. The PCR reactions were performed in two steps to restrict amplification and high
239 throughput sequencing indexing related biases as described previously (Berry et al., 2011). The
240 primers mentioned above were used in the first step while the indexed primer versions (for
241 multiplexing prior to sequencing) provided in Table S1 were used in the second step. Briefly, 2 μl
242 of DNA extracts diluted to $0.1 \text{ ng}\cdot\mu\text{l}^{-1}$ were added as templates to 18 μl mixtures containing 10 μl
243 Phusion Flash High-Fidelity PCR Master mix (Finnzymes, ThermoFisher-Scientific, Waltham,
244 Massachusetts, USA), 1 μl of each primer solution (10 μM), 0.4 μl of bovine serum albumin (BSA –
245 ThermoFisher-Scientific – Waltham, Massachusetts, USA – $20 \text{ mg}\cdot\text{ml}^{-1}$) and 5.6 μl PCR grade water.
246 The resulting 20 μl reaction mixtures were subject to the first PCR comprising: an initial step of 3
247 minutes at 95°C for enzyme activation/DNA-denaturation; followed by 28 cycles of 95°C for 15
248 seconds for DNA denaturation, 50°C for 15 seconds for primer annealing and 72°C for 15 seconds
249 for DNA elongation; followed by 10 minutes at 72°C for the final elongation step. Two microliters
250 of the resulting amplification products were used as DNA template in the second PCR cycle (same
251 mixture as above; 7 cycles only) to add indexed primers (Table S1) in preparation for sequencing.
252 Equimolar amounts of the PCR products were then multiplexed and sequenced with the Illumina

253 HiSeq 2500 platform for 300 cycles using the paired-end reads module by Fasteris SA (Geneva,
254 Switzerland).

255 Output sequences were analysed with the LotuS v1.44 software (Hildebrand et al., 2014) as
256 follows: reads were de-multiplexed and quality controlled with the LotuS native simple de-
257 multiplexer (smd) v1.26 using the default parameters with the exception of a minimum good
258 quality sequence length of 170 bp; read pairs were then merged with FLASH v1.2.8 (Magoč and
259 Salzberg, 2011); clustering of reads into 97% identity operational taxonomic units (OTUs) was
260 performed with the USEARCH/UPARSE v8.0.1623 algorithm (Edgar, 2013); chimeric OTUs were
261 removed with UCHIME v4.2 (Edgar et al., 2011) and the ribosomal database project (RDP) gold
262 database version; and representative OTU sequences were classified into known taxa using the
263 RDP naïve Bayesian classifier v2.11 (Wang et al., 2007). Selected OTU sequences were further
264 analysed to obtain phylogenetic information according to a maximum likelihood approach,
265 whereby closely related sequences were obtained from the National Center for Biotechnology
266 Information (NCBI) 16S rRNA gene type strain collection using the basic local alignment search tool
267 (BLAST) (Altschul et al., 1990). The best BLAST hits were clustered with Cdhit v4.6 (Li et al., 2001)
268 to reduce sequence redundancy and the cluster representative sequences were aligned with
269 Muscle v3.8.31 (Edgar, 2004). Non-properly aligned and uninformative alignment blocks were
270 removed using Gblocks v0.91b (Talavera and Castresana, 2007) and the remaining concatenated
271 alignment blocks were subjected to maximum likelihood phylogenies with the RAxML software
272 v8.1.24 (Stamatakis, 2014) using the general time-reversible (GTR) model for gamma rate
273 heterogeneity and considering invariable sites using 1000 bootstrap replicates. The sequences
274 obtained were submitted to the NCBI (NCBI - <https://www.ncbi.nlm.nih.gov>) Sequence Read
275 Archive (SRA - <https://www.ncbi.nlm.nih.gov/sra/>) and are currently available under the accession
276 numbers SRR4424247-55.

277

278

279 2.5. Plant growth experiment and plant analysis

280

281 At the end of the 5 months incubation, soils from each microcosm were used to grow *Helichrysum*
282 *italicum* (Roth) G.Don plantlets (approx. height 5 cm) in plastic pots [23 cm (L) x 15 cm (H) x15 cm
283 (D)] filled with ~3 kg of soil. We selected this plant species as it was growing abundantly at the site
284 investigated suggesting an apparent tolerance to the metal(loid)s present and good adaptability to
285 the low fertility conditions which characterised the soil. A total of thirty-six *H. italicum* plantlets
286 were grown on each treated and untreated soil in 6 pots (6 plants x pot; 2 pots x microcosm) for
287 16 weeks under controlled conditions (20°C; 60–70% relative humidity). At harvest, shoots and
288 roots were separated, thoroughly washed with distilled water, and oven-dried at 70°C for 96 h for
289 dry weight determination.

290 Metal(loid)s in shoots and roots were determined, after mineralization of the plant material (EPA
291 Method 3052), using a flame atomic absorption spectrometer equipped with a HGA-600 graphite
292 furnace (Perkin Elmer Analyst 600). Peach leaf was used as standard reference material (NIST-SRM
293 1515).

294

295

296 2.6. Data analysis

297

298 Chemical (i.e. sequential extraction) and (micro)biological data were subjected to One-way
299 analysis of variance and when significant *P* values were obtained ($P<0.05$) differences between
300 individual means were compared with the post-hoc Tukey Kramer multiple comparison test

301 ($P < 0.05$) using the NCSS software (Keyville, Utah). The 16S rRNA gene sequence data were
302 analysed using the R v3.2.3 software (R Core Team, 2015). Tests performed included: Hellinger
303 transformation of the OTU matrix as suggested by Legendre and Gallagher (Legendre and
304 Gallagher, 2001) followed by hierarchical clustering using the unweighted pair group method with
305 arithmetic mean (UPGMA) and principal component analysis with modelling of the
306 physical/chemical variables with the Vegan package v2.4-0 (Oksanen et al., 2016). Treatment
307 group related OTU differential abundances were assessed with Fisher's exact tests as
308 implemented by the Edge R v3.12.0 package using the Holm correction for multiple hypothesis
309 testing (Robinson et al., 2010).

310

311

312 **3. Results and Discussion**

313

314 *3.1. Influence of the amendments on physico-chemical soil features and metal(loid) mobility in soil*

315

316 In this study we evaluated the combined application of Fe-WTRs and MSWC, at two different rates
317 (i.e. at 1 and 2%), as potential amendments for the remediation of an alkaline (pH 8.0) degraded
318 soil polluted by Sb and co-occurring metals. The soil was characterized by a significant content of
319 sand (~83% coarse and fine sand), a medium cation exchange capacity (CEC, ~17 $\text{cmol}_{(+)}\cdot\text{kg}^{-1}$ soil)
320 and very low organic matter content (OM, ~0.4%) (Table 2). Moreover, the total concentrations of
321 Sb, Cd, Zn and Pb all exceeded the national (Italian) threshold values for private, residential and
322 public green areas and for commercial and industrial sites (Tables 2 and S2).

323 The combined addition of Fe-WTRs and MSWC clearly improved some fertility characteristics of
324 the soil such as the CEC (>25 and 97% increase at 1 and 2% amendment rates, respectively), OM

325 (>50 and 90%), and N content (>15 and 45%) (Table 2). DOC also increased significantly after soil
326 amendment (>400 and 700% at 1 and 2% amendment rates, respectively) while pH, EC, total and
327 active carbonate were little affected (Table 2).

328 The amendment additions did not change the total concentrations of metal(loid)s in the soil (Table
329 2). On the other hand, the sequential extraction data indicated a relatively low content of readily
330 labile and surface-bound Sb in the untreated polluted soil, i.e. the water-soluble and easily
331 exchangeable Sb extracted in Step 1 and the Sb forming inner-sphere complexes extracted in Step
332 2 respectively (Fig. 1). This may be encouraging from an environmental point of view since these
333 Sb fractions can be considered to have the greatest impact on soil biota (Wenzel et al., 2001;
334 Garau et al., 2014). Most of the Sb in the polluted soil (~ 85%) was instead associated with
335 amorphous and especially crystalline Al- and/or Fe-oxides as highlighted in Step 3 and 4
336 respectively (Fig. 1).

337 The added amendments had no obvious effects on the readily labile Sb but significantly reduced
338 the amount of metalloid extracted in Step 2 (Fig. 1). This is relevant as this Sb fraction can be
339 simply mobilised (i.e. becoming bioavailable) following a pH change (e.g. due to plant and/or
340 microbial activity) or phosphate increase (Wenzel et al., 2001). The Sb fraction associated with
341 amorphous Al- and Fe-oxides (Step 3) increased significantly after soil amendment while the
342 opposite was true for the metalloid fraction associated to crystalline Al- and Fe-oxides (Step 4; Fig.
343 1). Finally, the residual Sb fraction (i.e. that is strongly retained and not expected to be readily
344 released) increased by approximately 3- and 4-fold in the soils amended at 1 and 2% rate
345 respectively.

346 Overall, these data show that the combined addition of Fe-WTRs and MSWC induced a significant
347 redistribution of Sb in soil, i.e. a reduction of its more labile and bioavailable forms and an increase
348 of the residual (i.e. not readily bioavailable) forms. This can be the result of different mechanisms

349 induced by the amendments, likely including Sb mobilisation from soil and its redistribution within
350 the soil/amendment components. In particular, Sb mobilisation in the treated soils could have
351 occurred through competitive exchange phenomena involving organic and inorganic components
352 of both amendments, but also through partial dissolution of Sb-containing Al and Fe-(hydr)oxides
353 (amorphous and crystalline) due to solubilisation of Fe/Al nuclei. As recently highlighted (Chen et
354 al., 2016), fulvic acids within Fe-WTRs and MSWC may have played an important role in this (Table
355 1). On the other hand, the formation of soluble complexes between the organic matter within Fe-
356 WTRs/MSWC and Sb is not expected to be relevant at the pH value of this soil (Filella and Williams,
357 2012).

358 As supported by the sequential extraction data (Step 3; Fig.1), sorption of Sb in the amended soils
359 may have involved existing amorphous Al- and Fe-(hydr)oxides in soil and, above all, the
360 amorphous Al- and Fe-(hydr)oxides added with Fe-WTRs (Table 1). This could explain the
361 significant increase of the Sb detected in Step 3 and the reduction of that extracted in Step 4 (Fig.
362 1). Moreover, the precipitation of part of the released Sb(OH)_6^- with soluble Ca^{2+} (abundantly
363 present within MSWC; Table 1) may also have contributed to the increase of the residual Sb pool
364 in the treated soils (Johnson et al., 2005 and Conesa et al., 2010).

365 The sequential extraction of Basta and Gradwohl (2000) showed a limited amount of water soluble
366 and exchangeable Cd, Zn and Pb in the contaminated soil (Fig. 2). This was somewhat expected in
367 this alkaline (carbonate-rich) soil where a substantial amount of the metals are likely to be
368 precipitated as metal carbonates and/or as metal (hydr)oxides (Kabata-Pendias, 2011).

369 Accordingly, approx. 40, 50 and 66% of total Zn, Cd and Pb respectively were extracted with EDTA
370 (Fig. 2) suggesting that these relatively immobile (and not readily bioavailable) metal fractions
371 were likely involved in inner-sphere surface complexes and/or were in the form of precipitates
372 (Basta and Gradwohl, 2000). The residual metal fractions in the contaminated soil, i.e. very

373 insoluble and/or occluded metal pools, were approx. 30, 45 and 50% of total Pb, Cd and Zn
374 respectively (Fig. 2). The most important amendment effect was the significant reduction of water
375 soluble and exchangeable Cd, Zn and Pb, i.e. the more mobile and bioavailable fractions. This
376 could be mainly explained with the capacity of the organic and inorganic components of both the
377 amendments to sorb the metals considered as previously highlighted (Castaldi et al., 2015, Silveti
378 et al., 2015 and Manzano et al., 2016). A reduction of the acid-soluble and/or weakly complexed
379 Pb (extracted with NaOAc) was also observed after amendment addition together with a
380 significant increase of the residual Cd (Fig. 2).

381 Overall, the addition of Fe-WTRs and MSWC to the contaminated soil contributed to lower the
382 environmental impact of the metal(loid)s present by limiting the amount of their soluble and
383 exchangeable forms and in some cases (especially for Sb and Cd) by substantially increasing their
384 residual fraction. As previously pointed out, this is expected to have relevant positive effects on
385 the soil microbial component and its functionality (Garau et al., 2007, Garau et al., 2011 and Garau
386 et al., 2014).

387

388

389 *3.2. Influence of the amendments on the soil microbial abundance, enzyme activities and* 390 *community level physiological profile*

391

392 The combined addition of Fe-WTRs and MSWC induced significant increases in all targeted soil
393 microbial groups. In the case of total culturable heterotrophic bacteria and actinomycetes (but not
394 fungi) the increase in CFUs (colony forming units) g^{-1} soil was also positively correlated with the
395 amendment rate (Table 3). This increase of readily culturable microorganisms in the treated soils
396 was likely due to the presence of additional easily metabolisable carbon sources deriving from the

397 OM within MSWC and Fe-WTRs (Garau et al., 2011). This was supported by the significant increase
398 of the DOC and OM in the amended soils (Table 2). The reduction of labile metal(loid) fractions in
399 the treated soils (Figs. 1-2) could have also contributed to microbial growth by increasing the
400 metabolic energy dedicated by microorganisms to cell growth (and multiplication) instead of high-
401 energy demanding metal detoxification processes (Garau et al., 2011). The number of readily
402 culturable bacteria and fungi can provide an estimate of the potentially active microbial
403 populations in soil, i.e. microbial populations involved in the prompt degradation of soil organic
404 matter and the release (and cycling) of mineral elements essential for plant growth, e.g. N and P
405 (Timms-Wilson et al., 2006). In this sense, the increase of these microbial populations in the
406 amended soils can be considered as positive as it suggests an improved fertility status while
407 confirming a reduced environmental impact of the contaminants.

408 Enzyme activity data indicated functional improvements in the treated soils. In particular, higher
409 DHG values were recorded in amended soils compared to the control (approx. 6 and 12-fold higher
410 at 1 and 2% amendment rate). Also URE and GLU significantly increased after 2% amendment
411 (2.17 and 2.9-fold higher respectively compared to controls) (Table 3). All this indicated a higher
412 microbial activity in the amended soils and possibly an improved C and N turnover. Pearson
413 correlation analysis indicated significant ($P < 0.05$) positive correlations between the number of
414 culturable microorganisms and all the enzyme activities ($r = 0.69-0.94$; Table S3). This may support
415 the view that culturable microorganisms may play a role in soil functioning and nutrient turnover.
416 However, it should be noted that the relationship between soil functioning and microbial
417 community is only partially explored by culturable techniques and it cannot be excluded that
418 unculturable microorganisms also play a very important role.

419 The Biolog Community level physiological profile (CLPP) showed significant differences between
420 untreated and treated soils. In particular, the microbial populations extracted from treated soils

421 displayed a significantly higher potential carbon source utilisation compared to those extracted
422 from control soil (Fig. 3). This increase also appeared proportional to the amendment rate. The
423 same was recorded for richness values, or the number of carbon sources metabolised by the
424 microbial communities (Fig. 3). As pointed out in earlier studies, fast-growing culturable bacteria
425 are the most relevant contributors of colour development in Biolog Ecoplates (Pinna et al., 2012
426 and Garau et al., 2014) and in this sense, Biolog data support the higher bacterial counts recorded
427 in the treated soils (Table 3). Moreover, CLPP analysis also indicates that the amendment addition
428 had a positive effect on the catabolic versatility of the microbial communities as indicated by the
429 richness values (Fig. 3). For instance, only the microbial communities of treated soils were able to
430 catabolise i-erythritol, L-serine and D-glucosaminic and 4-hydroxy benzoic acids (other than the
431 substrates catabolised by the control soil microbial community) while glycogen and itaconic acid
432 were exclusively metabolised by the microbial populations extracted from the 2% amended soil.
433 The appearance in the amended soils of microbial communities with higher and more diverse
434 catabolic potentials could be partly the result of reduced environmental pressure (i.e. reduced
435 metal(loid) bioavailability) on microbial taxa that were poorly represented in the contaminated
436 soil (e.g. because they are more sensitive to contaminants). All this combined with the larger
437 availability in the treated soils of easily metabolisable carbon sources could have sustained the
438 growth of these rare taxa and allowed for the appearance of the new phenotypes.

439

440

441 *3.3. Amendment impact on soil bacterial diversity*

442

443 Most often, the selection of an amendment for the in-situ treatment of contaminated soils is
444 primarily based on its ability to reduce the concentration of labile metal(loid)s. By contrast,

445 additional effects such as the impact of the amendment on the soil microbial abundance and soil
446 functionality are often neglected as well as the impact of the amendment on the soil microbial
447 diversity. Nowadays it is widely accepted that soil microbial diversity affects many key ecosystem
448 processes such as the biogeochemical cycle of plant nutrients as well as the evolutionary dynamics
449 in plant communities. Since soil microbial diversity allows for the preservation of such functional
450 traits, even in the face of external perturbations (Fitter et al., 2005), its maintenance and/or
451 improvement following amendment addition should be of primary importance. Accordingly, soil
452 bacterial diversity in the treated soils was evaluated by high throughput partial 16S rRNA gene
453 sequencing.

454 The sequencing effort was suitable for screening the vast majority of the existing bacterial
455 diversity as indicated by the Good's coverage estimate (Good, 1953) with a range of 98.4-99.4% of
456 the soil bacterial SSU diversity being uncovered (Table 4). Both inverse Simpson ($1/\lambda$) and Gini-
457 Simpson ($1-\lambda$) indexes were significantly higher in the treated soils while the other indexes did not
458 reveal significant differences (Table 4). In this regard, it should be noted that the Simpson's index
459 and its derivatives, also known as 2nd order diversity indexes, have been shown to better capture
460 the diversity of dominant OTUs in environmental samples (Mendes et al., 2008 and Gihring et al.,
461 2011) as opposed to other indexes like the Fisher's α diversity (Fisher et al., 1943), the effective
462 number of OTUs based on a generalised Tsallis entropy (Tsallis, 1988) as implemented by Mendes
463 et al. (2008) and, of course, the observed richness (S) or richness estimates (Chao, 1984 and Chao
464 and Lee, 1992). PCA of the Hellinger transformed OTU matrix using OTUs with relative
465 participation of 1% or above (13 OTUs in total), and grouping the rest OTUs as a single group of
466 "rare" OTUs, showed a treatment-wise grouping of samples (Figure 4a). Furthermore, modelling of
467 selected soil physico-chemical parameters showed strong correlations of the community with the
468 pH_{pZC} , DOC and OM, CEC and the concentration of the more labile fractions of Sb, Pb, Cd and Zn

469 (Fig. 4a). Three OTUs, namely OTU 2, 4 and 8 were dominantly associated with the treated soils as
470 shown in Figure 6b. These were classified as *Bacilli* (OTUs 2 and 4) and *Acidobacteria* (OTU 8)
471 according to the RDP classifier with the maximum likelihood phylogenies showing evolutionary
472 associations with halo-philic/-tolerant or metal resistant *Bacilli* for OTUs 2 and 4. OTU 2 was
473 placed next to the *Bacillus persicus* B48 and *B. luteolus* YIM93174 strains (Figure S1a), previously
474 shown to be able to thrive or tolerate NaCl of up to 10 % (w/v) (Shi et al., 2011 and Didari et al.,
475 2013). OTU 4 was placed next to *B. beringiensis* BR035 (Figure S1b), a psychrotolerant isolate of
476 the Bering Sea (Yu et al., 2011), and species like *B. megaterium* which encompasses several metal
477 resistant isolates (Yu et al., 2011 and Fierros-Romero et al., 2016). Maximum likelihood phylogeny
478 showed relation with a versatile *Acidobacterium* named *Holophaga foetida* TMBS4 (Figure S1c)
479 able to degrade aromatic compounds, which, however, did not show tolerance to high NaCl
480 concentrations (up to 1.5 %) (Anderson et al., 2012). Nevertheless, lack of a sufficient number of
481 isolates and the poor 16S rRNA marker gene evolutionary signal of *Acidobacteria* (as shown by the
482 elimination of most acidobacterial representative sequences via the Cd-hit clustering) suggest that
483 deeper investigations are necessary to gain further insights about the niche of the OTU 8 related
484 strains.

485 Taken together these data highlight a clear impact of the amendments on the bacterial diversity,
486 with amended soils showing significantly higher α -diversity indices (i.e. inverse Simpson and Gini-
487 Simpson indexes; Table 4). At the same time, β -diversity was also affected by soil treatment as
488 pointed out by the significant differences in the bacterial community composition of treated and
489 untreated soils (Fig. 6a,b). This is likely due to a combination of reduced metal(loid) mobility and
490 enhanced availability of C sources in the amended soils which eventually lead to the increase of
491 rare taxa (possibly more sensitive to metal(loid)s in soil). Moreover, this can also explain the

492 appearance of new metabolic potentials (i.e. higher Biolog richness values) in the microbial
493 communities of treated soils (Fig. 3).

494

495

496 3.4. Influence of the amendments on plant growth and metal(loid) uptake

497

498 In order to obtain a comprehensive overview of the treatment suitability we carried out a pot
499 experiment to evaluate the combined impact of Fe-WTRs and MSWC on the growth and
500 metal(loid)s uptake of *Helichrysum italicum* plants.

501 Adding Fe-WTRs and MSWC to the polluted soil had a positive impact on plant growth. Root dry
502 weight increased by approx. 45 and 73 % after amendment addition at 1 and 2% rate respectively
503 and very similar increases were recorded for shoot dry weight (Fig. 5a). This was apparently due to
504 the improved fertility of the amended soils (Table 1) and also to the reduced metal(loid)
505 bioavailability, as indicated by the sequential extraction data (Figs. 1-2). Plants grown in the
506 amended soils showed approximately 50% reduction in Sb concentration in roots at both the
507 amendment rates while Sb concentration in shoots (i.e. stem + leaves) was reduced by 40 and 60%
508 at 1 and 2% amendment rate respectively (Fig. 5b).

509 Significant reductions in Cd, Pb and Zn uptake were also recorded for amended soils (Fig. 5c).

510 Irrespective of the amendment rate, metal concentrations in roots were consistently reduced (by
511 more than 50% compared to the untreated soil on average) while the addition of the 2%
512 amendment brought the concentration of Cd in plant shoots below the instrumental detection
513 limit (i.e. $<0.2 \mu\text{g}\cdot\text{kg}^{-1}$; Fig. 5c). Moreover, the amendment addition did not significantly change the
514 translocation factor of Sb (i.e. the roots to shoots metal(loid) concentration ratio) which remained
515 close to 0.1 while decreased significantly that relative to Cd (from 0.50 to 0.14). On the other

516 hand, an increase of Zn translocation factor was recorded at the 2% amendment rate (from 0.14 to
517 0.28). Taken together, these data highlight the overall effectiveness of the applied amendments
518 for reducing the mobility and bioavailability of both Sb and selected co-occurring metals and
519 improving soil fertility. Moreover, they also indicated the potential use of *H. italicum* for aided
520 phytostabilisation of metal(loid) contaminated soils.

521

522

523 **4. Conclusions**

524 This study showed that Fe-WTRs and MSWC can be used as amendments for the chemical and
525 micro-biological recovery of degraded subalkaline soils contaminated with Sb and co-occurring
526 metals such as Pb, Cd and Zn. The amendments addition had a clear positive impact on the soil
527 fertility attributes and reduced the labile fractions of the metal(loid)s in soil, especially Sb. All this
528 likely had a substantial influence on the microbial community, and functionality, of the amended
529 soils. Culturable microorganisms were more abundant in the treated soils and dehydrogenase, β -
530 glucosidase and urease activities were also significantly enhanced. Moreover, microbial
531 communities extracted from the amended soils showed higher metabolic potential and catabolic
532 versatility as well as greater α -diversity values based on high throughput partial 16S rRNA gene
533 sequencing. Plant growth in the treated soils was also greatly stimulated while metal(loid)s uptake
534 was significantly reduced. These results suggest that Fe-WTRs and MSWC can be effectively used
535 to alleviate the impact of inorganic xenobiotics on soil microbiota and promote a recovery of
536 ecosystem functioning.

537

538

539 **Acknowledgements**

540 This work was supported by the Comune di Sassari within the framework of the PO FESR 2007-
541 2013 Regione Autonoma della Sardegna “Interventi di bonifica, messa in sicurezza d'emergenza e
542 permanente e recupero ambientale del sito ex estrattivo minerario dell'argentiera”.

543

544 **References**

545 Ahmad, M., Lee, S.S., Lim, J.E., Lee, S.E., Cho, J.S., Moon, D.H., Hashimoto, Y., Ok, Y.S., 2014. Speciation and
546 phytoavailability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and
547 biochar: EXAFS spectroscopy and chemical extractions. *Chemosphere* 95, 433-441.

548

549 Altschul, S., Gish, W., Miller, W., Myers, E., Lipman, D., 1990. Basic local alignment search tool. *Journal of*
550 *Molecular Biology* 215, 403-410.

551

552 Alvarez-Ayuso, E., Otones, V., Murciego, A., Garcia-Sanchez, A., 2013. Evaluation of different amendments
553 to stabilize antimony in mining polluted soils. *Chemosphere* 90, 2233-2239.

554

555 Anderson, I., Held, B., Lapidus, A., Nolan, M., Lucas, S., Tice, H., Del Rio, T.G., Cheng, J.F., Han, C., Tapia, R.,
556 Goodwin, L.A., Pitluck, S., Liolios, K., Mavromatis, K., Pagani, I., Ivanova, N., Mikhailova, N., Pati, A., Chen,
557 A., Palaniappan, K., Land, M., Brambilla, E.M., Rohde, M., Spring, S., Goker, M., Detter, J.C., Woyke, T.,
558 Bristow, J., Eisen, J.A., Markowitz, V., Hugenholtz, P., Klenk, H.P., Kyrpides, N.C., 2012. Genome sequence of
559 the homoacetogenic bacterium *Holophaga foetida* type strain (TMBS4(T)). *Stand Genomic Sci* 6, 174-184.

560

561 Appel, C., Mab, L. Q., Rhueb, R. D., Kennelley, E., 2003. Point of zero charge determination in soils and
562 minerals via traditional methods and detection of electroacoustic mobility. *Geoderma* 113, 77-93.

563

564 Basta, N., Gradwohl, R., 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soils by a
565 sequential extraction procedure. *Journal of Soil Contamination* 9, 149-164.

566

567 Berry, D., Mahfoudh, K.B., Wagner, M., Loy, A., 2011. Barcoded primers used in multiplex amplicon
568 pyrosequencing bias amplification. *Applied and Environmental Microbiology* 77, 7846-7849.

569

570 Biondi, E.G., Tatti, E., Comparini, D., Giuntini, E., Mocali, S., Giovannetti, L., Bazzicalupo, M., Mengoni, A.,
571 Viti, C., 2009. Metabolic capacity of *Sinorhizobium (Ensifer) meliloti* strains as determined by phenotype
572 microarray analysis. *Applied and Environmental Microbiology* 75, 5396-5404.

573

574 Brandstetter, A., Sletten, R.S., Mentler, A., Wenzel, W., 1996. Estimating dissolved organic carbon in natural
575 waters by UV absorbance (254 nm). *Zeitschrift für Pflanzenernährung und Bodenkunde* 159, 605-607.

576

577 Castaldi, P., Melis, P., Silvetti, M., Deiana, P., Garau, G., 2009. Influence of pea and wheat growth on Pb, Cd,
578 and Zn mobility and soil biological status in a polluted amended soil. *Geoderma* 151, 241-248.

579

580 Castaldi, P., Silvetti, M., Garau, G., Deiana, S., 2015. Influence of the pH on the accumulation of phosphate
581 by red mud (a bauxite ore processing waste). *Journal of Hazardous Materials* 182, 266-272.

582

583 Castaldi, P., Silvetti, M., Garau, G., Demurtas, D., Deiana, S., 2015. Copper(II) and lead(II) removal from
584 aqueous solution by water treatment residues. *Journal of Hazardous Materials* 283, 140-147.

585

586 Chao, A., 1984. Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of*
587 *Statistics* 11, 265-270.

588

589 Chao, A., Lee, S.-M., 1992. Estimating the Number of Classes via Sample Coverage. *Journal of the American*
590 *Statistical Association* 87, 210-217.

591

592 Chen, Z.Y., Liu, F., Bu, T.D., Liu, Y.S., Zhu, J., 2016. Effects of organic acids on dissolution of Fe and Mn from
593 weathering coal gangue. *Acta Geochimica* 35, 316-328.

594

595 Ciavatta, C., Govi, M., Antisari, L. V., Sequi, P., 1990. Characterization of humified compounds by extraction
596 and fractionation on solid polyvinylpyrrolidone. *Journal of Chromatography A* 509, 141-146.

597

598 Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkila, J., Marchesi, J.R., Smidt, H., de Vos, W.M., Ross, R.P.,
599 O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring
600 microbial community structures in the human distal intestine. *PLoS ONE* 4, e6669.

601

602 Conesa, H.M., Wieser, M., Gasser, M., Hockmann, K., Evangelou, M.W.H., Studer, B., Schulin, R., 2010.
603 Effects of three amendments on extractability and fractionation of Pb, Cu, Ni and Sb in two shooting range
604 soils. *Journal of Hazardous Materials* 181, 845-850.

605

606 Day, P.R., 1965. Particle fractionation and particle-size analysis. In: Black, C.A. (Ed.), *Methods of Soil*
607 *Analysis: Part 1*. American Society of Agronomy, Madison, WI, pp. 545-566.

608

609 Didari, M., Amoozegar, M.A., Bagheri, M., Mehrshad, M., Schumann, P., Sproer, C., Sanchez-Porro, C.,
610 Ventosa, A., 2013. *Bacillus persicus* sp. nov., a halophilic bacterium from a hypersaline lake. *International*
611 *Journal of Systematic and Evolutionary Microbiology* 63, 1229-1234.

612

613 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic*
614 *Acids Research* 32, 1792-1797.

615

616 Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*
617 10, 996-998.

618

619 Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed
620 of chimera detection. *Bioinformatics* 27, 2194-2200.

621

622 Fierros-Romero, G., Gomez-Ramirez, M., Arenas-Isaac, G.E., Pless, R.C., Rojas-Avelizapa, N.G., 2016.

623 Identification of *Bacillus megaterium* and *Microbacterium liquefaciens* genes involved in metal resistance
624 and metal removal. *Canadian Journal of Microbiology* 62, 505-513.

625

626 Filella, M., Williams, P.A., 2012. Antimony interactions with heterogeneous complexants in waters,
627 sediments and soils: A review of binding data for homologous compounds. *Chemie Der Erde-Geochemistry*
628 72, 49-65.

629

630 Filella, M., Williams, P.A., Belzile, N., 2009. Antimony in the environment: knowns and unknowns.
631 *Environmental Chemistry* 6, 95-105.

632

633 Fisher, R.A., Corbet, A.S., Williams, C.B., 1943. The relation between the number of species and the number
634 of individuals in a random sample of an animal population. *Journal of Animal Ecology* 12, 42-58.

635

636 Fitter, A.H., Gilligan, C.A., Hollingworth, K., Kleczkowski, A., Twyman, R.M., Pitchford, J.W., Programme,
637 N.S.B., 2005. Biodiversity and ecosystem function in soil. *Functional Ecology* 19, 369-377.

638

639 Garau, G., Castaldi, P., Santona, L., Deiana, P., Melis, P., 2007. Influence of red mud, zeolite and lime on
640 heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a
641 contaminated soil. *Geoderma* 142, 47-57.

642

643 Garau, G., Silvetti, M., Castaldi, P., Mele, E., Deiana, P., Deiana, S., 2014. Stabilising metal(loid)s in soil with
644 iron and aluminium-based products: microbial, biochemical and plant growth impact. *Journal of*
645 *Environmental Management* 139, 146-153.

646

647 Garau, G., Silvetti, M., Deiana, S., Deiana, P., Castaldi, P., 2011. Long-term influence of red mud on As
648 mobility and soil physico-chemical and microbial parameters in a polluted sub-acidic soil. *Journal of*
649 *Hazardous Materials* 185, 1241-1248.

650

651 *Gazzetta Ufficiale della Repubblica Italiana* n. 248, 21 ottobre 1999. Metodi ufficiali di analisi chimica dei
652 suoli (1999).

653

654 *Gazzetta Ufficiale della Repubblica Italiana* n. 84 del 10 aprile 2002. Metodi ufficiali di analisi chimica dei
655 suoli (2002).

656

657 Gihring, T.M., Green, S.J., Schadt, C.W., 2011. Massively parallel rRNA gene sequencing exacerbates the
658 potential for biased community diversity comparisons due to variable library sizes. *Environmental*
659 *Microbiology* 14, 285-290.

660

661 Good, I.J., 1953. The population frequencies of species and the estimation of population parameters.
662 *Biometrika* 40, 237-264.

663

664 Guo, X.J., Wu, Z.J., He, M.C., Meng, X.G., Jin, X., Qiu, N., Zhang, J., 2014. Adsorption of antimony onto iron
665 oxyhydroxides: Adsorption behavior and surface structure. *Journal of Hazardous Materials* 276, 339-345.

666

667 Hildebrand, F., Tadeo, R., Voigt, A., Bork, P., Raes, J., 2014. LotuS: an efficient and user-friendly OTU
668 processing pipeline. *Microbiome* 2, 30.

669

670 Johnson, C.A., Moench, H., Wersin, P., Kugler, P., Wenger, C., 2005. Solubility of antimony and other
671 elements in samples taken from shooting ranges. *Journal of Environmental Quality* 34, 248-254.

672

673 Kabata-Pendias, A., 2011. Trace elements in soils and plants 4th Ed. CRC Press, Boca Raton FL.

674

675 Legendre, P., Gallagher, E., 2001. Ecologically meaningful transformations for ordination of species data.
676 *Oecologia* 129, 271-280.

677

678 Li, W., Jaroszewski, L., Godzik, A., 2001. Clustering of highly homologous sequences to reduce the size of
679 large protein databases. *Bioinformatics* 17, 282-283.

680

681 Luo, J.M., Bai, Y.H., Liang, J.S., Qu, J.H., 2014. Metagenomic approach reveals variation of microbes with
682 arsenic and antimony metabolism genes from highly contaminated soil. *Plos One* 9.

683

684 Magoč, T., Salzberg, S.L., 2011. FLASH: Fast Length Adjustment of Short Reads to Improve Genome
685 Assemblies. *Bioinformatics*.

686

687 Manzano, R., Silveti, M., Garau, G., Deiana, S., Castaldi, P., 2016. Influence of iron-rich water treatment
688 residues and compost on the mobility of metal(loid)s in mine soils. *Geoderma* 283, 1-9.

689

690 Mendes, R.S., Evangelista, L.R., Thomaz, S.M., Agostinho, A.A., Gomes, L.C., 2008. A unified index to
691 measure ecological diversity and species rarity. *Ecography* 31, 450-456.

692

693 Okkenhaug, G., Amstatter, K., Bue, H.L., Cornelissen, G., Breedveld, G.D., Henriksen, T., Mulder, J., 2013.
694 Antimony (Sb) contaminated shooting range soil: Sb mobility and immobilization by soil amendments.
695 Environmental Science & Technology 47, 6431-6439.
696
697 Okkenhaug, G., Grasshorn Gebhardt, K.-A., Amstaetter, K., Lassen Bue, H., Herzel, H., Mariussen, E.,
698 Rossebø Almås, Å., Cornelissen, G., Breedveld, G.D., Rasmussen, G., Mulder, J., 2016. Antimony (Sb) and
699 lead (Pb) in contaminated shooting range soils: Sb and Pb mobility and immobilization by iron based
700 sorbents, a field study. Journal of Hazardous Materials 307, 336-343.
701
702 Okkenhaug, G., Zhu, Y.G., He, J.W., Li, X., Luo, L., Mulder, J., 2012. Antimony (Sb) and arsenic (As) in Sb
703 mining impacted paddy soil from Xikuangshan, China: differences in mechanisms controlling soil
704 sequestration and uptake in rice. Environmental Science & Technology 46, 3155-3162.
705
706 Oksanen, J., Blanchet, G.F., Friendly, M., Kindt, R., Legendre, P., McGilinn, D., Minchin, P.R., O'Hara, R.B.,
707 Simpson, G.L., Solymos, P., Henry, M., Stevens, R., Szoecs, E., Wagner, H., 2016. Vegan: Community Ecology
708 Package. R package version 2.4-0.
709
710 Pan, X.L., Zhang, D.Y., Chen, X., Bao, A.M., Li, L.H., 2011. Antimony accumulation, growth performance,
711 antioxidant defense system and photosynthesis of *Zea mays* in response to antimony pollution in soil.
712 Water Air and Soil Pollution 215, 517-523.
713
714 Pinna, M.V., Castaldi, P., Deiana, P., Pusino, A., Garau, G., 2012. Sorption behavior of sulfamethazine on
715 unamended and manure-amended soils and short-term impact on soil microbial community. Ecotoxicology
716 and Environmental Safety 84, 234-242.
717

718 Pirri, I.V., 1996. The Pb-Zn-Ag-Sb deposit of Argentiera (Nurra, NW Sardinia): An example of direct
719 mineralogical inheritance. *Neues Jahrbuch fur Mineralogie, Monatshefte*, 223-240.
720
721 R Core Team, 2015. R: a language and environment for statistical computing, reference index version 3.2.2.
722 R Foundation for Statistical Computing.
723
724 Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. EdgeR: a Bioconductor package for differential
725 expression analysis of digital gene expression data. *Bioinformatics* 26, 139-140.
726
727 Sanderson, P., Naidu, R., Bolan, N., 2014. Ecotoxicity of chemically stabilised metal(loid)s in shooting range
728 soils. *Ecotoxicology and Environmental Safety* 100, 201-208.
729
730 Sanderson, P., Naidu, R., Bolan, N., 2015. Effectiveness of chemical amendments for stabilisation of lead
731 and antimony in risk-based land management of soils of shooting ranges. *Environmental Science and*
732 *Pollution Research* 22, 8942-8956.
733
734 Shi, R., Yin, M., Tang, S.K., Lee, J.C., Park, D.J., Zhang, Y.J., Kim, C.J., Li, W.J., 2011. *Bacillus luteolus* sp. nov., a
735 halotolerant bacterium isolated from a salt field. *International Journal of Systematic and Evolutionary*
736 *Microbiology* 61, 1344-1349.
737
738 Shtangeeva, I., Niemela, M., Peramaki, P., 2014. Effects of soil amendments on antimony uptake by wheat.
739 *Journal of Soils and Sediments* 14, 679-686.
740
741 Silvetti, M., Castaldi, P., Garau, G., Demurtas, D., Deiana, S., 2015. Sorption of Cadmium(II) and Zinc(II) from
742 Aqueous Solution by Water Treatment Residuals at Different pH Values. *Water Air and Soil Pollution* 226.
743

744 Stamatakis, A., 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large
745 phylogenies. *Bioinformatics*.
746

747 Strawn, D.G., Bohn, H.L., O'Connor, G.A., 2015. *Soil Chemistry Fourth Edition*. John Wiley & Sons, Ltd.
748 Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously
749 aligned blocks from protein sequence alignments. *Systematic Biology* 56, 564-577.
750

751 Timms-Wilson, T.M., Griffiths, R.I., Whiteley, A.S., Prosser, J.I., Bailey, M.J., 2006. Detection of Active
752 Bacterial Populations in Soil, In: van Elsas, J.D., Jansson, J.K., Trevors, J.T. (Eds.), *Modern Soil Microbiology -*
753 *Second Edition*. CRC Press, Boca Raton, FL, pp. 387-407.
754

755 Tsallis, C., 1988. Possible generalization of Boltzmann-Gibbs statistics. *Journal of Statistical Physics* 52, 479-
756 487.
757

758 Tschan, M., Robinson, B.H., Schulin, R., 2009. Antimony in the soil-plant system - a review. *Environmental*
759 *Chemistry* 6, 106-115.
760

761 Vasileiadis, S., Puglisi, E., Arena, M., Cappa, F., Cocconcelli, P.S., Trevisan, M., 2012. Soil bacterial diversity
762 screening using single 16S rRNA gene V regions coupled with multi-million read generating sequencing
763 technologies. *PLoS ONE* 7, e42671.
764

765 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA
766 sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73, 5261-5267.
767

768 Wang, Q.S., He, M.C., Wang, Y., 2011. Influence of combined pollution of antimony and arsenic on
769 culturable soil microbial populations and enzyme activities. *Ecotoxicology* 20, 9-19.

770

771 Wang, X.Q., He, M.C., Xie, J., Xi, J.H., Lu, X.F., 2010. Heavy metal pollution of the world largest antimony
772 mine-affected agricultural soils in Hunan province (China). *Journal of Soils and Sediments* 10, 827-837.

773

774 Wei, Y., Chen, Z.P., Wu, F.C., Hou, H., Li, J.N., Shangguan, Y.X., Zhang, J., Li, F.S., Zeng, Q.R., 2015. Molecular
775 diversity of arbuscular mycorrhizal fungi at a large-scale antimony mining area in southern China. *Journal of*
776 *Environmental Sciences-China* 29, 18-26.

777

778 Wenzel, W.W., Kirchbaumer, N., Prohaska, T., Stingeder, G., Lombi, E., Adriano, D.C., 2001. Arsenic
779 fractionation in soils using an improved sequential extraction procedure. *Analytica Chimica Acta* 436, 309-
780 323.

781

782 Yu, Y., Li, H.R., Zeng, Y.X., Chen, B., 2011. *Bacillus beringensis* sp. nov., a psychrotolerant bacterium isolated
783 from the Bering Sea. *Antonie van Leeuwenhoek* 99, 551-557.

784

785

786

787 **Figure caption**

788 Figure 1. Sequential extraction of Sb from untreated polluted and amended soils.

789

790 Figure 2. Sequential extraction of metals from untreated polluted and amended soils.

791

792 Figure 3. Average carbon source utilisation and richness values of microbial communities extracted
793 from untreated polluted and amended soils.

794

795 Figure 4. Triplot of the PCA scores of the S1-9 soil samples (black characters; percentage of
796 variance explained by each axis is also provided) according to their composition in dominant OTUs
797 using the Hellinger transformed values, the scores of the dominant OTUs (red characters) and the
798 fitting of selected soil physico-chemical parameters (blue characters and arrows; highly significant
799 fits with $P \leq 0.01$) (panel a), and differential abundance of the dominant OTUs (different letters
800 denote significant differences for $\alpha \leq 0.05$ using the Holm correction – panel b). Rare OTUs refers
801 to the group of sequences with relative participation of less than 1%. S1-3, untreated soils; S4-6,
802 soils + 1% amendment; S7-9, soils + 2% amendment ; OM, organic matter; DOC, dissolved organic
803 carbon; CEC, cation exchange capacity; pH_{PZC} , point of zero charge; Sb, Pb, Cd and Zn lab,
804 concentrations of metal(loid) labile fractions (i.e. Sb extracted in Step1+2 and Pb, Cd and Zn
805 extracted with $\text{Ca}(\text{NO}_3)_2 + \text{NaOAc}$).

806

807 Figure 5. Biomass (a), Sb (b) and metal uptake (c) of *H. italicum* plants grown in the untreated
808 polluted and amended soils.

809

810 Table 1. Selected physico-chemical characteristics of the amendments used in this study (dry
 811 matter basis).

<i>Physico-chemical parameters</i>	<i>Fe-WTR</i>	<i>MSWC</i>
pH(H ₂ O)	7.88±0.04	7.93±0.06
EC (mS·cm ⁻¹)	3.01±0.02	3.26±0.03
S _{BET} (m ² ·g ⁻¹)	35.00	2.78
pH _{PZC}	7.80	4.53
Organic matter (g·kg ⁻¹)	244.71±2.79	420.19±3.44
Fulvic and Humic acids (g·kg ⁻¹)	24.34± 1.05	153.24±2.76
DOC (mg·g ⁻¹)	0.101±0.00	0.573±0.04
Total N (g·kg ⁻¹)	8.04±0.28	21.84±0.42
Total P (g·kg ⁻¹)	0.68±0.03	7.16 ±0.41
Total metals (mg·kg ⁻¹)		
Fe	245,480±1,350	5,587±88
Mn	31,636±764	141±6
Ca	89±2	63,444±130
Mg	69±2	5,403±135
Na	32±1	993±24
K	99±3	1,709±21
Pb	12±0.3	3.7±0.1
Zn	246±7	30±3
Cd	0.2±0.0	n.d.
Cu	29±1	19±2

812

813 n.d., not detected

814

815

816 Table 2. Physico-chemical characteristics of the treated (amended) and untreated contaminated
 817 soils (dry matter basis). Shaded values are exceeding the national (Italian) threshold values for
 818 private, residential and public green areas and for commercial and industrial sites (see Table S2 in
 819 SI for details).

<i>Physico-chemical parameters</i>	<i>Untreated soil</i>	<i>Amended soils</i>	
		<i>Fe-WTR+MSWC (1.0 %)</i>	<i>Fe-WTR+MSWC (2.0 %)</i>
pH(H ₂ O)	8.01 ± 0.08	7.97 ± 0.02	7.92 ± 0.03
EC (mS·cm ⁻¹)	1.43 ± 0.23	1.65± 0.09	1.69± 0.10
Total carbonate (g·kg ⁻¹)	174.0 ± 5.0	185.7 ±3.4	199.3 ± 2.9
Active carbonate (g·kg ⁻¹)	64.0 ± 9.6	79.2 ±3.8	83.3 ± 1.4
CEC (cmol ₍₊₎ ·kg ⁻¹)	16.92 ± 0.17	21.31 ± 1.24	33.46 ± 2.43
pH _{PCZ}	6.90	7.42	7.49
Organic matter (g·kg ⁻¹)	3.89±0.03	5.93±0.03	7.48±0.06
DOC (mg·g ⁻¹)	0.029 ± 0.001	0.123 ± 0.002	0.213 ± 0.006
Total N (g·kg ⁻¹)	0.70± 0.03	0.81± 0.06	1.02± 0.06
Total metal(loid)s (mg·kg ⁻¹)			
Fe	40,533 ± 1,197	43,382 ± 470	43,972 ± 698
Mn	1,758 ± 34	1,837 ± 315	2,054 ± 68
Ca	50,132 ± 2,301	49,162 ± 467	50,653 ± 728
K	4,269 ± 362	4,114 ± 394	4,331 ± 321
Sb	108.86 ± 1.74	106.46 ± 0.84	105.85 ± 0.97
As	7.67 ± 0.76	7.44± 0.25	7.42± 0.21
Pb	1,214 ± 31	1,184 ± 4	1,146 ± 25
Zn	5,400 ± 70	5,468 ± 482	5,385 ± 137
Cd	23.17 ± 2.87	22.38 ± 1.24	24.86 ± 4.54
Cu	48.86 ± 2.48	45.65 ± 3.62	46.86 ± 2.62

820

821

Table 3. Counts of heterotrophic microorganisms and selected enzyme activities in the untreated polluted (UP) and amended soils. All values are expressed on a soil dry matter basis. DHG, dehydrogenase; GLU, β -glucosidase; URE, urease. Within each column, values which share a common letter do not differ significantly (Fisher's LSD test, $P < 0.05$).

	<i>Microbial counts (log CFU·g⁻¹ soil)</i>			<i>Enzyme activities</i>		
	<i>Bacteria</i>	<i>Fungi</i>	<i>Actinomycetes</i>	<i>DHG</i> ($\mu\text{g TPF}\cdot\text{g}^{-1}$ soil)	<i>GLU</i> ($\mu\text{g } \rho\text{-nitrophenol}\cdot\text{g}^{-1}$ soil)	<i>URE</i> ($\mu\text{g NH}_4\text{-N}\cdot\text{g}^{-1}$ soil)
UP soil	5.81±0.07 ^a	4.79±0.09 ^a	5.57±0.03 ^a	2.93±0.29 ^a	70.67±10.06 ^a	2.37±0.36 ^a
UP soil+1% amend.	6.36±0.07 ^b	4.89±0.03 ^a	5.97±0.01 ^b	18.86±1.72 ^b	95.06±1.79 ^a	3.25±0.06 ^a
UP soil+2% amend.	6.61±0.01 ^c	5.00±0.05 ^b	6.19±0.02 ^c	37.41±6.72 ^c	208.65±16.07 ^b	4.96±0.44 ^b

Table 4. Number of partial 16S rRNA gene sequences investigated and related diversity indexes. For each diversity index different letters denote significant differences between treatment (ANOVA F and P values are reported).

Sample*	Seq# used	Good's Coverage	Gini-Simpson $1-\lambda$	ANOVA: F=118, P=1.51e-05	Inverse Simpson $1/\lambda$	ANOVA: F=35.26, P=4.82e-04	Fisher's α	ANOVA: F=4.156, P=0.073	Effective number of OTUs at q*	ANOVA: F=4.405, P=0.066	Observed S	ANOVA: F=1.864, P=0.235	Chao 1	ANOVA: F=2.401, P=0.171
S1	22889	0.988	0.977		43.8		384		674		1615		1814	
S2	21849	0.992	0.977	a	42.7	a	307	a	602	a	1339	a	1452	a
S3	20865	0.990	0.979		46.6		360		688		1509		1646	
S4	21092	0.990	0.988		82.8		376		790		1561		1693	
S5	17760	0.987	0.989	b	92.5	b	378	a	801	a	1495	a	1664	a
S6	14921	0.984	0.987		75.8		353		704		1359		1508	
S7	22258	0.994	0.991		117.0		293		725		1295		1384	
S8	22520	0.993	0.989	b	93.2	b	329	a	758	a	1422	a	1511	a
S9	18541	0.990	0.990		99.0		303		684		1272		1412	

* S1-S3, untreated soils; S4-S6, soils amended at 1% (Fe-WTR+MSWC) rate; S7-S9, soils amended at 2% (Fe-WTR+MSWC) rate

Figure 1

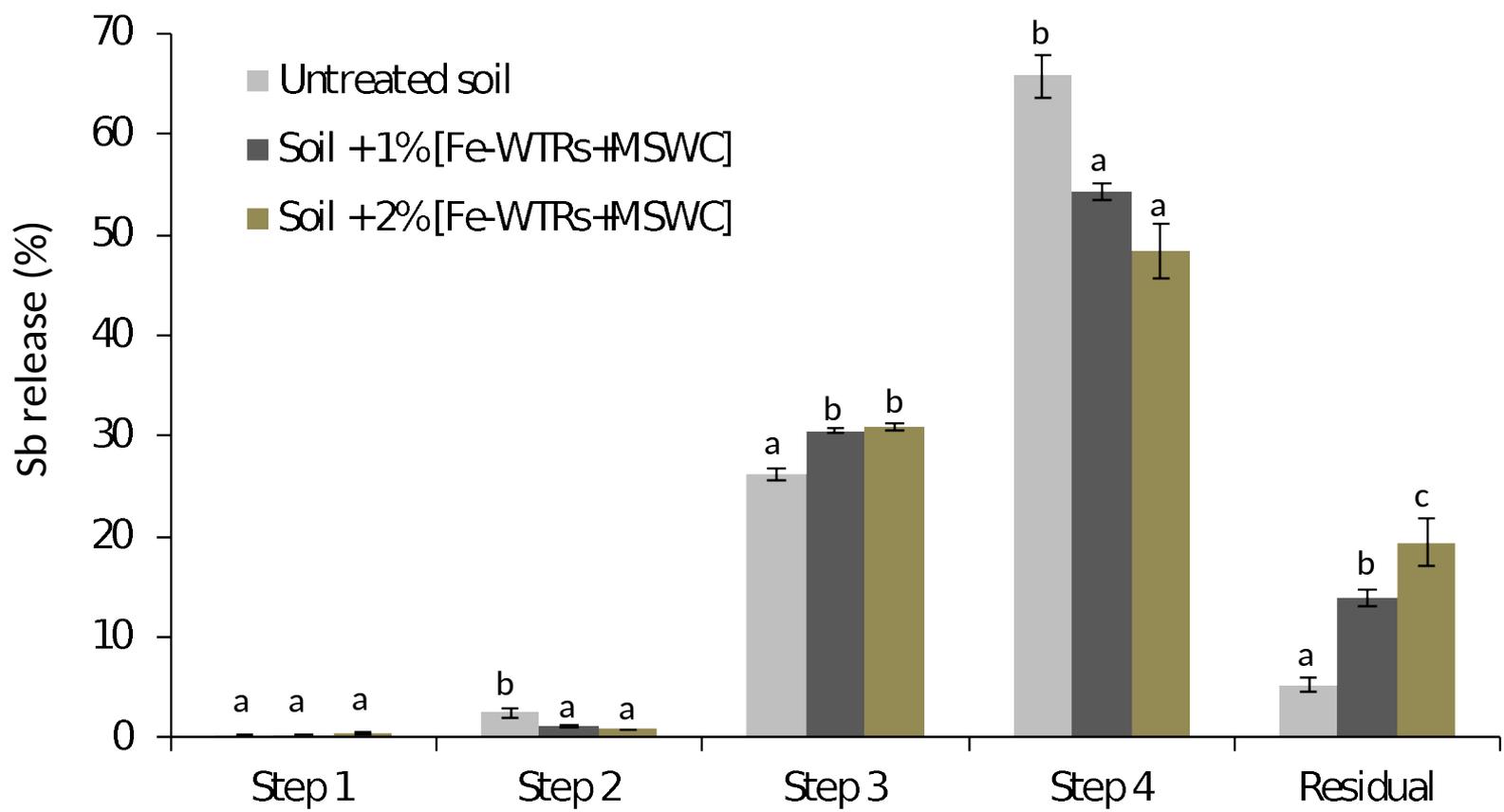


Figure 2

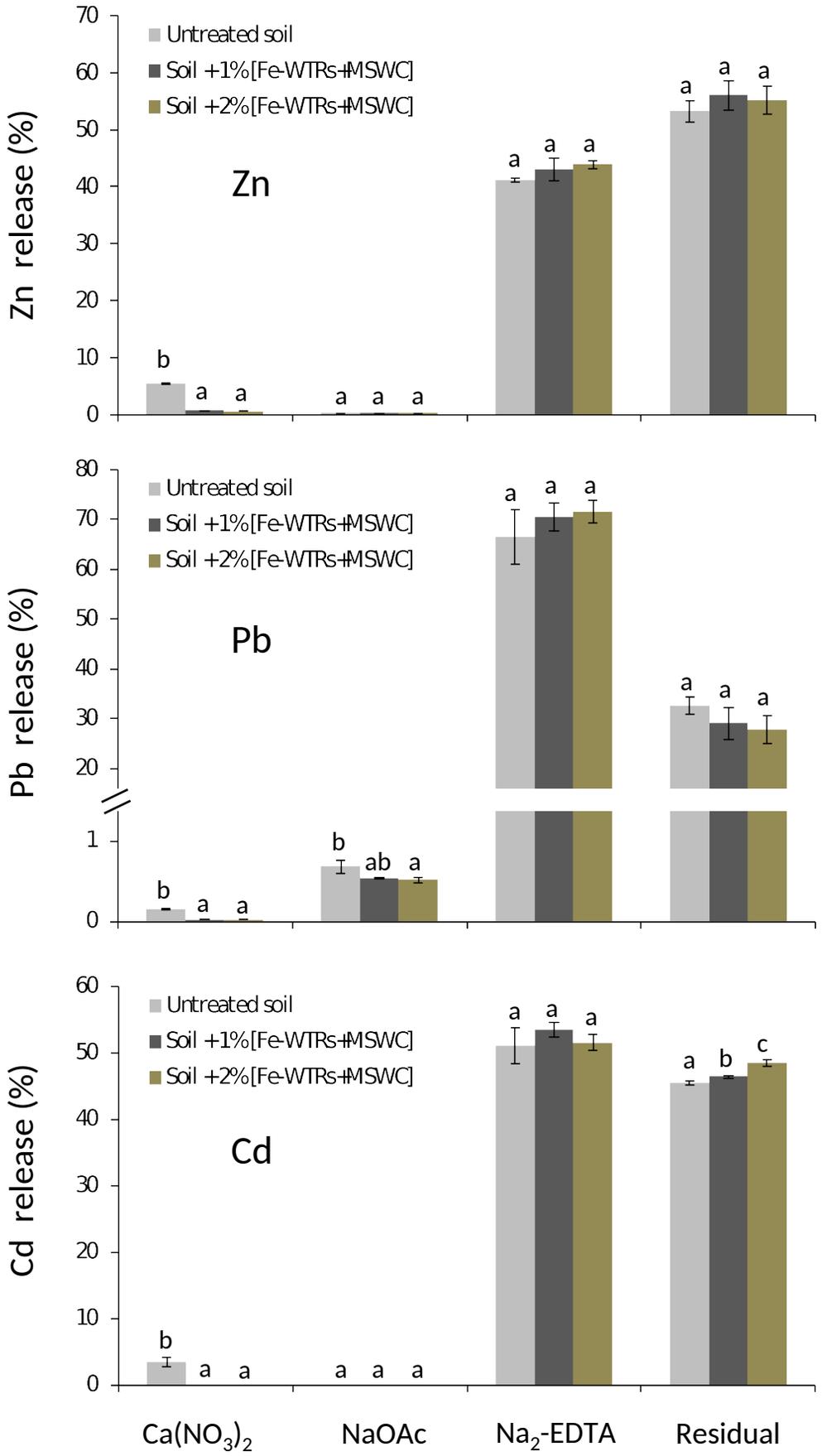


Figure 3

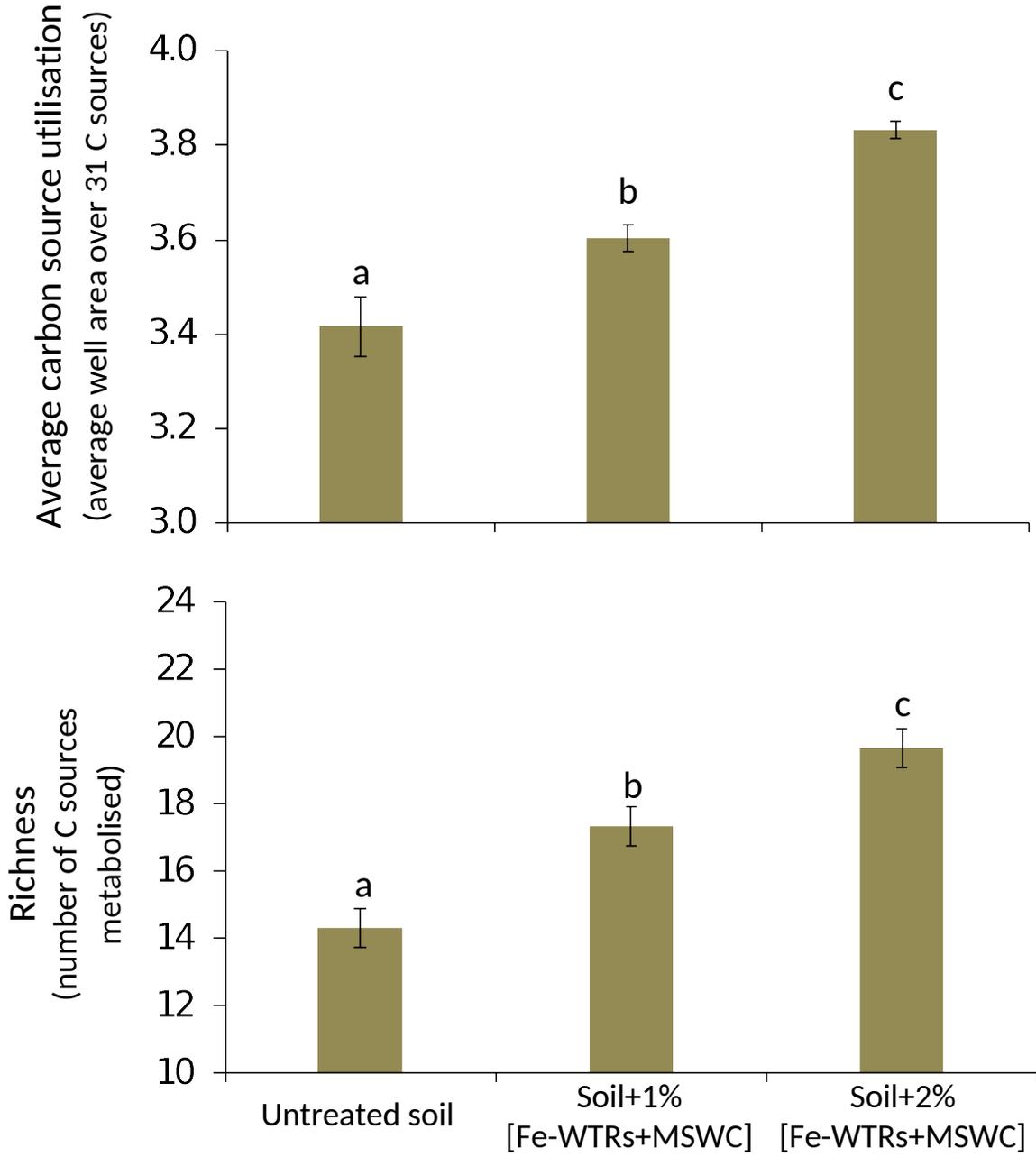


Figure 4

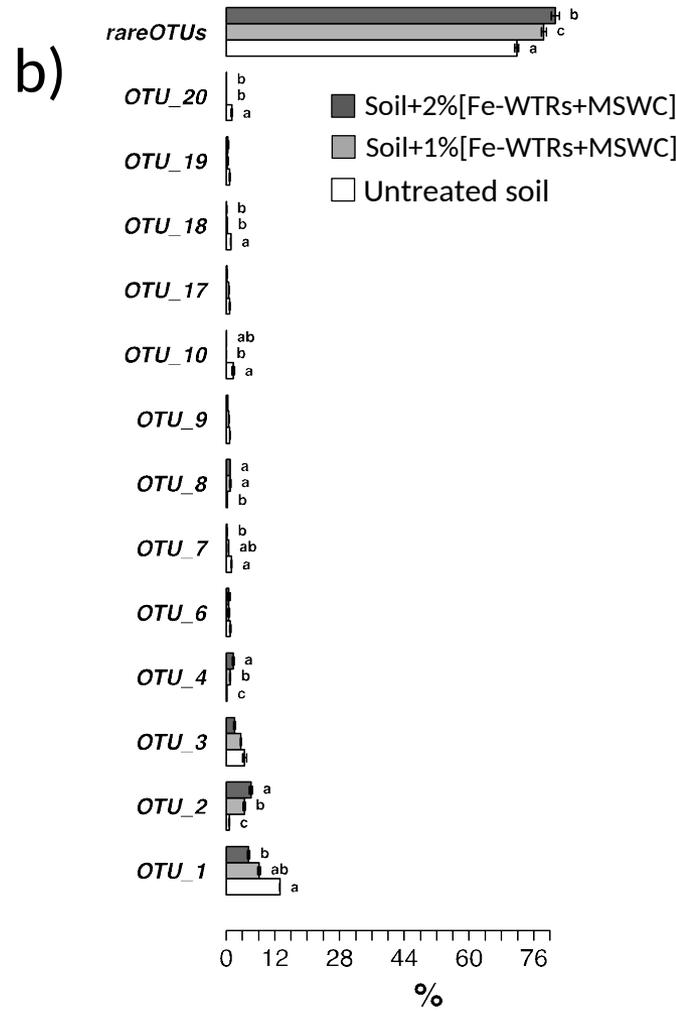
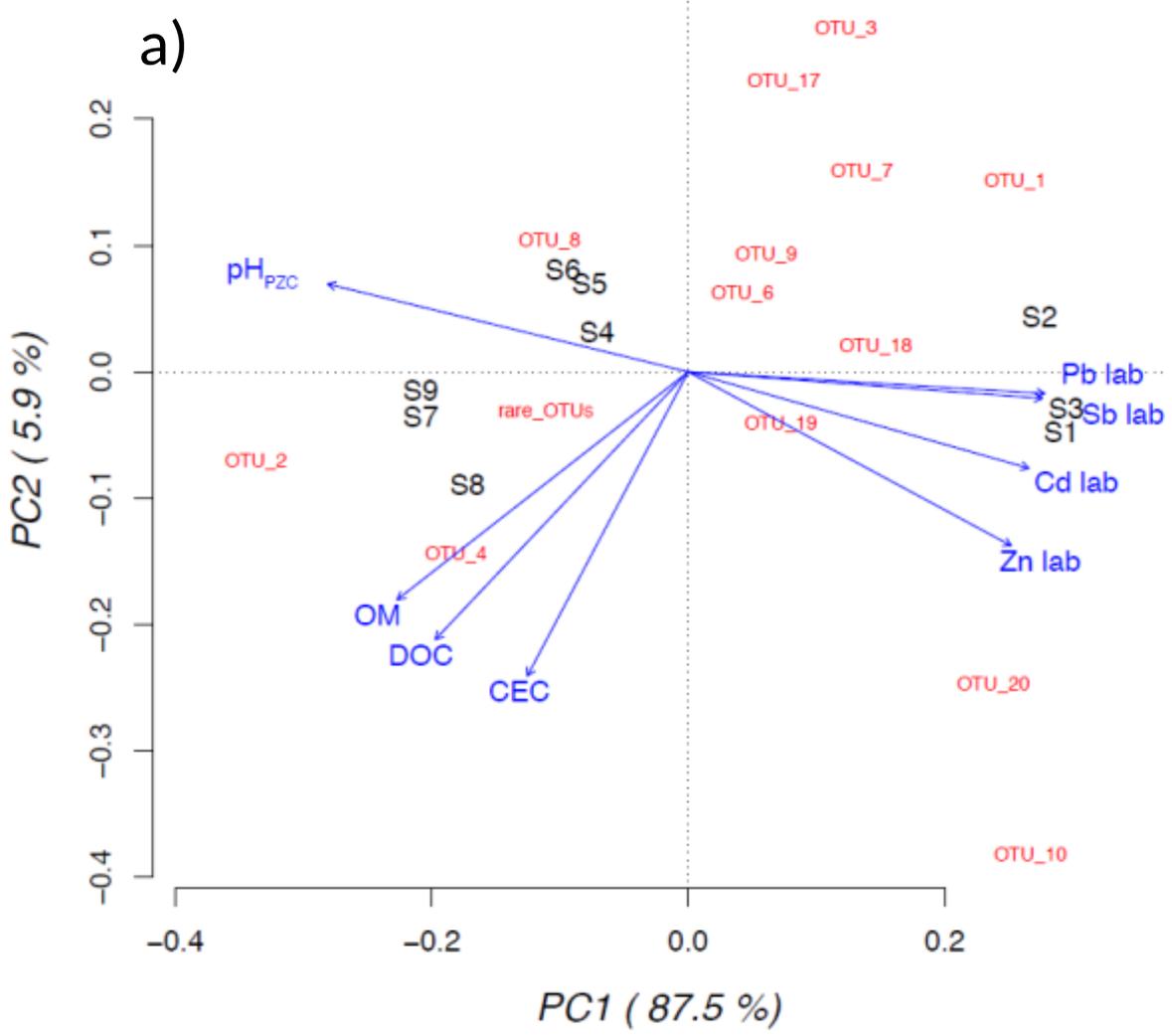
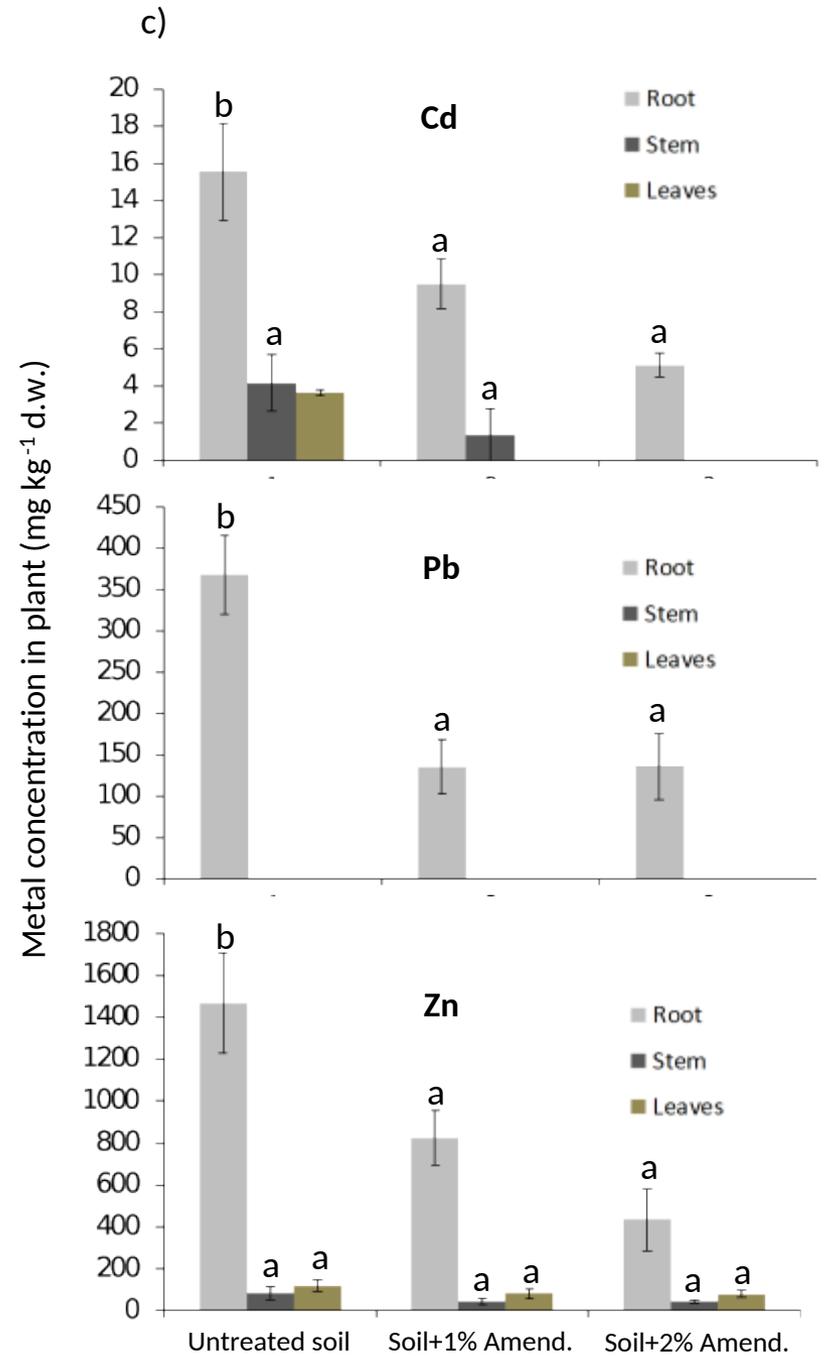
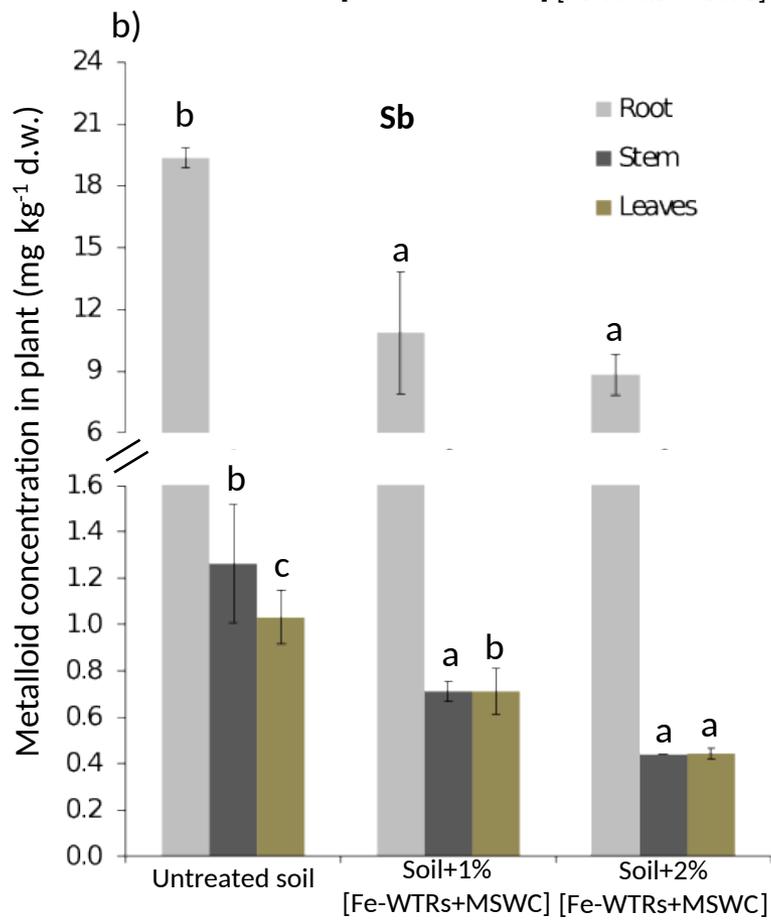
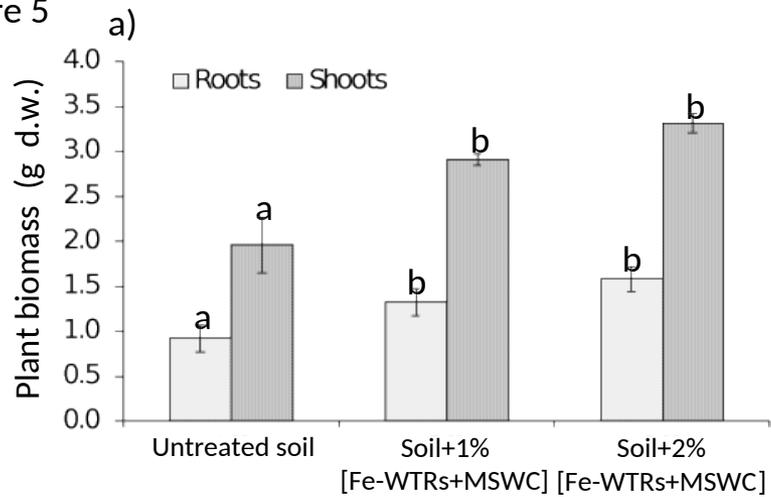


Figure 5



Use of municipal solid wastes for chemical and microbiological recovery of soils contaminated with metal(loid)s

Giovanni Garau^{1*}, Margherita Silveti¹, Sotirios Vasileiadis², Erica Donner², Stefania Diquattro¹, Enzo Lombi², Paola Castaldi^{1*}

1 Dipartimento di Agraria, University of Sassari, Viale Italia 39, 07100 Sassari, Italy, **2** Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

*ggarau@uniss.it (GG); castaldi@uniss.it (PC)

Highlights

- FeWTRs and MSWC were used as amendments to recover a metal(loid)s-contaminated soil
- FeWTRs and MSWC reduced the labile fractions of Sb, Pb, Cd and Zn in soil
- Treated soils showed higher abundance of culturable heterotrophic microorganisms
- Treated soils showed improved enzyme activity, metabolic potential and α -diversity
- Plant growth in the treated soils was greatly stimulated

Supplementary Information – Tables S1-S3

Table S1. Index sequences, linker (TA) primer (TACGGRAGGCAGCAG) sequences and complete construct of the indexed primers used for the PCR based sequencing bacterial diversity analysis of the V3-V4 hypervariable 16S rRNA gene region together with the reverse primer TACNVGGGTWTCTAATCC as described in the materials and methods.

#Sample ID*	Index Sequence	Linker-Primer Sequence	Index-Linker-Primer Sequence
S1	AACATGC	TATACGGRAGGCAGCAG	AACATGCTATACGGRAGGCAGCAG
S2	AACCGAT	TATACGGRAGGCAGCAG	AACCGATTATACGGRAGGCAGCAG
S3	AACCTGT	TATACGGRAGGCAGCAG	AACCTGTTATACGGRAGGCAGCAG
S4	AACGAGA	TATACGGRAGGCAGCAG	AACGAGATATACGGRAGGCAGCAG
S5	AACGCAA	TATACGGRAGGCAGCAG	AACGCAATATACGGRAGGCAGCAG
S6	AACGCTT	TATACGGRAGGCAGCAG	AACGCTTTATACGGRAGGCAGCAG
S7	AACGTAC	TATACGGRAGGCAGCAG	AACGTACTATACGGRAGGCAGCAG
S8	AACTAGC	TATACGGRAGGCAGCAG	AACTAGCTATACGGRAGGCAGCAG
S9	AACTGAC	TATACGGRAGGCAGCAG	AACTGACTATACGGRAGGCAGCAG

* S1-S3, untreated soils; S4-S6, soils amended at 1% (Fe-WTR+MSWC) rate; S7-S9, soils amended at 2% (Fe-WTR+MSWC) rate

Table S2. Concentration thresholds of selected metal(loid)s in soil as related to the soil specific use*.

Metal(oid)s	Private, residential and public green areas (mg·Kg ⁻¹)	Commercial and industrial sites (mg·Kg ⁻¹)
Sb	10	30
Cd	2	15
Zn	150	1,500
Pb	100	1,000

*From: Gazzetta Ufficiale della Repubblica Italiana. Decreto Legislativo 3 aprile 2006, No. 152 — Norme in materia ambientale. Supplemento Gazzetta Ufficiale No. 88 del 14 aprile 2006. Roma, Italy: Istituto Poligrafico dello Stato; 2006. [in Italian].

Table S3. Pearson correlation values/coefficients (*r*) between microbial counts of fast-growing culturable bacteria, fungi and actinomycetes in amended and unamended soils and DHG, GLU and URE activities. *P* values are also reported.

	Microbial counts		
	Bacteria	Fungi	Actinomycetes
DHG	0.924565	0.853516	0.944112
<i>P</i>	0.00036	0.003422	0.000129
GLU	0.816829	0.696705	0.860052
<i>P</i>	0.007204	0.037031	0.002936
URE	0.892011	0.83243	0.919447
<i>P</i>	0.001224	0.005361	0.000451

Figure S1

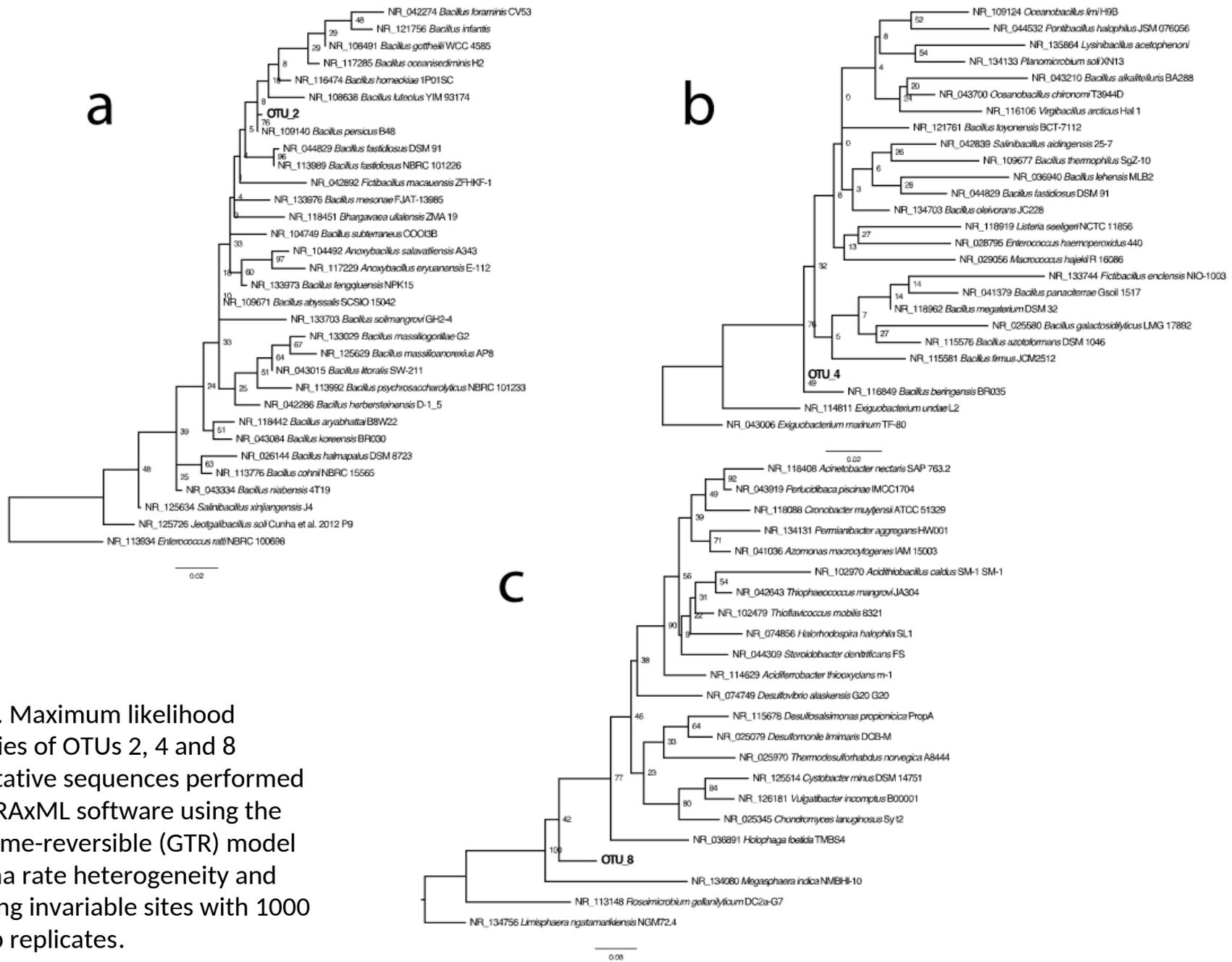


Figure S1. Maximum likelihood phylogenies of OTUs 2, 4 and 8 representative sequences performed with the RAxML software using the general time-reversible (GTR) model for gamma rate heterogeneity and considering invariable sites with 1000 bootstrap replicates.