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1   **Does ochre have the potential to be a remedial treatment for As-contaminated soils?**

2

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12

13   **Abstract**

14   Ochre is an iron oxyhydroxide-rich waste that accumulates in water bodies associated with

15   disused mines. Laboratory experiments were conducted to examine the potential of four

16   different ochres to be used as remedial agents for As contaminated soils. The ochres

17   removed As from solution (200 and 500 mg L^{-1}) in adsorption experiments at pH 3 and 8 and

18   when added to As contaminated soil (5 % w/w) significantly reduced As release to solution.

19   In both these experiments the highest surface area ochres performed best. The impact of

20   ochre amendments on uptake of As from soil by plants and humans and release of As to

21   ground water was assessed in a year-long incubation. Ochres increased soil pH and

22   reduced $CaCl_2$ extractable As but had no consistent effect on plant growth, plant As uptake

23   or As extraction in physiologically based extraction tests. Ochre may be better used for water

24   treatment than soil remediation.

25   **Keywords**: arsenic; ochre; contaminated soil; incubation; bioassays

26

27 **Capsule:** Ochre amendments to As-contaminated soil increase pH and reduce CaCl$_2$

28 extractable As but have no impact on plant growth, As uptake or PBET extractable As.

29

30 **Introduction**

31 High concentrations of As in soils and water bodies occur throughout the world due to

32 anthropogenic activities such as mining, smelting and wood preservative usage (Abrahams

33 and Thornton; Álvarez-Ayuso et al., 2012; Eapaea et al., 2007; Lin et al., 2004; Mench and

34 Bes, 2009; Nriagu et al., 2007; Ritchie et al., 2013; Warren et al., 2003). The main routes of

35 arsenic poisoning from contaminated soils are accidental ingestion or inhalation of

36 contaminated soil or water or consumption of plants grown on As-contaminated soils

37 (Miretzky and Cirelli, 2010). There is a need for affordable remedial solutions that can be

38 applied to As-contaminated soil. It is increasingly recognised that the remedial methods of

39 disposal or isolation of contaminated soil are not sustainable. This had led to a growing

40 willingness to use organic or mineral amendments to alter soil chemistry and break

41 pathways between pollutant sources and receptors (e.g. Hodson, 2010; Jones and Healey,

42 2010; O'Day and Vlassopoulos, 2010).

43

44 A large literature reports the sorption of many potential contaminants to Fe oxides, e.g.

45 Cornell and Schwertmann, 2003 and references therein, Jambor and Dutrizac, 1998 and

46 references therein. Much work has investigated As adsorption by Fe oxides (e.g. Bowell,

47 1994; Matis et al.,1997; Sun and Doner, 1998; Jain et al., 1999; Garcia-Sanchez et al., 2002;

48 Goldberg, 2002; Ford, 2002; Jackson and Miller, 2000; Grafe et al., 2001;Waltham and Eick,

49 2002; Sun and Doner, 1996; Manning et al., 1998; Goldberg and Johnston, 2001; Livesey

50 and Huang, 1981; Elkhatib et al.,1984a,b; Manning and Suarez, 2000; Smith et al., 2002)

51 and this had led to investigations into using Fe oxides to remediate As-contaminated soil

2

(e.g. Boisson et al., 1999; Garcia-Sanchez et al., 1999; Warren et al., 2003; Warren and
Alloway, 2003; Nielsen et al., 2011; Kumpiene et al., 2008; Lee et al., 2011). Ochre is the
name given to Fe(III) oxyhydroxide precipitates that accumulate in the outflows of mine
systems. In the United Kingdom the Coal Authority is responsible for over 68 (as of August
2014) mine water treatment schemes that remove c. 4000 tonnes of iron per year from water
courses resulting in ochre production (UK Government, 2014). Some of this ochre is used in
brick production to partially offset the waste management costs (Clean Rivers Trust, 2012).
Additionally, research has been carried out, with mixed success, into using ochre to limit
phosphate concentrations in water and soil (Fenton et al., 2012; Heal et al., 2005; Dobbie et
al. 2009; Sibrell et al. 2009). However, there is still no mature market for ochre in the UK; its
accumulation poses a waste disposal problem.

Previously (Doi et al., 2005) we showed that ochre may be an appropriate remedial
amendment. However, properties of ochres are site specific. Here we characterise a further
4 ochres to further demonstrate the ability of ochres to adsorb As. We then report a year
long incubation study investigating whether ochre amendments can break the most
significant pathways (leaching to ground water, ingestion of soil, uptake by plants) between
As-contaminated soil and receptors. We also examine the impact on ochre amendment on
soil microbial functioning via assay of hydrolytic enzyme activity.

Materials and methods

As-bearing soils were collected from the upper 20 cm of profiles located at a former mine
site (Devon Great Consols, SX 72878 96419, soil DGC), a former As calciner (Tresavean,
Lanner, Redruth, SW 72423 39743, soils RRT1, RRT2) and an allotment site (Scunthorpe,
SE 89344 10835, soil SCP). Soils were air-dried, sieved to $\leq$ 2 mm and stored prior to
characterisation and use in experiments.

3

78

79 Four ochres were provided by the UK Coal Authority: Bull House (SE 421192 402506, BH)

80 and Woolley (SE 41586 78838, WY) from passive treatment works, Old Meadows from an

81 active treatment works (SJ 43496 95991, OM) and Six Bells from a combined passive and

82 active treatment works (SO 22250 03039, SB). All ochres were supplied moist in sealed,

83 plastic containers and were air-dried and crushed to $\leq$ 2 mm prior to use in experiments.

84

85 pH (ISO, 2005), loss on ignition (for organic matter content, Rowell, 1994), particle size

86 distribution, BET surface area (de Boer et al., 1987), total As and Fe by aqua regia digest,

87 acid ammonium oxalate and citrate-bicarbonate-dithionite extractable Fe (Loeppert and

88 Inskeep, 1996), point of zero charge (Zelazny et al., 1996) and mineralogy by X-ray

89 diffraction were determined following standard established methods (details in

90 Supplementary material).

91

92 In adsorption experiments to investigate sorption of As by ochres 0.1g ochrewas added to

93 0.1 M sodium nitrate (40 mL); pH was adjusted to 3 or 8 using 0.1 M NaOH or 0.1M HCl.

94 The mixture was shaken on an end-over-end shaker at 20$\pm$1 °C for 24 hours in darkness

95 then 40 μL of 200 or 500 mg L^{-1} NaAsO$_3$ solution was added; pH was readjusted to pH 3 or 8

96 using NaOH or HCl and the mixtures were returned to the shaker. 15 replicates of each

97 ochre-As combination were used and 15 ochre-free controls. After 0.5, 1, 3, 6 and 24 hours,

98 three sacrificial replicates were removed, pH adjusted back to 3 or 8, samples were

99 centrifuged at 2113 g for 15 minutes and the centrifugate filtered through Whatman no. 2

100 filter papers. Arsenic concentrations were determined using ICP-OES.

101

102 In batch experiments to investigate reduction of As release from contaminated-soil into

103 solution due to ochre addition 1g As-contaminated soil was mixed with ochre (0.05, 0.1, 0.2

4

104 and 0.5 g) and added to 0.01 M CaCl$_2$ solution (20 mL). The mixture was shaken on an end-

105 over-end shaker at 20 $\pm$ 1 °C for 24 hours in darkness then centrifuged at 2113 g for 10

106 minutes. The supernatant was filtered (Whatman no. 2) and analysed for As by ICP-OES

107 and pH.

108

109 Incubation experiments investigated the impact of ochre amendments on As mobility under

110 pseudo-field conditions. Ochre (60 g) was added to soil (1200 g) (DGC, RRT2). Treatments

111 and controls were moistened to 100 % water holding capacity and incubated at 30 °C in

112 sealed plastic bags for 52 weeks in darkness. The soil was mixed weekly. Five replicates

113 were established per treatment and subsamples taken after 3, 12, 24 and 52 weeks of

114 incubation for analysis. As Fe and As are redox sensitive elements we measured Eh at

115 weeks 24 and 52 (there being insufficient resource to measure it at the other sampling

116 points) in addition to pH (ISO, 2005) at each sampling point to determine potential changes

117 in Fe and As speciation. Acid ammonium oxalate and citrate-bicarbonate-dithionite

118 extractable Fe and a fluorescein diacetate (FDA) hydrolysis assay to measure microbial

119 activity following the method of Adam and Duncan (2001) (Supplementary materials) were

120 carried out on the subsamples.

121

122 We assessed the effect of ochre amendments on the most likely pathways for As

123 contamination in soils to impact on the environment and human health. To determine

124 possible As leaching into ground water 1 g of air-dried soil was added to 20 mL of 0.01M

125 CaCl$_2$ solution (to represent soil solution; Houba et al., 1990) and shaken on an end-over-

126 end shaker for 24 hours at 20 °C. Samples were centrifuged at 2113 g for 15 minutes at 20

127 °C. Supernatant pH was measured.. The supernatant was filtered (Whatman No 2) and

128 analysed for As by ICP-OES. In a plant growth and As uptake bioassay , rye grass (*Lolium*

129 *perenne* . L., 0.5 g seeds per pot) was grown in 150g of incubated soil in a plant growth

130 room for 40 days. Plants were harvested and shoots cut 1 cm above ground level; roots

131 were washed in deionised water to remove attached soil. Samples were dried at 70 °C to a

132 constant mass, digested in nitric acid and analysed for As by ICP-OESFor week 3 and 52

133 subsamples, a PBET extraction to assess As availability to humans on ingestion of the soil

134 (Intawongse and Dean, 2008) was carried out. Air dried, $\leq$ 250 μm soil (1g) was shaken with

135 simulated stomach and intestine fluids which were analysed for As by ICP-OES. Analytical

136 and methodological details are given in the Supplementary materials.

137

138 **Quality control and statistical analysis**

139 An in-house 500 ppb reference solution was analysed by ICP-OES at the start of each

140 analytical run and returned values within 10% of established concentrations. The detection

141 limit for the adsorption and batch experiment As solutions and CaCl$_2$ extractions was 8 μg L^{-1}

142 calculated from the mean plus 6 times the standard deviation on ten replicate analyses of

143 the blank calibration standard (Gill, 1997). Detection limits were 1.133 $\pm$ 0.198 mg kg^{-1} for

144 plant digests and 61.6 mg kg^{-1} and 121.95 mg kg^{-1} for the stomach and small intestine phase

145 of the PBET analysis. Method blanks were run for all extractions and results were blank

146 corrected where appropriate. For aqua regia digests an in-house reference material (SS39)

147 traceable to CRM 143R sewage sludge-amended soil (Commission of European

148 Communities Community Bureau of Reference BCR) was digested. Recoveries were 95 –

149 105 % for As. For plant digests an in-house reference material (Hay 2) was digested with

150 each batch of digests. Recoveries were 98 – 102 %. Analytical precision for the different

151 matrices by duplicate analysis of 10 % of the samples (Gill, 1997) was > 95%.

152

153 Statistical analysis was carried out using SigmaStat 12.0.

154

155 **Results and discussion**

156   The soils and ochres showed a range of properties (Table 1). The ochres contained

157   relatively low concentrations of As, had a range of surface areas and the crystalline material

158   present was goethite.

Table 1. Mean soil and ochre properties used in the adsorption and batch experiments (n = 3 ± standard deviation)

Parameter	Soil DGC	RRT1	RRT2	SCP		Ochre BH	OM	SB	WY	
pH	$3.74^a \pm 0.06$	$4.70^{ab} \pm 0.05$	$5.26^{ab} \pm 0.04$	$7.27^b \pm 0.09$	NP	$5.63^a \pm 0.07$	$7.16^{ab} \pm 0.11$	$6.65^{ab} \pm 0.13$	$7.48^b \pm 0.08$	NP
LOI / %	$4.18^a \pm 0.12$	$7.52^b \pm 0.02$	$4.65^c \pm 0.24$	$8.14^d \pm 0.19$	P	$11.15^a \pm 0.18$	$9.95^b \pm 0.75$	$12.46^c \pm 0.08$	$13.67^d \pm 0.39$	P
Clay / %	$1.31^a \pm 0.09$	$3.19^{ab} \pm 0.16$	$1.94^{ab} \pm 0.10$	$5.09^b \pm 0.44$	NP	$22.8^a \pm 7.17$	$22.00^a \pm 1.11$	$10.74^b \pm 2.26$	$15.10^{ab} \pm 1.87$	P
Silt / %	$9.78^a \pm 0.63$	$32.57^{ab} \pm 2.76$	$15.00^{ab} \pm 0.61$	$39.93^b \pm 1.94$	P	$45.63^a \pm 7.82$	$54.47^{ab} \pm 3.84$	$26.40^c \pm 3.56$	$64.87^b \pm 3.62$	P
Sand / %	$88.93^a \pm 0.74$	$64.23^b \pm 2.87$	$83.07^c \pm 0.67$	$54.97^d \pm 2.12$	P	$31.57^a \pm 15.00$	$23.57^a \pm 4.92$	$62.80^b \pm 5.80$	$20.03^a \pm 5.47$	P
Textural class	Sand	Sandy loam	Loamy sand	Sandy loam		Loam	Silt loam	Sandy loam	Silt loam	
Total As / mg kg^{-1}	$33200^{ab} \pm 3020$	$310^{ab} \pm 29.5$	$1810^{ab} \pm 47.6$	$124^b \pm 9.15$	NP	$2.03^a \pm 0.07$	$< 0.02^b$	$4.24^c \pm 0.05$	$< 0.02^b$	P
Total Fe / %	$11.2^a \pm 0.31$	$2.67^b \pm 0.09$	$3.36^c \pm 0.15$	$13.0^d \pm 0.17$	P	$60.57^a \pm 0.31$	$47.20^b \pm 1.23$	$59.87^{ab} \pm 3.26$	$57.41^{ab} \pm 0.42$	NP
AO Fe / %	ND	ND	ND	ND		$12.84^a \pm 0.43$	$24.19^b \pm 0.54$	$25.25^b \pm 0.61$	$25.00^b \pm 0.35$	P
CBD Fe / %	ND	ND	ND	ND		$94.21^{ab} \pm 1.20$	$79.28^b \pm 0.78$	$96.15^a \pm 1.46$	$83.23^{ab} \pm 17.43$	NP
PZNC	ND	ND	ND	ND		$5.36^a \pm 0.29$	$6.15^a \pm 0.20$	$5.94^a \pm 0.46$	$4.26^a \pm 0.31$	NP
BET / m^2 g^{-1}	ND	ND	ND	ND		$170^a \pm 5.70$	$261^b \pm 1.71$	$65.4^c \pm 0.72$	$79.9^d \pm 0.50$	P
Mineralogy / %										
Goethite	BDL	BDL	BDL	16		100	100	100	100	
Quartz	43	70	65	84		BDL	BDL	BDL	BDL	
Chlorite + kaolinite	45	7	11	BDL		BDL	BDL	BDL	BDL	
Mica	6	11	13	BDL		BDL	BDL	BDL	BDL	
Microcline	BDL	7	9	BDL		BDL	BDL	BDL	BDL	
Fluorite	5	1	1	BDL		BDL	BDL	BDL	BDL	
Albite	Trace	4	1	BDL		BDL	BDL	BDL	BDL	
Siderite	1	Trace	Trace	BDL		BDL	BDL	BDL	BDL	

AO = ammonium oxalate extractable Fe; CBD = citrate bicarbonate dithionite extractable Fe; ND = not determined; PZNC = point of net zero charge; BDL = below detection limit of ~ 5 %. Across the soils and across ochres, values were compared by Analysis of Variance (ANOVA) if normally distributed (P) or Kruskal-Wallis Analysis of Variance on Ranks if not normally distributed (NP), values with different subscripts are significantly different ($p \leq 0.05$; Tukey test).

Adsorption experiments

Since Fe oxides can adsorb As (Dixit and Hering, 2003; Kanematsu et al., 2013; Miretzky and Cirelli, 2010) it was expected that the ochres would sorb As and this was verified in initial adsorption experiments conducted at pHs above and below the PZNC (Fig. 1). The majority of adsorption happened within the first 30 minutes of the experiment (Fig. 1), as in other experiments using ochres (Doi et al., 2005). The ochre PZNC values (Table 1) are lower than reported literature values of 7.5 – 9.0 for goethite (Stumm and Morgan, 1981; Bigham et al., 2002). This is probably due to organic matter in the ochres (Appel et al. 2003). Significant adsorption occurred at both pH 3 and 8, below and above the PZNC, suggesting that sorption was dominated by chemisorption rather than electrostatic interactions. Consistent with other anion adsorption studies, adsorption was generally greater at pH 3 than pH 8 (Giménez et al., 2007; Dixit and Hering, 2003; Matis et al., 1997). 3-way analysis of variance (ANOVA) indicated that at initial As concentrations of 200 and 500 mg L^{-1} there were significant interactions between ochre type, pH and duration of experiment ($p \leq 0.01$) (Tables S1 and S2). Generally there was little change in adsorption between 6 and 24 hours. Considering the 24 hour data, 3-way ANOVA indicated a significant interaction between ochre type, pH and initial As concentration ($p \leq 0.01$) (Table S3). At pH 3 and 8 there were significant differences between the adsorption that occurred on BH and OM between the initial As concentrations of 200 and 500 mg L^{-1}; more adsorption occurred for initial As concentrations of 500 mg L^{-1} for BH but less for OM. The greatest adsorption, at both 200 and 500 mg L^{-1} As was shown by OM which had the highest surface area; SB and WY showed the least adsorption and had the lowest surface areas. Thus it seems likely that differences in adsorption between the ochres were primarily driven by surface area and therefore availability of adsorption sites.

27 **Fig. 1.** Arsenic adsorption by the four different ochres (BH, OM, SB and WY) at a) pH 3,
28 initial As concentration of 200 mg L^{-1}, b) pH 3, initial As concentration of 500 mg L^{-1}, c) pH 8,
29 initial As concentration of 200 mg L^{-1}, d) pH 8, initial As concentration of 500 mg L^{-1}.
30 Adsorption values are means of 3 replicate analyses, vertical error bars are standard
31 deviations.
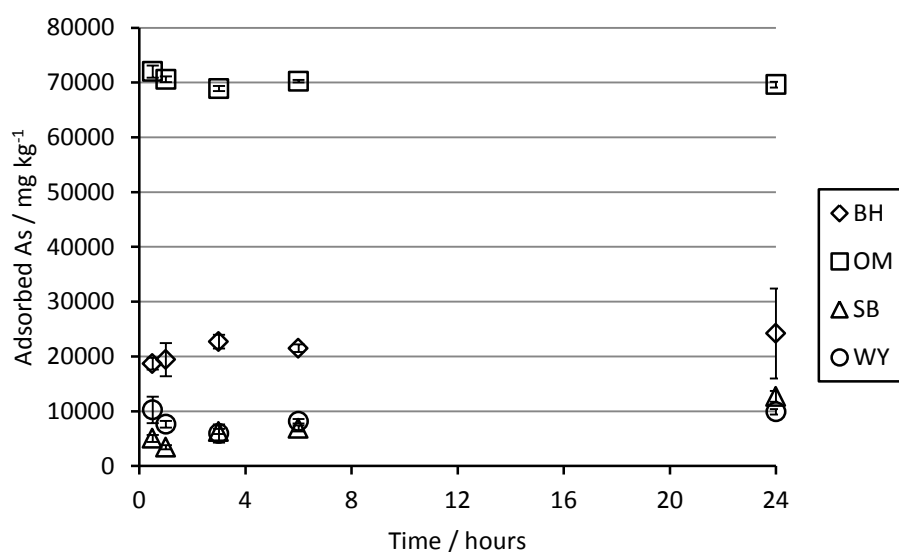
32

33   a) pH 3, 200 mg/L As

34

35

36   b) pH 3, 500 mg / L As

37
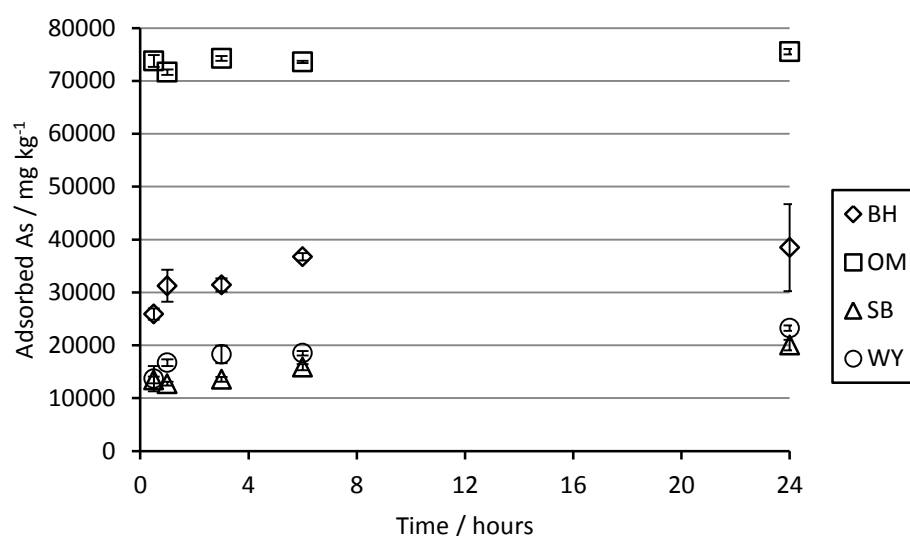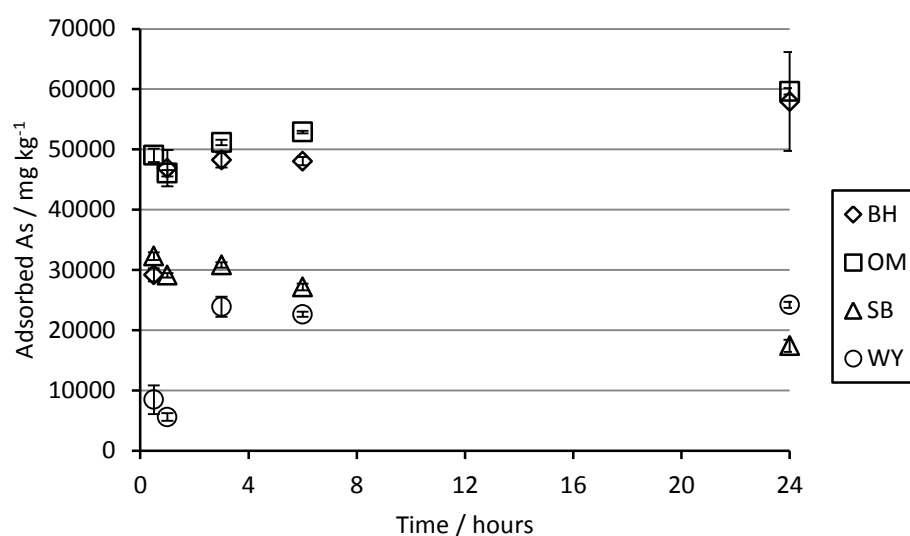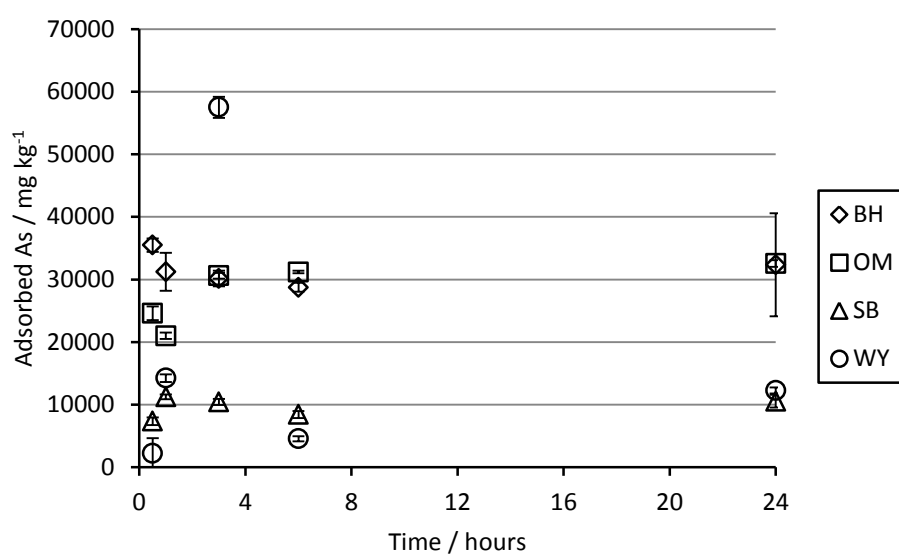
38   c) pH 8, 200 mg/L As

39

40

41   d) pH 8, 500 mg / L As

42

43

Batch experiments

Arsenic concentrations in batch experiments using RRT1 and SCP soils were below

detection (8 μg L^{-1}) and are not discussed further. Unamended RRT2 soil released less As

47   into solution than DGC (Fig. 2) though as a proportion of total As, RRT2 released more,

48   highlighting the importance of determining mobile or available contaminant concentrations

49   rather than total concentrations in pollution studies and risk assessment. Addition of even a

50   small amount of ochre reduced As release into solution. The decrease in As release with

51   increasing ochre addition was presumably due to provision of more sorption sites (Fig. 2).

52   Arsenic concentration in solution was below detection in the RRT2 experiment at all levels of

53   ochre addition.

54

55   Two-way ANOVA of the DGC data indicates a statistically significant effect of both ochre

56   type and mass of ochre added on As concentration in solution and a significant interaction

57   between the two ($p \leq 0.001$) (Table S4). OM and WY remove significantly more As from

58   solution than BH and SB at ochre loadings of 0.05 and 0.1 g ($p \leq 0.01$) but at the higher

59   loadings effects of the different ochres are not significantly different. Perhaps at these

60   masses of ochre, adsorption sites are not a limiting factor for As removal. Increasing ochre

61   loadings does not increase As removal from solution by OM and WY but has a significant

62   effect on As removal for BH and SB. OM has a higher surface area than the other ochres

63   (Table 1) and was the most adsorptive in the adsorption experiments. However, WY has a

64   relatively low surface area and, in adsorption experiments showed relatively low adsorption,

65   together with BH. This suggests that interaction with the soil played an important role in

66   determining the level of As removal. The OM and WY suspensions both had higher pHs

67   (Table S5) than the BH and SB suspensions but it seems unlikely that higher pH causes

68   reduced As release since typically As adsorption is greater at lower pH, as observed in our

69   adsorption experiments and elsewhere (Giménez et al., 2007; Dixit and Hering, 2003; Matis

70   et al., 1997).

71

72   The adsorption and batch experiments demonstrate that ochres adsorb As and therefore

73   have the potential to be used to remediate As-contaminated soils. However, in both cases,

74   there was a high ratio of solution to solid, maximising interaction between As in solution and

75   potential sorbing surfaces. If mineral amendments are to be used in the field they will be

76   mixed with soils and the level of contaminant – mineral interaction will be less. Therefore

77   further experiments, with more realistic ochre – soil mixtures are necessary to fully assess

78   the merits of mineral amendments for soil remediation.

79

Fig. 2. Arsenic concentration in solution after 24 hour batch experiment solutions using 1 g

of As-bearing soil and varying masses of ochre for a) DGC soil and b) RRT2 soil. Values are

mean of three replicates, error bars are standard deviations. The dashed horizontal line

indicates the detection level of 8 μg L^{-1}.

84

85   a) DGC soil

86

87   b) RRT2 soil

88
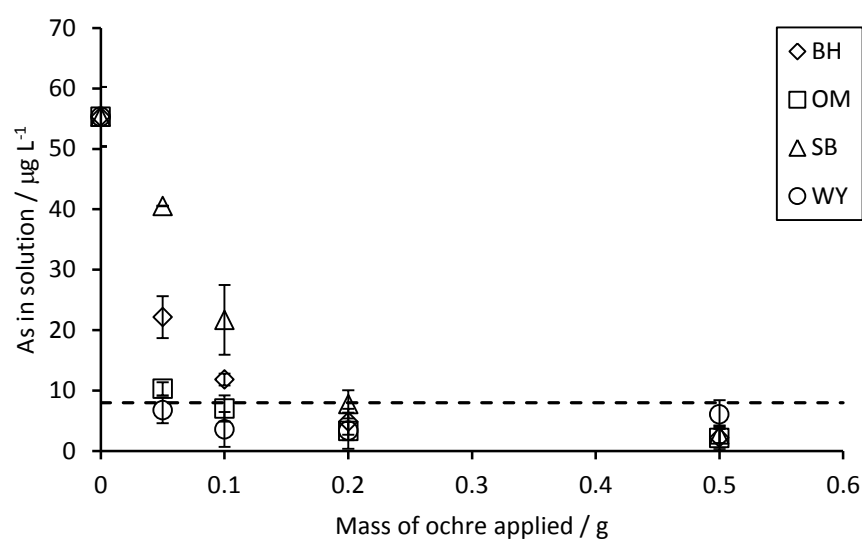
Incubation experiments

90	Ochre amendments reduced the amount of 0.01 M $CaCl_2$ extractable As (Table 2a, S6). For

91	RRT2 soil the amendments reduced extractable As from 0.27 ± 0.08 mg kg^{-1} to below

92	detection (8 μg L^{-1}, 0.16 mg kg^{-1}). For DGC soil, similar to the adsorption and batch

93	experiments, OM ochre had the most significant impact. Extractable As increased over time

94	for the unamended and amended DGC soil ($p \leq 0.01$). However, the ratio of extractable As in

95	the unamended to ochre amended soils (Table 2b) either remained the same or increased

96	($p \leq 0.01$) suggesting that the efficacy of ochre treatments in reducing As mobility was

97	constant or increased with respect to incubation time.

98

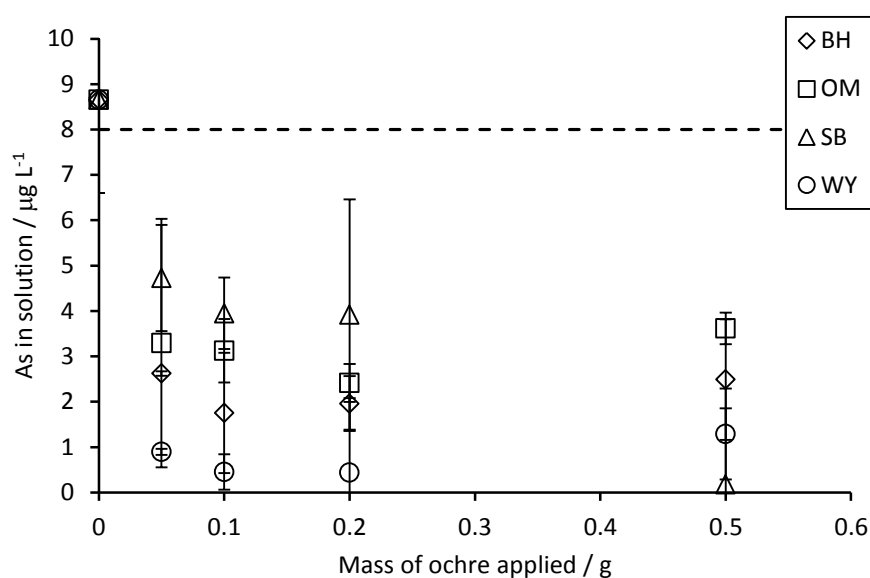99	Table 2a. 0.01 M $CaCl_2$ extractable As in the DGC soils.

Ochre	Week 3	Week 12	Week 24	Week 52
None	2.25 ± 0.41aA	3.85 ± 0.33 aB	6.27 ± 0.33 aC	6.4 ± 0.30 aC
BH	1.69 ± 0.07 bcA	3.44 ± 0.28 bB	5.02 ± 0.19 bC	4.38 ± 0.16 bD
OM	0.79 ± 0.04 dA	1.33 ± 0.08 cB	1.41 ± 0.11 cB	2.01 ± 0.09 cC
SB	1.82 ± 0.11 bA	3.26 ± 0.13 bB	4.21 ± 0.21 dC	3.47 ± 0.24 dB
WY	1.45 ± 0.26 cA	2.65 ± 0.18dB	2.79 ± 0.09eB	5.25 ± 0.08 eC

100	Values are means of 5 replicates $\pm$ standard deviation. Same letters = not significantly
101	different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
102	specific weeks, capital letters within specific ochre treatments.

103

104	Table 2b. Ratio of 0.01 M $CaCl_2$ extractable As in the unamended to amended DGC soils.

	Week 3	Week 12	Week 24	Week 52
None / BH	1.33 ± 0.06 ab	1.12 ± 0.02 a	1.25 ± 0.01 a	1.46 ± 0.01 a
None / OM	2.85 ± 0.29 a	2.90 ± 0.09 a	4.44 ± 0.17 b	3.18 ± 0.04 b
None / SB	1.24 ± 0.06 ab	1.18 ± 0.01 a	1.49 ± 0.01 c	1.84 ± 0.02 c
None / WY	1.56 ± 0.16 ab	1.45 ± 0.03 a	2.25 ± 0.02 d	1.22 ± 0.00 a

105	Uncertainties in ratios are propagated through the calculations from the standard deviations
106	about mean As extraction. Ratios are compared using Least squared difference. Same
107	letters = treatment not significantly different between weeks

108

109	Addition of ochre amendments typically increased pH in both soils (Tables 3, S7) though

110	there are no consistent trends in pH change over time. pH increases due to ochre addition

111 have been observed previously (Doi et al., 2005; Nielsen et al., 2011). Doi et al. (2005)

112 attributed the increases to dissolution of calcite present as an impurity in the ochres. No

113 calcite was detected in the present ochres but the ochre pH (Table 1) suggests that calcite

114 might be present, buffering pH, at concentrations below the XRD level of detection (c. 5%).

115 Alternatively the pH increase may be due to the release of OH^- due to sorption of anionic As

116 species to the ochre (Jain et al., 1999).

117

118 Table 3. pH of a) DGC and b) RRT2 soil.

Ochre	Week 3	Week 12	Week 24	Week 52
None	2.82 ± 0.02 aA	2.56 ± 0.11 aB	2.90 ± 0.03 aC	2.96 ± 0.03 aD
BH	2.79 ± 0.02 aA	2.58 ±0.07 aB	2.88 ± 0.03 aC	2.97 ± 0.02 aD
OM	3.58 ± 0.09 bA	3.30 ± 0.04 cB	3.39 ± 0.02 bC	3.33 ± 0.02 bB
SB	2.91 ± 0.02 cA	2.79 ± 0.03 dA	2.96 ± 0.02 cAB	3.00 ± 0.03 aB
WY	3.38 ± 0.03 dA	3.25 ± 0.03 cB	3.30 ± 0.02 dAC	3.26 ± 0.03 cC

119 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
120 different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
121 specific weeks, capital letters within specific ochre treatments.

122

123 Table 3b.

Ochre	Week 3	Week 12	Week 24	Week 52
None	4.28 ± 0.09 aA	4.05 ± 0.13 aB	4.54 ± 0.09 aC	4.55 ± 0.04 aC
BH	4.45 ± 0.13 bA	4.44 ± 0.10 bA	4.77 ±0.07 bB	4.70 ± 0.04 bB
OM	5.12 ± 0.10 cAB	5.02 ± 0.06 cA	5.23 ± 0.04 cB	5.16 ± 0.04 cB
SB	4.60 ± 0.05 dAB	4.58 ± 0.07 dA	4.71 ± 0.05 bB	4.65 ± 0.03 bAB
WY	5.08 ± 0.06 cAB	4.92 ± 0.04 eC	5.13 ± 0.04 cA	5.02 ± 0.01 dBC

124 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
125 different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
126 specific weeks, capital letters within specific ochre treatments.

127

128 Eh was positive at weeks 24 and 52 in the incubation experiment (Table 4, S8). For DGC

129 soil there were slight differences between treatments and, when significant changes

130 occurred between 24 and 52 weeks Eh became more oxidising. Only in week 52 did one

131 treatment (WY) result in a lower Eh than the unamended soil. In contrast there was a

132 significant decrease in Eh between weeks 24 and 52 for all the RRT2 treatments. The Eh-pH

133 conditions recorded fall around the $Fe^{2+}_{(aq)}$ - FeOOH and H_3AsO_3 (i.e. As^{III}) - $H_2AsO_4^-$ (i.e.

134 As^V) stability field divides in Eh-pH diagrams (e.g. Scheffer and Schachtschabel, 1989; Lu

135 and Zhu, 2011) making it hard to be certain of the speciation of either the Fe or As in the

136 systems or the stability of the ochres. The ochres may have undergone reductive dissolution

137 during the experiment. At the start of the experiment As may have been present as As^V in

138 the soil with the reduction in Eh in the RRT2- OM, SB and WY mixtures causing reduction to

139 As^{III}. The reduction in Eh could be driven by oxidation of organic matter. RRT2 contains

140 slightly more organic matter than DGC which may explain why no Eh reductions were seen

141 in the DGC mixes. However, if the Eh reduction is due to organic matter oxidation it is not

142 clear why this occurred in the RRT2- OM, SB and WY mixtures but not the RRT2-BH mixture

143 or untreated soil.

144

145 Table 4. Eh of a) DGC and b) RRT2 soil (mV).

Ochre	Week 24	Week 52
None	438.2 ± 28.0 abA	506.0 ± 8.8 aB
BH	418.2 ± 19.9 abA	454.4 ± 47.8 abA
OM	383.4 ± 28.5 aA	484.6 ± 17.7 abB
SB	453.4 ± 41.7 bA	469.8 ± 10.6 abA
WY	433.4 ± 53.9 abA	439.6 ± 14.1 bA

146 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
147 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons within
148 specific weeks, capital letters within specific ochre treatments.

149

150 Table 4b.

Ochre	Week 24	Week 52
None	439.6 ± 49.7 aA	476 ± 9.6 aA
BH	474.2 ± 42.6 aA	397.6 ± 54.5 bB
OM	469.0 ± 25.8 aA	204.0 ± 34.0 cB
SB	471.2 ± 30.4 aA	188.0 ± 22.8 cB
WY	402.0 ± 57.7 bA	216.6 ± 4.9 cB

151 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
152 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons within
153 specific weeks, capital letters within specific ochre treatments.

154

155 Ammonium oxalate extractions were used as a proxy for amorphous and poorly crystalline

156 iron oxides. For the DGC soils the amount of amorphous and poorly crystalline iron oxide

157   was generally greatest for the OM-amended soil and increased over time (Tables 5a,

158   S9a).The other ochre amendments didn't significantly increase the amount of extractable

159   iron. A similar trend of increasingly extractable iron over time occured for the RRT2 soil

160   (Table 5b, S9b). The amount of extractable iron was lower for the RRT2 than the DGC soil,

161   despite the same level of Fe amendments. However for the RRT2 soil the ochre

162   amendments did increase the amount of extractable Fe. The DGC control soil contains more

163   ammonium extractable Fe than RRT2 (Table 5) suggesting that background iron levels in the

164   soils might be dominating the results of this extraction for DGC but that for RRT2, iron levels

165   are sufficiently low for ochre amendments to have a significant impact.

166

167   Table 5a. Acid ammonium extractable Fe in a) DGC and b) RRT2 soil (mg kg^{-1}).

Ochre	Week 3A	Week 12B	Week 24 C	Week 52 D
None a	4.96 ± 1.21	4.92 ± 1.0	7.73 ± 0.73	6.77 ± 0.12
BH a	5.75 ± 0.05	4.46 ± 0.10	7.32 ± 0.09	6.01 ± 0.36
OM b	5.61 ± 0.55	5.11 ± 0.19	9.61 ± 0.07	8.10 ± 0.28
SB a	4.98 ± 0.08	4.30 ± 0.18	8.16 ± 1.1	5.75 ± 0.37
WY a	5.18 ± 1.13	4.31 ± 0.16	8.23 ± 2.6	5.79 ± 0.19

168   Values are means of 5 replicates ± standard deviation. Same letters = not significantly
169   different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons between
170   treatments taking account of all weeks, capital letters between weeks taking account of all
171   treatments.

172

173   Table 5b.

Ochre	Week 3	Week 12	Week 24	Week 52
None	0.35 ± 0.039 aA	0.33 ± 0.032 aA	0.83 ± 0.57 aB	0.51 ± 0.48 aA
BH	0.74 ± 0.16 bA	0.85 ±0.048 bA	1.8 ± 0.038 bB	1.7 ± 0.074 bB
OM	1.8 ± 0.16 cA	1.8 ± 0.033 cA	3.9 ± 0.052 cB	4.1 ± 0.080 cB
SB	0.58 ± 0.091 abA	0.46 ± 0.031 aA	1.0 ± 0.0068 aB	0.97 ± 0.0051 dB
WY	0.76 ± 0.069 bA	0.87 ± 0.048 bA	1.4 ± 0.015 dB	1.5 ± 0.039 b B

174   Values are means of 5 replicates ± standard deviation. Same letters = not significantly
175   different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
176   specific weeks, capital letters within specific ochre treatments.

177

178   Ochre amendments increased the citrate dithionite extractable iron, a proxy for crystalline

179   Fe, in both soils, though this increase became less over time (Tables 6, S10) suggesting a

180 gradual loss of crystalline material, possibly as goethite in the ochre degraded, producing

181 more amorphous material. Assuming that the ochres were pure goethite, the level of ochre

182 amendment would have resulted in goethite concentrations in the mixtures at or below the

183 limit of detection of XRD (c. 5%), thus no attempt was made to track changes in Fe

184 mineralogy in the mixtures using XRD. However, the operationally defined decrease in

185 crystalline and increase in amorphous Fe oxides is consistent with the Eh-pH data. Goethite

186 dominated the ochre mineralogy as determined by XRD during material characterisation and

187 Eh-pH measurements suggest the potential for this phase to be unstable in the ochre

188 amended soils. Fe oxides are dynamic species that change in soils over time (e.g. Bigham et

189 al, 2002; Schwertmann and Cornell, 1991). Our incubation study was a year long but it is

190 possible, given the slow kinetics of many redox reactions, that the mineralogy of the mixtures

191 was not in steady state but was still changing. This highlights an important consideration for

192 studies on soil amendments. In addition to laboratory testing, modelling should be carried

193 out to try and predict the long term stability of the amendments and their impact on

194 contaminant mobility.

195

196 Table 6a. Citrate dithionite extractable Fe in a) DGC and b) RRT2 soil (mg kg^{-1}).

Ochre	Week 3	Week 12	Week 24	Week 52
None	5.95 ± 0.28 aA	6.92 ± 0.22 aB	5.89 ± 0.61 abA	5.54 ± 0.23 aA
BH	7.34 ± 0.20 bcA	11.3 ± 0.26 bB	6.06 ± 0.50 aC	6.16 ± 0.09 aC
OM	7.21 ± 0.26 bcA	10.5 ± 0.38 cB	5.62 ± 0.16 abC	6.31 ± 0.11 aC
SB	6.66 ± 0.17 abA	9.12 ± 0.56 dB	4.98 ± 0.13 bC	5.74 ± 0.23 aD
WY	8.03 ± 0.15 cA	10.6 ± 0.77 cB	5.33 ± 0.36 abC	6.29 ± 1.7 aD

197 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
198 different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
199 specific weeks, capital letters within specific ochre treatments.

200

201

202   Table 6b.

Ochre	Week 3A	Week 12 B	Week 24A	Week 52 C
None a	3.68 ± 0.90	3.45 ± 0.10	2.84 ± 0.09	2.63 ± 0.08
BH b	6.89 ± 0.09	7.97 ± 0.27	7.12 ± 0.24	5.53 ± 0.14
OM c	6.17 ± 1.8	6.93 ± 0.64	6.27 ± 0.49	5.26 ± 0.19
SB d	5.01 ± 0.24	5.97 ± 0.50	5.18± 0.47	4.42 ± 0.20
WY b	7.71 ± 0.33	8.53 ± 1.68	7.28 ± 0.56	5.84 ± 0.13

203   Values are means of 5 replicates ± standard deviation. Same letters = not significantly

204   different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons between

205   treatments taking account of all weeks, capital letters between weeks taking account of all

206   treatments.

207

208   Typically the adsorption capacity of less well crystalline material is greater than that of more

209   crystalline material (e.g. Jambor and Dutrizac, 1998; Cornell and Schwermann, 2003;

210   Guzman et al., 1994). Thus the conversion of free oxides to amorphous material should lead

211   to an increase in As sorption. This is consistent with the increased efficacy of the ochre

212   treatments (Table 2b). However 0.01 M $CaCl_2$ extractable As increased with time. This may

213   be due to reductive dissolution of the ochres to Fe^{2+}, with amorphous material being an

214   intermediate reaction product. Alternatively or additionally an increase in dissolved organic

215   carbon due to break down of organic matter leading to increased competition for sorption

216   sites between As species and either, or both, dissolved organic matter (DOM) (e.g. Garcia-

217   Sánchez et al., 2010; Gustafsson, 2006; Weng et al, 2009) and phosphate (e.g. Mamindy-

218   Pajany et al., 2011; Sharma and Kappler, 2011; Smith et al., 2002) would lead to an

219   increase in As release. Similar to the possible reductive dissolution of goethite in the ochre,

220   the change in Eh-pH conditions for the RRT2-OM, SB and WY mixtures might cause a

221   change in As speciation from As^V to As^{III}. At the pH of the mixtures, adsorption of As^V is

222   more favourable than that of As^{III} (e.g. Dixit and Hering, 2003; Miretsky and Cirelli, 2010).

223   Thus reduction of As^V could lead to an increase in 0.01 M $CaCl_2$ extractable As. Such

224   changes could be driven by microbial activity (Páez-Espino et al. 2009; Yamamura and

225   Amachi, 2014). At present we are unable to differentiate between these possible

226   mechanisms. Dissolved organic carbon and phosphate were not measured in our

227   extractions. Although we measured Eh and pH in our mixtures, conditions plot too close to

228 stability field boundaries to be certain of the oxidation state or stability of the phases present.

229 A more detailed spectroscopic investigation to determine Fe and As speciation would be

230 required to resolve this. This highlights the importance of Eh-pH conditions when considering

231 Fe amendments and As remediation. Additionally the possible increase in sorption capacity

232 of the ochre (Table 2) due to conversion of crystalline to amorphous Fe oxyhydroxides

233 coupled with potential desorption of As from ochres due to interaction with DOM, phosphate

234 and changing As speciation highlights the difficulties in extrapolating from simple laboratory-

235 based adsorption experiments to interactions in the field.

236

237 Microbial activity, determined using a FDA hydrolysis assay, which estimates the total

238 hydrolytic capacity of soils, was greatest after 3 weeks of incubation ($p \leq 0.01$) for both soils

239 (Table 7). Prior to incubation the soil had been air-dried and sieved. Initial peaks in microbial

240 activity are commonly observed when dry, sieved soil is moistened since microbial

241 metabolism is no longer constrained by desiccation and there is enhanced substrate

242 availability due to: (i) production of cytoplasmic solutes by the microbial biomass in response

243 to the rapid increase in soil water potentials (Fierer and Schimel, 2003) and (ii) exposure of

244 previously physically protected organic matter as a result of sieving (Franzluebbers, 1999)

245 and rewetting (Fierer and Schimel, 2003). Initially, activity was greater in the WY amended

246 soils but from Week 12 onwards there were no significant differences between control and

247 ochre-amended soils (Table 7, S11).

248

249 Table 7a. Microbial activity (μg fluorescein per gram dry soil per 0.5 h) in a) DGC and b)
250 RRT2 soil.

Ochre	Week 3	Week 12	Week 24	Week 52
None	29.6 ± 9.21 aA	16.1 ± 3.89 aB	5.05 ± 1.06 aC	7.51 ± 1.96 aC
BH	30.2 ± 6.23 aA	10.9 ± 1.37 aB	9.02 ± 0.950 aB	7.00 ± 1.46 aB
OM	41.2 ± 9.79 bA	12.8 ± 0.782 aB	15.3 ± 7.29 aB	15.0 ± 2.60 aB
SB	29.0 ± 8.45 aA	10.1 ± 1.97 aB	6.54 ± 1.83 aB	10.3 ± 0.798 aB
WY	48.8 ± 17.0 bA	15.3 ± 2.65 aB	14.3 ± 2.64 aB	16.2 ± 3.57 aB

251   Values are means of 5 replicates $\pm$ standard deviation. Same letters = not significantly
252   different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
253   specific weeks, capital letters within specific ochre treatments.

254

255

256   Table 7b.

Ochre	Week 3	Week 12	Week 24	Week 52
None	38.4 ± 9.44aA	19.2 ± 3.00aB	24.9 ± 3.40aB	22.7 ± 2.47aB
BH	37.6 ± 6.16aA	18.1 ± 5.18aB	20.8 ± 5.56aB	21.0 ± 2.19aB
OM	41.0 ± 6.17aA	16.9 ± 3.38aB	27.5 ± 2.86aC	24.3 ± 2.61aC
SB	35.9 ± 8.11aA	19.7 ± 7.41aB	21.5 ± 2.84aB	20.8 ± 4.74aB
WY	51.6 ± 9.27bA	15.7 ± 2.05aB	22.2 ± 3.13aB	18.8 ± 3.51aB

257   Values are means of 5 replicates ± standard deviation. Same letters = not significantly
258   different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within
259   specific weeks, capital letters within specific ochre treatments.

260

261   Our initial extractions suggest that ochre amendments can reduce the amount of 0.01 M

262    $CaCl_2$ extractable As from As-contaminated soils, most likely due to sorption of As to iron

263   oxyhydroxides. However, ochre amendment, with the exception for OM (DGC soil) and WY

264   (DGC and RRT2 soil) ochre at week 3 (Table 7a), does not cause a significant increase in

265   microbial activity. In week 3, the increased microbial activity in the OM and WY ochre

266   amended DGC soils corresponded to the most pronounced ochre-induced reductions in As

267   mobility. Arsenic can cause microbial toxicity (Páez-Espino et al. 2009; Yamamura and

268   Amachi, 2014) so this result could be interpreted as ochre-mediated alleviation of As toxicity.

269   However, in subsequent weeks As mobility increases whilst microbial activity remains

270   constant and As mobility is greater in the non-amended soils whilst there is no difference in

271   microbial activity between amended and non-amended soils (Tables 2a, 7). The differential

272   response in week 3 might be related to the rehydration of the soil at the start of the

273   experiment. As previously explained, this would likely have resulted in a flush of available

274   substrate and it is possible that microorganisms in OM and WY ochre amended DGC soils

275   were more able to respond to this flush as a result of reduced As toxicity (or elevated pH) in

276   these treatments; once rehydration effects subsided effects of ochre amendment on

277   microbial activity were no longer detectable.

278

279   At 3, 12, 24 and 52 weeks subsamples of soil were taken and used in plant bioassays. Rye

280   grass was grown in the soil for 40 days, harvested and then biomass and plant As content

assessed. For both soils generally there was no difference in root biomass with treatment (Table S12, S13) whereas shoot biomass generally increased in the WY and, for RRT2, OM amended soils (Tables 8, S14). Arsenic concentrations in shoots and roots showed a large amount of variation within replicates, potentially indicating adhesion of soil particles to the plant material used in the digestions (e.g. Markert, 1995) as has been found to be problematic in previous studies (e.g. Doi et al., 2005; Walsh and Keeny, 1975). Arsenic concentrations were greater in roots than shoots (Tables 9, 10). For the DGC soil, WY amendments initially reduced As uptake into shoots but over time uptake of As from untreated DGC soil decreased and by week 24 there was no significant effect of the ochre amendments (Tables 9a, S15a). For the RRT2 soil there is a similar decrease in As uptake into shoots over time but no significant impact of ochre on As uptake (Tables 9b, S15b). For roots, there is a similar decrease in As uptake with duration of incubation for the DGC soil so that initially significant reductions in As uptake due to addition of ochre ($p \leq 0.01$) are not significant after 52 weeks incubation (Tables 10a, S16a). For the RRT2 roots uptake was significantly higher in week 3 compared to weeks 12, 24 and 52 ($p \leq 0.01$) but there was no significant effect of the ochre amendments (Table 10b, S16b).

Table 8a. Shoot biomass (mg) for *Lolium perenne* grown in a) DGC and b) RRT2 soil.

Ochre	Week 3	Week 12	Week 24	Week 52
None	20.4 ± 9.90 aA	62.0 ± 17.6 aB	68.0 ± 17.2 aB	15.0 ± 3.20 aA
BH	48.2 ± 20.8 abA	46.2 ± 14.5 aA	57.0 ± 29.0 aA	12.4 ± 5.60 aB
OM	65.0 ± 10.8 bcA	58.6 ± 22.5 aA	70.2 ± 4.30 aA	18.0 ± 8.50 aB
SB	86.2 ± 7.50 cA	73.4 ± 30.1 aA	70.0 ± 40.7 aA	8.20 ± 4.40 aB
WY	132 ± 10.4 dA	211 ± 43.7 bB	133 ± 15.0 bA	33.2 ± 8.60 aC

Values are means of 5 replicates ± standard deviation. Same letters = not significantly different ($p \leq 0.05$, Holm-Sidak method). Lower case letters are for comparisons within specific weeks, capital letters within specific ochre treatments.

304 Table 8b.

Ochre	Week 3	Week 12	Week 24	Week 52
None	37.2 ± 27.7 aA	178 ± 18.5 aB	109 ± 30.0 aAB	71.8 ± 10.0 aA
BH	65.2 ± 30.8 abAB	182 ± 68.4 aC	144 ± 53.5 aAC	84.8 ± 14.2 aB
OM	109 ± 68.7 abA	313 ± 55.5 bB	307 ± 81.9 bB	267 ± 32.5 bB
SB	52.6 ± 39.4 aA	58.8 ± 21.2 cA	76.2 ± 17.7 aA	26.8 ± 5.60 aA
WY	142.2 ± 20.0 bA	284 ± 24.4 bB	289 ± 85.1 bB	246 ± 43.6 bB

305 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
306 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons within
307 specific weeks, capital letters within specific ochre treatments.

308

309 Table 9a. Shoot As (mg kg^{-1}) for *Lolium perenne* grown in a) DGC and b) RRT2 soil.

Ochre	Week 3	Week 12	Week 24	Week 52
None	983± 594 aA	2005 ± 1081 aB	297 ± 95.4 aC	221 ± 153 aC
BH	368 ± 169 abAB	795 ± 309 bA	356 ± 131 aAB	46.4 ± 37.0 aB
OM	890 ± 723 aA	122 ± 43.2 cB	169 ± 95.7 aB	72.0 ± 76.0 aB
SB	745 ± 446 abA	523 ± 186 bcA	290 ± 184 aA	427 ± 338 aA
WY	171 ± 131 bA	350 ± 162 bcA	259 ± 114 aA	325 ± 334 aA

310 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
311 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons within
312 specific weeks, capital letters within specific ochre treatments.

313

314 Table 9b.

Ochre	Week 3 A	Week 12B	Week 24B	Week 52B
None a	255 ± 375	145± 192	19.1 ± 4.72	50.7± 40.4
BH a	144± 86.9	31.2± 27.1	13.2 ± 5.75	43.3± 55.3
OM a	105± 85.6	9.17 ± 6.00	9.69 ± 2.52	14.6± 8.17
SB a	89.9± 32.1	61.0 ± 56.7	16.6 ± 5.78	39.2± 19.3
WY a	23.9± 22.4	10.5 ± 3.96	9.63 ± 4.24	11.7± 7.88

315 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
316 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons between
317 treatments taking account of all weeks, capital letters between weeks taking account of all
318 treatments.

319

320

321 Table 10a. Root As (mg kg^{-1}) for *Lolium perenne* grown in a) DGC and b) RRT2 soil.

Ochre	Week 3	Week 12	Week 24	Week 52
None	5350± 2110 abcA	2460± 779 aB	1830± 346 aB	1350± 418 aB
BH	3130± 1400 dA	2100± 273 bA	1450± 817 aA	1560± 969 aA
OM	7470± 3260 bA	1640± 388 cB	1020± 235 aB	773± 457 aB
SB	3330± 2380 cdA	2180± 974 bcA	1340± 560 aA	1270± 614 aA
WY	4710± 1310 cdA	3630± 1478 bcA	963± 366 aB	641± 279 aB

322 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
323 different (p ≤ 0.05, Holm-Sidak method). Lower case letters are for comparisons within
324 specific weeks, capital letters within specific ochre treatments.

325

326 Table 10b.

Ochre	Week 3 A	Week 12B	Week 24B	Week 52B
None a	445± 214	171 ± 48.6	103 ± 36.2	96.3 ± 65.7
BH a	789 ± 883	138 ± 22.4	63.9 ± 44.2	48.9 ± 21.2
OM a	604 ± 286	121 ± 23.8	60.9 ± 11.5	56.1 ± 30.7
SB a	266 ± 65.0	107 ± 32.0	95.7 ± 25.8	148 ± 174
WY a	431 ± 199	83.7 ± 15.5	88.0 ± 9.84	71.6 ± 15.9

327 Values are means of 5 replicates ± standard deviation. Same letters = not significantly
328 different (p ≤ 0.05; Holm-Sidak method). Lower case letters are for comparisons between
329 treatments taking account of all weeks, capital letters between weeks taking account of all
330 treatments.

331

332 Ochre amendments had little benefit in terms of plant growth promotion and As uptake

333 reduction. Higher levels of As in the DGC soil and lower pH clearly had a significant impact

334 on plant growth compared to the RRT2 soil. Root biomass was similar between the two soils

335 despite the higher As content of the DGC roots whereas shoot biomass was lower and shoot

336 As concentration was higher in the DGC soil. This further suggests that some of the "root As"

337 in the DGC roots was actually due to adhering soil particles or that root growth is less

338 sensitive to As than shoot growth. Given the reduction in CaCl$_2$ extractable As due to ochre

339 addition and the increase in CaCl$_2$ extractable As over time the lack of a significant impact of

340 ochre on plant uptake and decrease in plant uptake from the soils that had been incubated

341 for longer was surprising. This suggests that As uptake may be dominated by rhizosphere

342 processes at a scale that the extraction of As from bulk soil is unable to resolve.

343

344   Due to time constraints the PBET was only applied to soils incubated for 3 and 52 weeks.

345   For the DGC and RRT2 stomach phase and the DGC intestine phase, significantly more As

346   was extracted after 52 weeks incubation compared to 3 weeks incubation for both soils and

347   all treatments ($p \leq 0.01$) (Tables 11, S17), consistent with the increase in $CaCl_2$ extractable

348   As (Table 2). For the RRT2 soil, ochre amendments reduced extractable As ($p \leq 0.01$) but this

349   wasn't the case for the DGC soil; this may be due to the pH differences of the soils. RRT2

350   had a higher pH than DGC and the soil-ochre mixtures may have buffered the low pH of the

351   PBET extraction to a greater extent, resulting in less As release. Extractable As in the

352   intestine phase for the RRT2 soil was below detection (121.95 mg kg^{-1}).

353

354   **Conclusions**

355   The adsorption and batch experiments suggest that waste ochre may have a role to play in

356   treating As-contaminated water. However further research would be required to establish the

357   Eh-pH stability field of the ochres, the impact of time on ochre composition and sorption

358   capacity, the impact of water chemistry e.g. ionic strength, dissolved organic carbon on

359   sorption, and a means of deploying the ochre in water courses, possibly via incorporation

360   into a semi-permeable membrane.

361

362   On the basis of the soil incubation study it is not possible to recommend ochre amendments

363   to As-contaminated soils as a remedial treatment. Although the amendments may reduce

364   transfer of As to water courses they do not impact reliably on other significant pathways of

365   As transfer through the environment, i.e. uptake by plants and release of As following

366   ingestion of As-contaminated soil by humans. Additionally it is not clear that the ochres are

367   stable in the amended soils and therefore the long term impacts on extractable As are not

368   clear. A more detailed investigation into soil Eh and both ochre and As speciation would be

369   required coupled with modelling studies to cast further light on this.

370

371   The well documented sorption of a variety of elements to Fe oxides does suggest that the

372   use of ochres for the remediation of multi-element contaminated waters and soils may be

373   worth investigating for situations with appropriate Eh-pH conditions.

374

383

Table 11. As concentration (mg kg^{-1}) in the stomach phase of the PBET extraction.

Soil Ochre	DGC - stomach Week 3 A	Week 52 B	DGC – intestine Week 3 A	Week 52 B	RRT2 - stomach Week 3A	Week 52B
None	93.7 ± 2.73a	122 ± 32.9	517 ± 168 a	707 ±96.0	8.24 ± 0.326a	25.0 ± 3.62
BH	120 ± 2.45a	164 ± 49.3	623 ± 112 a	685 ± 53.2	3.53 ± 1.76b	14.1 ± 1.66
OM	112 ± 1.82ab	185 ± 25.4	474 ± 10.3 a	796 ± 190	3.54 ± 1.23b	16.5 ± 4.06
SB	97.7 ± 7.97a	146 ± 27.9	423 ± 51.3 a	582 ± 187	1.95 ± 1.30b	18.0 ± 1.14
WY	133 ± 5.97b	264 ± 49.6	585 ± 106 a	879 ± 102	2.61 ± 0.953b	16.1 ± 0.290

Values are means of 3 replicates ± standard deviation. For each set of data (DGC- stomach, DGC – intestine, RRT2 – stomach) same letters = not significantly different ($p \leq 0.05$; Holm-Sidak method); lower case letters are for comparisons between treatments taking account of both weeks 3 and 52 (letters shown in week 3 column for clarity), capital letters between weeks 3 and 52 taking account of all treatments.

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220

221   **Supplementary material**

222

223   **Does ochre have the potential to be a remedial treatment for As-contaminated soils?**

224

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234

235   **Characterisation of soils and ochres**

236   pH was measured on a suspension of 10 g material in 25 mL deionised water that was

237   shaken for 15 minutes at 20 ± 1 °C on an end-over-end shaker prior to measurement with a

238   Jenway 3310 pH meter calibrated using pH 7.00 and 4.00 buffers (ISO, 2005). Loss on

239   ignition was determined as a proxy for organic matter content by oven drying the soils and

240   ochres at 105 °C overnight and then igniting overnight at 500 °C in a muffle furnace (Rowell,

241   1994). It should be noted that calcium carbonate and clays can degrade at this temperature

242   potentially giving misleadingly high values for the ochre samples. Particle size distribution

243   was determined using a Beckman Coulter LS230 laser granulometer with a variable fluid

244   module and Polarisation intensity differential scattering (PIDS) system. Particle size

245   calculations were made using the Fraunhofer optical model system (de Boer et al., 1987).

246   BET surface area was determined by gas adsorption and application of the BET isotherm

247   (Brunauer et al., 1938) using a Gemini III 2375 surface area analyser; samples were

248   degassed overnight at 60 °C with a N_2 purge. Total As and Fe of the samples was

249   determined by aqua regia digestion (Arnold et al., 2008) followed by analysis using a Perkin

250   Elmer Optima 3000 inductively coupled plasma-optical emission spectrometer (ICP-OES).

251   The iron content of the ochre was further characterised by acid ammonium oxalate and

252   citrate-bicarbonate-dithionite extractions (Loeppert and Inskeep, 1996) to determine

253   amorphous / poorly crystalline forms of Fe oxides and free Fe oxides respectively. Resulting

254   solutions were analysed by ICP-OES. The point of zero charge of the ochres was

255   determined after the method of Zelazny et al. (1996) which was adapted from Uehara and

256   Gillman (1982). In brief, the ochres were allowed to adsorb K^+ and Cl^- in an electrolyte of 1 M

257   KCl over a range of pH values; the amount of adsorbed K^+ and Cl^- were taken as the

258   quantities of negative and positive surface charge at each pH and the PZNC taken as the pH

259   at which these two values were equal and opposite. Mineralogy was determined on

260   randomly oriented samples of ground material using a Siemens D5000 X-ray diffractometer

261   using Cu Kα radiation at 40 keV and 40 mA, with a scanning range of 4° – 64°, 20 steps per

262   degree and a dwell time of 2 seconds.

263

264   **Analytical details for measurements made on the incubation experiment soils**

265   ***Eh measurement***

266   The redox potential of the incubated soil samples was monitored using a platinum electrode,

267   redox solution and a millivolt meter. An initial test was conducted by inserting the platinum

268   electrode into the standard redox solution to ascertain that the meter and the electrode were

269   working within the normal range (200 to 275 mV) (Hanna Instrument). Inserting the electrode

270   into redox standard solution for about 1 hour before measurement helps to ensure stable

271   reading and prevents fluctuation. To take the redox measurement, the platinum electrode

272   was inserted into the wet soil and allowed to stabilize for one minute before recording the

273   reading from a millivolt meter (Hanna pH 21 pH/mV). The electrode was rinsed with

274   deionised water and then wiped with soft tissue between measurements.

275

276   ***Plant bioassays***

277   For the plant bioassays, plant pots lined with filter paper to aid soil retention were filled with

278   150 g of the wet incubated soil. Rye grass seeds (0.5 g per pot, purchased from Herbiseed,

279   New Farm, Mire Lane, West End, Twyford, England) were added to the surface of the soil.

280   Plant pots were set out in a completely randomized design in a plant growth room subject to

281   un-monitored ambient temperature and a lighting regime of 150 – 300 micromoles m^{-1} s^{-1}

282   with a photoperiod of 17 hours. The average amount of water lost from each pot over two

283   days due to evaporation and transpiration was assessed by mass loss as 15 mL. This

284   volume of deionised water was added to the pots every other day. After 40 days plants were

285   harvested. Shoots were cut 1 cm above ground level. Roots were washed in deionised water

286   to remove attached soil. Plant samples were dried at 70 °C to a constant mass which was

287   recorded and then ground using an agate pestle and mortar prior to acid digestion using an

288   in house nitric acid digestion method. This method involved addition of 5 mL of 1M HNO_3 to

289    $\leq$ 0.25 g of plant material in digestion tubes. Following HNO_3 addition, samples were left

290   overnight and subsequently heated to 60 °C and left for 3 hours. The temperature was

291   raised to 110 °C and the samples digested for a further 6 hours. After cooling samples were

292   filtered, diluted as necessary and analysed for As by ICP-OES.

293

294   ***PBET extraction***

295   The PBET extraction followed that of Intawongse and Dean, (2008). In brief 1 g of air dried,

296    $\leq$ 250 μm soil was shaken with 100 mL simulated gastric acid solution at 150 oscillations per

297   hour for one hour at 37 ºC. 5 mL of solution was filtered through a 0.45 μm cellulose filter

298 and analysed for As by ICP-OES. This was the stomach phase. The gastric acid solution

299 comprised 2.5 g pepsin, 1 g sodium malate, 1 g sodium citrate, 1 mL acetic acid and 0.84

300 mL lactic acid made up to 2 L with ultra pure water and with the pH adjusted to 2.5 using

301 concentrated hydrochloric acid (stomach phase). Saturated sodium bicarbonate solution was

302 added dropwise to the remaining solution until a pH of 7 was reached. Bile salt (0.175 g) and

303 pancreatin (0.05 g) were added and the solution shaken at 37 ºC for a further 4 hours after

304 which time 5 mL of solution was filtered and analysed for As by ICP-OES. This was the small

305 intestine phase. In initial tests a sample was taken at 2 and 4 hours but comparison of the 2

306 and 4 hour samples indicated that equilibrium had not been reached after 2 hours.

307

308 **_Fluorescein diacetate (FDA) hydrolysis assay_**

309 The FDA hydrolysis assay followed the method of (Adam and Duncan, 2001). Soil samples

310 (1 g wet weight) in sterile McCartney bottles were amended with 7.5 ml of warmed (26 $^\circ$C)

311 sterile potassium phosphate buffer (60 mM, pH 7.6) and allowed to equilibrate at 26 ºC on a

312 reciprocating shaker for 2 minutes. The assay was initiated by addition of 0.1 ml FDA

313 (Sigma-Aldrich) substrate solution (1000 µg/ml in acetone) to each tube and tubes were

314 incubated (26 ºC) with shaking for 30 minutes after which time the assay was stopped by

315 addition of 7.5 ml of chloroform:methanol (2:1). Tubes were vortex mixed (10 s) and then

316 centrifuged at low speed (~300 g, 2 mins) to clarify the phases. The upper phase (2 ml) was

317 further centrifuged (13,000 x g, 5 mins) to remove suspended fines prior to determination of

318 absorbance at 490 nm (Cecil CE292 Spectrophotometer). Absorbance readings were

319 compared to a calibration curve for fluorescein disodium salt (0-5 µg ml^{-1} in potassium

320 phosphate buffer, 60 mM, pH 7.6). To correct for extraction of soil compounds absorbing at

321 490 nm, blank samples amended with 0.1 ml of acetone instead of FDA solution were

322 included. To check for abiotic hydrolysis of FDA, the above assay was also conducted for

323 autoclaved soil samples (15 minutes at 15 psi) but negligible abiotic hydrolysis was

324 recorded.

326 Supporting Table S1. 3 way ANOVA table for 200 mg L^{-1} adsorption experiment.

Source of variation	df	SS	MS	F	P-value
Ochre	3	7.24×10^{10}	2.41×10^{10}	7152.985	< 0.001
Time	4	4.33×10^{8}	1.08×10^{8}	32.102	< 0.001
pH	1	2.03×10^{9}	2.03×10^{9}	602.118	< 0.001
Ochre x time	12	2.11×10^{8}	1.76×10^{7}	5.214	< 0.001
Ochre x pH	3	2.64×10^{8}	8.81×10^{7}	26.107	< 0.001
Time x pH	4	8.95×10^{7}	2.24×10^{7}	6.630	< 0.001
Ochre x time x pH	12	1.06×10^{8}	8.82×10^{6}	2.614	0.005
Residual	80	2.70×10^{8}	3.37×10^{6}		
Total	119	7.58×10^{10}	6.37×10^{8}		

327

328

329   Supporting Table S2. 3 way ANOVA table for 500 mg L^{-1} adsorption experiment.

Source of variation	df	SS	MS	F	P-value
Ochre	3	1.37×10^{10}	4.56×10^{9}	347.362	< 0.001
Time	4	2.04×10^{9}	5.10×10^{8}	38.867	< 0.001
pH	1	5.63×10^{9}	5.63×10^{9}	428.674	< 0.001
Ochre x time	12	3.75×10^{9}	3.13×10^{8}	23.838	< 0.001
Ochre x pH	3	2.55×10^{9}	8.52×10^{8}	64.893	< 0.001
Time x pH	4	6.47×10^{8}	1.62×10^{8}	12.331	< 0.001
Ochre x time x pH	12	3.11×10^{9}	2.59×10^{8}	19.753	< 0.001
Residual	80	1.05×10^{9}	1.31×10^{7}		
Total	119	3.25×10^{10}	2.73×10^{8}		

330

331

332   Supporting Table S3. 3 way ANOVA for 24 hour data from the As – ochre adsorption
333   experiment.

Source of variation	df	SS	MS	F	P-value
As concentration	1	1.35×10^8	1.35×10^8	13.443	< 0.001
Ochre	3	1.54×10^{10}	5.12×10^9	510.746	< 0.001
pH	1	2.37×10^9	2.37×10^9	235.838	< 0.001
As concentration x ochre	3	2.57×10^9	8.55×10^8	85.27	< 0.001
As concentration x pH	1	1.75×10^8	1.75×10^8	17.483	< 0.001
Ochre x pH	3	2.73×10^8	9.11×10^7	9.078	< 0.001
As concentration x ochre x pH	3	2.59×10^8	8.63×10^7	8.606	< 0.001
Residual	32	3.21×10^8	1.00×10^7		
Total	47	2.15×10^{10}	4.57×10^8		

334

335

336 Supporting Table S4. 2 way ANOVA for batch experiment study using DGC soil.

Source of variation	df	SS	MS	F	P-value
Ochre type	3	1063.729	354.576	33.740	< 0.001
Ochre mass	4	21993.122	5498.280	523.190	< 0.001
Ochre type x ochre mass	12	1653.643	137.804	13.113	< 0.001
Residual	40	420.366	10.509		
Total	59	25130.860	425.947		

337

338 Supporting Table S5. pH of DGC batch experiment suspensions. Mean $\pm$ standard deviation,
339 n = 3.

Ochre	Mass / g	pH	
-	-	2.46	0.04
BH	0.05	3.02	0.08
BH	0.1	3.44	0.11
BH	0.2	3.65	0.23
BH	0.5	4.16	0.09
OM	0.05	4.16	0.09
OM	0.1	4.08	0.08
OM	0.2	5.13	0.09
OM	0.5	5.91	0.03
SB	0.05	3.27	0.08
SB	0.1	3.56	0.02
SB	0.2	3.98	0.07
SB	0.5	4.63	0.13
WY	0.05	4.28	0.26
WY	0.1	4.48	0.07
WY	0.2	5.25	0.09
WY	0.5	5.92	0.14

340

341

342 Supporting Table S6. Two way repeated measures ANOVA for 0.01M $CaCl_2$ extractable As
343 from DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	114.752	28.688	843.962	<0.001
Rep(Ochre)	20	0.680	0.0340		
Week	3	110.236	36.745	788.742	<0.001
Ochre x Week	12	38.614	3.218	69.070	<0.001
Residual	60	2.795	0.0466		
Total			99	267.077	2.698

344

345

346 Supporting Table S7a. Two way repeated measures ANOVA for pH of DGC soil with ochre
347 and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	6.391	1.598	574.789	<0.001
Subject(Ochre)	20	0.0556	0.00278		
Week	3	0.743	0.248	162.694	<0.001
Ochre x Week	12	0.553	0.0461	30.293	<0.001
Residual	60	0.0913	0.00152		
Total	99	7.833	0.0791		

348

349 Supporting Table S7b. Two way repeated measures ANOVA for pH of RRT2 soil with ochre
350 and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	8.457	2.114	311.268	<0.001
Subject(Ochre)	20	0.136	0.00679		
Week	3	1.085	0.362	77.878	<0.001
Ochre x Week	12	0.473	0.0394	8.488	<0.001
Residual	60	0.279	0.00464		
Total	99	10.428	0.105		

351

352

353 Supporting Table S8a. Two way repeated measures ANOVA for Eh of DGC soil with ochre
354 and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	12309	3077	3.542	0.03
Subject(Ochre)	4	3685	921		
Week	1	25946	25946	24.126	< 0.01
Ochre x Week	4	15194	3798	3.663	0.027
Residual	16	16590	1036.895		
Total	49	91924.5	1876.01		

355

356 Supporting Table S8b. Two way repeated measures ANOVA for Eh of RRT2 soil with ochre
357 and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	235357	58839	54.105	< 0.001
Subject(Ochre)	4	13752	3438		
Week	1	342626	342626	148.145	< 0.001
Ochre x Week	4	134818	33704	34.877	< 0.001
Residual	16	15462	966		
Total	49	768667	15687		

358
359

360　Supporting Table S9a. Two way repeated measures ANOVA for acid ammonium oxalate
361　extractable Fe in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	23.732	5.933	12.734	<0.001
Subject(Ochre)	20	9.318	0.466		
Week	3	185.719	61.906	86.968	<0.001
Ochre x Week	12	16.240	1.353	1.901	0.052
Residual	60	42.710	0.712		
Total	99	277.718	2.805		

362

363　Supporting Table S9b. Two way repeated measures ANOVA for acid ammonium oxalate
364　extractable Fe in RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	69.066	17.266	580.176	<0.001
Subject(Ochre)	20	0.595	0.0298		
Week	3	20.989	6.996	212.395	<0.001
Ochre x Week	12	11.666	0.972	29.513	<0.001
Residual	60	1.976	0.0329		
Total	99	104.292	1.053		

365

366

367   Supporting Table S10a. Two way repeated measures ANOVA for citrate dithionite
368   extractable Fe in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	39.359	9.840	52.429	<0.001
Subject(Ochre)	20	3.754	0.188		
Week	3	255.968	85.323	281.600	<0.001
Ochre x Week	12	39.693	3.308	10.917	<0.001
Residual	60	18.180	0.303		
Total	99	356.953	3.606		

369

370   Supporting Table S10b. Two way repeated measures ANOVA for citrate dithionite
371   extractable Fe in RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	221.786	55.447	95.180	<0.001
Subject(Ochre)	20	11.651	0.583		
Week	3	42.857	14.286	37.666	<0.001
Ochre x Week	12	8.250	0.687	1.813	0.066
Residual	60	22.756	0.379		
Total	99	307.300	3.104		

372

373

374 Supporting Table S11a Two way repeated measures ANOVA for microbial activity in DGC
375 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	1640.811	410.203	10.438	<0.001
Subject(Ochre)	20	785.987	39.299		
Week	3	11224.440	3741.480	111.141	<0.001
Ochre x Week	12	854.317	71.193	2.115	0.029
Residual	60	2019.850	33.664		
Total	99	16525.404	166.923		

376
377 Supporting Table S11b Two way repeated measures ANOVA for microbial activity in RRT2
378 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	164.970	41.243	1.269	0.315
Subject(Ochre)	20	649.971	32.499		
Week	3	7859.502	2619.834	102.950	< 0.001
Ochre x Week	12	918.275	76.523	3.007	0.002
Residual	60	1526.864	25.448		
Total	99	11119.583	112.319		

379
380

381　Supporting Table S12a Root biomass (mg) for *Lolium perenne* in DGC soil. Values are mean

382　± standard deviation, n = 5

Ochre	Week 3	Week 12	Week 24	Week 52
None	236 ± 43.5 aA	231 ± 21.3 aA	239 ± 50.7 aA	211 ± 44.0 abA
BH	204 ± 34.2 aA	309 ± 77.1 aB	206 ± 30.4 aA	312 ± 65.5 cB
OM	280 ± 61.8 aAB	318 ± 83.4 aA	228 ± 61.5 aB	238 ± 74.8 abcAB
SB	196 ± 42.6 aA	298 ± 31.0 aB	175 ± 66.8 aA	288 ± 55.6 acB
WY	217 ± 11.6 aA	321 ± 42.2 aB	223 ± 13.9 aA	171 ± 47.7 bA

383　Same letters = not significantly different ($p \leq 0.05$, Holm-Sidak method). Lower case letters

384　are for comparisons within specific weeks, capital letters within specific ochre treatments.

385

386　Supporting Table S12b Two way repeated measures ANOVA for root biomass in DGC soil

387　with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	0.0205	0.00513	1.840	0.161
Subject(Ochre)	20	0.0557	0.00279		
Week	3	0.0954	0.0318	11.892	<0.001
Ochre x Week	12	0.107	0.00888	3.320	<0.001
Residual	60	0.160	0.00267		
Total	99	0.439	0.00443		

388
389

390   Supporting Table S13a Root biomass (mg) for *Lolium perenne* in RRT2 soil. Values are
391   mean $\pm$ standard deviation, n = 5

Ochre	Week 3 AB	Week 12 C	Week 24 A	Week 52 BC
None a	246 $\pm$ 117	301 $\pm$ 60.1	274 $\pm$ 64.1	302 $\pm$ 50.3
BH a	246 $\pm$ 25.2	312 $\pm$ 52.3	224 $\pm$ 25.0	302 $\pm$ 13.7
OM a	298 $\pm$ 70.8	350 $\pm$ 72.5	273 $\pm$ 40.9	351 $\pm$ 58.8
SB a	266 $\pm$ 49.7	364 $\pm$ 47.6	208 $\pm$ 10.7	237 $\pm$ 83.2
WY a	266 $\pm$ 83.0	306 $\pm$ 38.5	296 $\pm$ 10.8	367 $\pm$ 23.6

392   Same letters = not significantly different ($p \leq 0.05$, Holm-Sidak method). Lower case letters
393   are for comparisons between treatments taking account of all weeks, capital letters between
394   weeks taking account of all treatments.

395

396   Supplementary Table S13b Two way repeated measures ANOVA for root biomass in RRT2
397   soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	0.0406	0.0101	2.440	0.080
Subject(Ochre)	20	0.0831	0.00416		
Week	3	0.0927	0.0309	5.463	0.002
Ochre x Week	12	0.0646	0.00539	0.952	0.504
Residual	60	0.339	0.00566		
Total	99	0.621	0.00627		

398

399

400 Supplementary Table S14a. Two way repeated measures ANOVA for shoot biomass in DGC
401 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	0.104	0.0261	68.646	<0.001
Subject(Ochre)	20	0.00760	0.000380		
Week	3	0.0789	0.0263	65.619	<0.001
Ochre x Week	12	0.0452	0.00377	9.401	<0.001
Residual	60	0.0240	0.000401		
Total	99	0.260	0.00263		

402

403 Supplementary Table S14b. Two way repeated measures ANOVA for shoot biomass in
404 RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	0.615	0.154	117.064	<0.001
Subject(Ochre)	20	0.0262	0.00131		
Week	3	0.223	0.0742	34.467	<0.001
Ochre x Week	12	0.0888	0.00740	3.437	<0.001
Residual	60	0.129	0.00215		
Total	99	1.081	0.0109		

405

406

407 Supplementary Table S15a. Two way repeated measures ANOVA for shoot As
408 concentration in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	4681477.751	1170369.438	9.271	<0.001
Rep(Ochre)	20	2524764.918	126238.246		
Week	3	5282175.276	1760725.092	12.267	<0.001
Ochre x Week	12	9273522.998	772793.583	5.384	<0.001
Residual	60	8612054.281	143534.238		
Total	99	30373995.225	306808.033		

409

410 Supplementary Table S15b. Two way repeated measures ANOVA for shoot As
411 concentration in RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	120724.599	30181.150	1.822	0.164
Rep(Ochre)	20	331377.515	16568.876		
Week	3	173700.230	57900.077	7.217	<0.001
Ochre x Week	12	95351.017	7945.918	0.990	0.469
Residual	60	481387.017	8023.117		
Total	99	1202540.378	12146.873		

412

413

414 Supplementary Table S16a. Two way repeated measures ANOVA for root As concentration
415 in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	9642079.845	2410519.961	1.807	0.167
Subject(Ochre)	20	26676327.492	1333816.375		
Week	3	212977061.332	70992353.777	42.979	<0.001
Ochre x Week	12	69018419.756	5751534.980	3.482	<0.001
Residual	60	99107737.411	1651795.624		
Total	99	417421625.836	4216380.059		

416

417 Supplementary Table S16b. Two way repeated measures ANOVA for root As concentration
418 in RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	135865.248	33966.312	0.739	0.576
Subject(Ochre)	20	918747.592	45937.380		
Week	3	3185131.596	1061710.532	20.756	<0.001
Ochre x Week	12	708377.885	59031.490	1.154	0.336
Residual	60	3069064.889	51151.081		
Total	99	8017187.211	80981.689		

419

420

421   Supplementary Table S17a. Two way repeated measures ANOVA for stomach extractable
422   As in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	28738.914	7184.728	9.872	0.002
Col 1(Ochre)	10	7277.793	727.779		
Week	1	31558.150	31558.150	40.713	<0.001
Ochre x Week	4	9592.818	2398.204	3.094	0.067
Residual	10	7751.420	775.142		
Total	29	84919.095	2928.245		

423

424   Supplementary Table 17b. Two way repeated measures ANOVA for stomach extractable As
425   in RRT2 soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	245.125	61.281	11.616	<0.001
Col 1(Ochre)	10	52.754	5.275		
Week	1	1459.669	1459.669	499.707	<0.001
Ochre x Week	4	36.869	9.217	3.155	0.064
Residual	10	29.211	2.921		
Total	29	1823.627	62.884		

426

427   Supplementary Table 17c. Two way repeated measures ANOVA for intestine extractable As
428   in DGC soil with ochre and week of incubation as factors.

Source of Variation	DF	SS	MS	F	P
Ochre	4	164467.850	41116.963	2.545	0.105
Subject(Ochre)	10	161562.078	16156.208		
Week	1	316454.588	316454.588	23.567	<0.001
Ochre x Week	4	66255.039	16563.760	1.234	0.357
Residual	10	134276.232	13427.623		
Total	29	843015.788	29069.510		

429