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Composition, biomass and activity of microflora, and leaf yields and foliar elemental concentrations of lettuce, after in situ stabilization of an arsenic-contaminated soil

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ABSTRACT

Beringite (B) and zerovalent iron grit (Z), singly and in combination (BZ), were added to a loamy sand soil contaminated by trace elements (Reppel, Belgium), mainly by arsenic (As), to reduce As labile fractions and phytoavailability. An uncontaminated sandy soil was studied for comparison. Soils were placed in large lysimeters cultivated with maize and vegetables for 6 years. pH, organic C and total N content increased in amended soils. The Z and BZ treatments reduced the $Ca(NO_3)_2$ extractable soil As and As uptake by lettuce. The BZ lettuces had also the lowest foliar Pb, Cd, Zn, and Mn concentrations. All amendments had positive effects on the soil microbial biomass and reduced the qCO_2. Glucose mineralization was increased in Z and BZ amended soils. Acid phosphomonoesterase activity was higher in the untreated soil than in the other soils; the alkaline phosphomonoesterase, phosphodiesterase and protease activities were increased by Z and BZ treatments, whereas B amendment had less positive effects. Genetic fingerprinting using Denaturing Gradient Gel Electrophoresis (DGGE) revealed shifts in the composition of eubacterial and fungal communities of the amended soils. Microbial species richness decreased rather than increased in the treated soils, regardless of reduced trace element availability and increased soil microbial biomass and activity.

1. Introduction

Trace amounts of arsenic (As) are ubiquitous in the pedosphere being inherited from soil parent materials, but As can accumulate in soils due to inputs through human activities, e.g. ore mining and smelting, combustion of fossil fuels, cement manufacture, wood preservation, and use of arsenical pesticides (Nriagu and Pacyna, 1988; Adriano, 2001; Cappuyns et al., 2002). Under aerobic conditions in well-drained soils, arsenate (+V) species dominate (mainly $H_2AsO_4^-$ and $HAsO_4^{2-}$) at a range of soil pH values from 5 to 8, in equilibrium with other As forms due to reaction with soil minerals such as Fe, Mn (hydr)oxides (Langner et al., 2001; Tournassat et al., 2002) and organic compounds (Smith et al., 1999). Arsenic uptake by plants depends on the chemical speciation, exposure pathway, and plant species (Adriano, 2001; Padmavathiamma and Li, 2007). Arsenate uptake and toxicity/tolerance have been characterised in plants (Hartley-Whitaker et al., 2001), with arsenate acting as a phosphate analogue with regard to membrane transporters. Arsenic is also a potent microbial toxicant, affecting several soil microbial species and functions (Hall, 2002; Lorenz et al., 2006).

The mobility of soil As is mainly controlled by adsorption/desorption processes and co-precipitation (Cappuyns et al., 2002; Kumpiene et al., 2008). Reduction of As leaching and impact on plants and soil organisms can be achieved by soil amendment with minerals, e.g. Fe compounds, Al and Mn oxides, and clays, making excessive As less soluble by either sorption or precipitation reactions, while the influence of organic compounds and alkaline materials on As mobility presents controversial results (Mench et al., 2000, 2006a; Adriano et al., 2004; Brown et al., 2005; Kumpiene et al., 2006, 2008). It is widely accepted that microbial activity and microbial species richness are affected by mobile and bioavailable As and other trace element fractions (Lorenz et al., 2006; Mench et al., 2006b; Renella et al., 2008). Because of As complex chemistry, remediation of As-contaminated soils may require combinations of amendments capable of immobilizing As on a long-term scale (Mench et al., 2007). While deletereous effects of soil As pollution on microbial communities and soil functionality have been reported (Edvantoro et al., 2003; Fernandez et al., 2005), potential effects of reducing the labile pool of As for biological action in soil have been seldom studied. Lombi et al. (2002, 2004) incorporated various iron oxy-hydroxides in As-contaminated

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soils. This reduced the labile As pool and had positive effects on soil microbial biomass and activity, and the growth of lettuce and ryegrass. In a short pot experiment, several hydrolase activities were recovered following aided phytostabilization of a chromated copper arsenate (CCA)-contaminated soil (Kumpiene et al., 2006). However, long-term remediation experiments are required, and the effectiveness of an ecological restoration strategy may depend on the co-occurrence and concentrations of contaminants and soil characteristics. Therefore, this work aimed to assess changes in the composition of soil eubacterial and fungal communities and in the soil functionality, in relation to changes in labile As pool and phytoavailability in As-contaminated soils amended with the coal fly ash beringite and zerovalent iron grit, singly and in combination under small scale semi-field conditions. The effect was studied 6 years after beringite and/or zerovalent iron grit phytostabilization.

2. Materials and methods

2.1. Soils and treatments

The contaminated soil was a loamy sand soil, Cambic Podzol with 66.2% sand, 28.8% loam and 5.0% clay. The soil (3000 kg) was collected in the surface layer (0–0.2 m soil depth) of an agricultural field annually cropped with maize adjacent to the derelict As smelter located in Reppel (Bocholt, Belgium), mainly contaminated by As (169 mg kg^{-1}), a concentration which was 14 times higher than pedogeochemical background values for As elements in Belgian soils (De Temmerman et al., 1984; Tack et al., 1997). Also total soil Cu (26.9 mg kg^{-1}), Ni (9.9 mg kg^{-1}) and Zn (70.1 mg kg^{-1}) indicated anthropogenic inputs but to a much lesser extent than As. The reference soil was an uncontaminated maize-cropped duric/humic Podzol with 94.2% sand, 2.5% loam and 3.3% clay, sampled (0–0.2 m) at the Pierroton INRA Farm (Cestas, France). The main chemical properties of the soils are given in Table 1. Soils were sieved (<5 mm) and homogenised with a cement mixer. The lysimeters used were 0.5 m × 0.5 m × 0.5 m vats provided with a 0.2 m compensation zone resulting in a 1 m^2 soil surface. The experimental set up comprised a reference soil (R; characterized by similar physico-chemical characteristics as the contaminated soil), an untreated soil (Unt; non remediated contaminated soil), and three in situ stabilization treatments (amendments with B, beringite; Z, zerovalent iron grit; BZ, beringite plus iron grit). Lysimeters were prepared in May 1997. In previous experiments BZ treatment had proven most effective and therefore four replicate lysimeters were prepared for this treatment while two replicate lysimeters were set up for the R, Unt, B and Z soils. The lysimeter numbers are reported in parentheses in order to relate the results of the present study to those of future studies from this long-term experiment. The contaminated soils were treated once (B, Z, BZ), prior to crop planting. Subsamples (275 kg air dried soil) were mixed using a cement mixer with 5% B, 1% zerovalent iron grit (Z), or 5% beringite plus 1% iron grit (BZ). Iron grit is an industrial material used for shaping metal surfaces that contains mainly zerovalent iron (97% α-Fe) and native impurities such as Mn (Table 1; Mench et al., 2006a). Beringite is a mixture of modified aluminosilicates originating from the burning of coal refuse (Vangronsveld et al., 1999). Trace elements were present in the amendments, particularly Cu and Ni in Z, and Zn and Pb in B (Table 1). Untreated Reppel soil (Unt) and reference soil (R) were kept in identical lysimeters under the same conditions. All lysimeters were randomly placed in a greenhouse to prevent atmospheric fallout from the surrounding urban area. The greenhouse was maintained at 10 °C during winter time at night. Otherwise, natural climatic conditions prevailed, with average temperature between 10 and 27 °C during the year, and 50–65% humidity maintained during summer by a fogging system. The lysimeters were cultivated with maize, radish, cabbage and lettuce (Mench et al., 2006a). Soil subsamples for biochemical and molecular analyses were collected in year 5, sieved (<2 mm), moistened at 50% of saturation and incubated at 25 °C for 7 days prior to analyses. The soil pH was measured and 0.01 M $Ca(NO_3)_2$ extractable As and metals were determined as previously described (Mench et al., 2003).

2.2. Plants

In year 6 after soil treatment, lettuces (cv. Divina) were raised in compost modules, transplanted into each lysimeter (3 plants/lysimeter), and harvested at market size after 2 months. The soils in the lysimeters were maintained at 50% water holding capacity (WHC) with deionised water. The seven oldest (basal) leaves on each lettuce were harvested with ceramic scissors and fresh weights determined, the remainder of the shoots being used to test snail herbivory (Mench et al., 2006a). Plant samples were washed in distilled water (5 L) containing 0.25 mL Triton X, rinsed twice in distilled water, oven dried at 50 °C, weighted for dry weight (DW) yield and milled in a planetary grinder coated with zirconium (Retsch P 400). Weighed aliquots (1 g DW) were wet digested with 5 mL supra-pure 14 M HNO_3 and 10 mL 30% (v/v) H_2O_2 not stabilized by phosphates in PFA (perfluoroalkoxy copolymer) sealed tubes using a microwave digestion system (CEM MarsX, 1200 °C, 20 min). Certified reference material (rye grass BCR 281) was included in each plant series. Mineral composition in digests was determined by Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES; Varian Liberty 200) and Graphite Furnace Atomic Absorption Spectrometry (GFAAS; Varian A400), depending on element concentration. All elements were recovered within the ranges of the standard certified values. The standard deviation of replicates was <5%.

2.3. Soil biochemical analysis

Soil respiration was measured by placing 100 g (DW) of soil in 1 L air-tight glass jars provided with 3-way valves and incubated at

Table 1
Main chemical properties of the reference (R), untreated (Unt) and treated soils (beringite, B; zerovalent iron grit, Z; B and Z, BZ). Numbers in brackets indicate the reference numbers of the replicate lysimeters.

	Soils											
	R (1)	R (18)	Unt (3)	Unt (2)	B (4)	B (15)	Z (5)	Z (14)	BZ (6)	BZ (7)	BZ (13)	BZ (25)
$pH_{(H_2O)}$	5.87	5.91	4.91	4.91	6.2	6.0	5.21	5.21	6.2	6.4	6.1	6.5
CEC[a] ($cmol^+ kg^{-1}$)	5.65	5.88	3.98	3.89	5.78	6.09	5.94	5.81	6.15	6.27	6.21	6.18
TOC[b] (%)	5.2	5.3	6.3	6.6	7.6	7.7	7.1	7.0	6.8	7.1	6.9	7.2
N_{tot}[c] (%)	0.72	0.68	0.10	0.12	0.79	0.81	0.72	0.72	0.63	0.67	0.74	0.69

[a] CEC is the cation exchange capacity determined according to Ciesielski and Sterckeman (1997).
[b] TOC is the soil total organic C determined according to Walkley and Black (1934).
[c] N_{tot} is the total N content determined by solid chromatography (PerkinElmer NA 1500).

25 °C in the dark for 7 days. Empty jars served as blanks accounting for the CO_2-C background concentration. Evolution of CO_2-C was measured by sampling the head-space and injecting the gas samples into a gas-chromatograph (Hewlett-Packard 6890) equipped with a packed column (Porapack Q) and a thermal conductivity detector (Blackmer and Bremner, 1977). After respiration measurement, soil microbial biomass was determined by measuring the ATP content of soils according to Ciardi and Nannipieri (1990).

The acid and alkaline phosphomonoesterase activities (Tabatabai and Bremner, 1969), the phosphodiesterase activity (Browman and Tabatabai, 1978), and the β-glucosidase activity (Tabatabai, 1982) were determined. Urease activity was measured using urea as substrate in phosphate buffer at pH 7.1 (Nannipieri et al., 1974), and the protease activity by the hydrolysis of N-benzoylargininamide (Ladd and Butler, 1972). All enzyme assays were carried out at 37 °C for 1 h, with centrifugation of soil slurries at 6000 × *g* at 4 °C. Concentrations of p-NP produced in the assays of phosphatase and β-glucosidase activities were calculated from a p-NP calibration curve after subtracting the absorbance of the controls at 400 nm wavelength. The NH_4^+-N produced by urease and N-benzoyl arginine amide (N-BAA) hydrolysing activities were determined using a flow injection analyzer (FIAStar, Tecator, S).

2.4. Molecular analysis

Bacterial and fungal communities were determined by Small SubUnit ribosomal RNA-Denaturing Gradient Gel Electrophoresis (SSU rRNA-DGGE). Total soil DNA was extracted from 0.5 g soil using the FastDNA SPIN Kit method (BIO 101, Qbiogen, Inc., USA). Double stranded DNA (*ds*DNA) was quantified fluorometrically (Hoefer DyNA Quant® 200, Hoefer Pharmacia Biotech, San Francisco, CA, USA) using bisbenzimide as fluorochrome (Hoechst H 33258). The quality of the extracted DNA was checked by electrophoresis on agarose gel (1 × Tris Acetate EDTA buffer; 1%, w/v; 60 V; 60 min). The composition of the soil eubacterial community was analysed by Denaturing Gradient Gel Electrophoresis (DGGE) fingerprinting. Therefore, 80 ng target DNA was amplified using the GC-clamped primer set GC968f/UNI1401r, specific for eubacterial 16S rRNA genes (Nübel et al., 1996), and 100 ng of the resulting PCR products were then analysed by DGGE on a 6% polyacrylamide gel with a urea–formamide denaturing gradient of 46–56% (100% denaturant contains 7 M urea and 40% formamide) at constant temperature (60 °C) and voltage (75 V) for 16 h using the DCode system (Biorad, Hercules, CA).

The composition of the soil fungal community was assessed by DGGE fingerprinting of 18S rRNA genes amplified by a nested PCR. The first round PCR was performed on 40 ng total soil DNA using the primer set NS1*f*/NS8*r* (Kowalchuk, 1999). The 1700 base pair PCR products (2 μl) were then amplified with the GC-clamped primer set EF4*f*/NS3*r*GC (Brodie et al., 2003). The resulting 500 bp amplicons were run on a 10% polyacrylamide gel (acrylamide–bisacrylamide 37.5:1, Biorad; 20 cm × 20 cm; 1 mm) with a urea–formamide denaturing gradient of 30–45%, at constant temperature (60 °C) and voltage (85 V) for 17 h using the DCode system (Biorad). The fingerprints of both the eubacterial and fungal DGGE patterns were visually scored after gel staining with SybrGreen I (FMC Bio Products, Rockland, ME, USA) from digital images (Polaroid Gel Cam, Elect; Polaroid Type 667 Film ISO 3000) by UV light gel transillumination (254/497 nm).

2.5. Data analysis

Lettuce leaf yields, extractable element concentrations, microbial biomass and respiration, glucose mineralization, and enzyme activities are reported as means of triplicate analyses for each replicate lysimeter. The data for microbial biomass and respiration, glucose mineralization, and enzyme activities were analysed by one-way ANOVA and the significance of the differences between the mean values was calculated by the Newman–Keuls test at *P*-level <5%. Lettuce shoot elemental analysis was conducted on two of the three plants from each lysimeter. Results of extractable element concentrations, lettuce leaf yields and lettuce elemental analysis are shown as means ± standard deviation. The metabolic quotient (qCO_2) was expressed as mg CO_2-C mmol ATP^{-1} d^{-1}. The similarity of microbial communities was assessed by calculating the similarity index of DGGE bands as proposed by Sørensen (1948) for plant communities: $S1,2 = 2a/(2a + b + c)$, where *a* is the number of DGGE bands (or plants) shared by both samples, *b* is the number of bands (or plants) in one sample, and *c* is the number of bands (or plants) in the other sample being compared. Samples were compared pairwise with each other and for DGGE analyses two bands were considered in common if they migrated at the same distance. The Sørensen index values approaching 0.5 indicate increasing similarity in the composition of microbial and plant communities.

3. Results

3.1. Soil chemical properties and extractable concentrations of trace elements

The B, Z and BZ amended soils showed higher pH values and total organic C and total N content as compared to untreated (Unt) and reference (R) soil (Table 1).

Extractable soil As was effectively reduced in Z and BZ amended soils as compared to the Unt soil, whereas its decrease was lower in B amended soils (Table 2). Extractable soil Cd, Mn, As and Ni were higher in Unt soil than in the R soil (Table 2). Extractable soil As was effectively reduced in Z and BZ amended soils, whereas it decreased less in B amended soils. Compared to Unt soil, the increase in soil pH in all B amended soils was associated with decreased extractable soil Mn, Cd, Ni, and Zn. Extractable soil Cu followed a similar trend. In Z amended soils, extractable soil Zn was slightly decreased while extractable soil Ni increased by a factor of two (Table 2).

3.2. Lettuce yields and foliar elemental concentrations

Shoots of plants from untreated soils were somewhat wilted, indicating higher transpiration or reduced water uptake. Their leaves showed interveinal chlorosis. Lettuces from all other treatments did not show these symptoms. Leaf yields of B and BZ plants were equivalent to that of the reference plants (Table 3). Compared to Unt plants, leaf yield was 2.7 and 2.4 times higher for B and BZ plants, whereas it was similar for Z plants.

The foliar As concentration was reduced by soil amendments in the following order: BZ > Z > B (Table 3). Highest decreases in foliar Cd, Zn, and Pb concentrations occurred in BZ plants. Foliar Mn and Fe concentrations were reduced in B and BZ amended soils. Foliar Ca, Mn, and Zn concentrations in BZ lettuce were either within or close to the expected ranges for mature, healthy greenhouse-grown lettuce (Mills and Jones, 1996), whereas foliar Fe concentrations were lower (Table 3).

3.3. Soil ATP and DNA content, respiration, qCO_2 values, and glucose mineralization

Basal respiration was not significantly influenced by the treatments, whereas the ATP contents were significantly higher in Z and BZ amended soils than in the Unt soil and the DNA yields followed the same trend (Table 4). Metabolic quotient values of the Unt soils were significantly higher than those of the BZ, B, Z

Table 2
Concentrations of 0.01 M $Ca(NO_3)_2$ extractable trace elements (μmol kg^{-1} soil DW) in reference, untreated and treated soils (means ± SD, $n = 3$).

Element	Soils											
	R (1)	R (18)	Unt (2)	Unt (3)	B (5)	B (14)	Z (4)	Z (15)	BZ (6)	BZ (7)	BZ (13)	BZ (25)
As	0.20 ± 0.18	0.12 ± 0.04	12.3 ± 0.6	11.4 ± 0.42	7.58 ± 0.54	8.14 ± 0.56	1.53 ± 0.22	0.63 ± 0.12	2.00 ± 0.52	2.11 ± 0.82	0.75 ± 0.41	0.88 ± 0.73
Cd	0.019 ± 0.005	0.009 ± 0.00	0.44 ± 0.01	0.63 ± 0.01	0.070 ± 0.004	0.092 ± 0.006	0.45 ± 0.02	0.40 ± 0.04	0.059 ± 0.006	0.032 ± 0.011	0.055 ± 0.013	0.038 ± 0.003
Cu	<dl	<dl	0.37 ± 0.15	0.94 ± 0.08	<dl	<dl	0.072 ± 0.016	0.33 ± 0.19	<dl	<dl	<dl	<dl
Mn	4.3 ± 0.03	3.85 ± 0.03	349 ± 25	263 ± 23	16.8 ± 0.4	27.2 ± 0.3	356 ± 57	330 ± 6.3	40.9 ± 2.0	26.5 ± 2.9	28.7 ± 1.7	31.7 ± 0.6
Ni	0.05 ± 0.02	0.02 ± 0.00	3.49 ± 0.26	5.98 ± 0.20	0.84 ± 0.10	0.53 ± 0.02	12.2 ± 0.3	12.0 ± 1.1	1.13 ± 0.10	0.87 ± 0.17	1.02 ± 0.13	0.63 ± 0.09
Pb	0.038	0.006	0.02 ± 0.01	0.005 ± 0	<dl	<dl	<dl	0.048 ± 0.026	0.049 ± 0	<dl	0.001	<dl
Zn	14.85 ± 0.16	3.15 ± 0.07	61.7 ± 1.8	104.5 ± 1.3	5.52 ± 0.08	5.29 ± 0.09	46.5 ± 0.9	46.4 ± 5.5	3.52 ± 0.25	1.30 ± 0.05	1.98 ± 0.21	1.17 ± 0.03

<dl: concentrations below detection limits.

amended and R soils (Table 4). Glucose mineralization after 48 h was significantly lower in the Unt and B treated soils than in R soil, Z and BZ treated soils (Table 4).

3.4. Soil hydrolase activity

Alkaline phosphomonoesterase was significantly increased by all treatments as compared to Unt soils, phosphodiesterase and protease activities were significantly increased in Z and BZ-amended soils, whereas soil amendment with singly B did not increase such activities (Figs. 1 and 2). The β-galactosidase and urease activities did not differ significantly between Unt and amended soils (Figs. 1 and 2). The acid phosphomonoesterase activity was significantly lower in BZ soils than in Unt, B and Z soils (Fig. 1).

3.5. Microbial community structure

Both the 16S and 18S rRNA-DGGE generated characteristic community fingerprints of dominant eubacterial (Fig. 3a) and fungal (Fig. 3b) populations in the R soil, and untreated and treated Reppel soils, with high reproducibility between the replicates. The 16S rRNA-DGGE fingerprint showed the most complex patterns for the Unt and BZ soils (plots 6 and 7), whereas R soil, BZ (plots 13 and 25), B and Z-amended soils showed less species richness (Fig. 3a). The 18S rRNA-DGGE fingerprint showed the most complex fungal community in the R soil (Fig. 3b). A reduced complexity occurred in the untreated and amended Reppel soils, except in the B-amended soil, which was characterized by a higher fungal species richness than in the Unt soil.

Values of the Sørensen similarity index for 16S rRNA-DGGE fingerprints showed similarities between the R, Unt and treated soils ($S = 0.250–0.300$). The lowest similarity was found between Unt and BZ (plots 6–7) profiles ($S = 0.170$) whereas the highest similarity occurred between BZ amended soils, plots 6–7 and plots 13–25 ($S = 0.429$).

Sørensen similarity indices for 18S rRNA-DGGE fingerprints showed generally low similarity between R soil, Unt and treated soils. The highest similarity values were found between the R soil and BZ (plots 13–25) ($S = 0.250$), B and BZ soils (plots 13–25) ($S = 0.286$) and Unt and BZ soils (plots 13–25) ($S = 0.308$).

4. Discussion

Soil amendment with B, singly or combined with Z, induced soil alkalinization (Table 1) due to the relatively high MgO and CaO contents in beringite (Vangronsveld et al., 1999). In BZ amended soils the increased TOC and total N content could be due to the higher plant rhizodeposition and possibly better protection by sorption with added mineral phases, as soils were not fertilized. High extractability of As in the Unt soil was likely due to its sandy texture (Pouschat and Zagury, 2006). The greatest decrease in extractable As in BZ and Z amended soils (Table 2) was likely due to its sorption via inner sphere complexation on the reactive surface of newly formed Fe and Mn oxides (Tournassat et al., 2002; Mench et al., 2006a). Basta and Casteel (2002) reported that soil extractable Fe oxides and pH were important soil properties controlling As adsorption. Beringite addition was less effective at reducing extractable As (Table 2). Arsenate sorption on illites, poorly crystalline Ca–Al-hydroxysulphate phases, and original Fe–Mn oxides from the soil may contribute to reduce extractable As in B amended soils. The increased pH in all B-amended soils reduced extractable Mn, Cd, and Zn through precipitation and sorption. Increased extractable Ni in Z amended soils likely originated from high Ni content in iron grit (Kumpiene et al., 2006), but such an increase was suppressed when B was mixed with Z, due to an

Table 3
Leaf yields and foliar elemental concentrations of lettuce. Values for leaf yields are mean ± standard deviation per treatment; $n = 6$ for R, Unt, B and Z soils, and $n = 12$ for BZ soils. Values for leaf elemental content are mean ± standard deviation of two plants analysed per lysimeter; $n = 4$ for R, Unt, B and Z soils, and $n = 8$ for BZ soils.

Soils	Yield (g FW leaf^{-1})	Elements								
		mg kg^{-1} DW							g kg^{-1} DW	
		As	Cd	Mn	Fe	Zn	Cu	Pb	Ca	P
R	6.9 ± 1.4	0.30 ± 0.29	0.94 ± 0.30	198 ± 90	110 ± 39	300 ± 125	21 ± 19	0.92 ± 0.82	19.3 ± 5.5	5.7 ± 0.4
Unt	3.0 ± 1.7	1.55 ± 0.95	3.20 ± 1.52	204 ± 73	104 ± 9	175 ± 7	29 ± 21	1.15 ± 0.77	13.8 ± 2.0	7.9 ± 2.7
B	8.2 ± 1.6	1.26 ± 0.32	2.05 ± 0.39	91 ± 30	75 ± 0.3	135 ± 15	30 ± 22	0.98 ± 0.79	18.0 ± 0.5	5.0 ± 1.1
Z	2.3 ± 1.6	0.64 ± 0.41	2.46 ± 0.03	105 ± 19	126 ± 49	160 ± 22	32 ± 17	1.35 ± 0.90	14.0 ± 3.4	4.6 ± 2.2
BZ	7.2 ± 2.0	0.33 ± 0.18	1.17 ± 0.24	73 ± 35	81 ± 9	128 ± 14	21 ± 2	0.34 ± 0.08	14.0 ± 1.5	5.3 ± 0.9
Survey[a]		0.02–1.5[b]	0.6–1.6[b]	55–110	168–223	33–196	6–16	0.28–0.71[b]	8–12	–

[a] Ranges for mature healthy lettuce in Mills and Jones (1996).
[b] Ranges for mature healthy lettuce in Mench and Baize (2004).

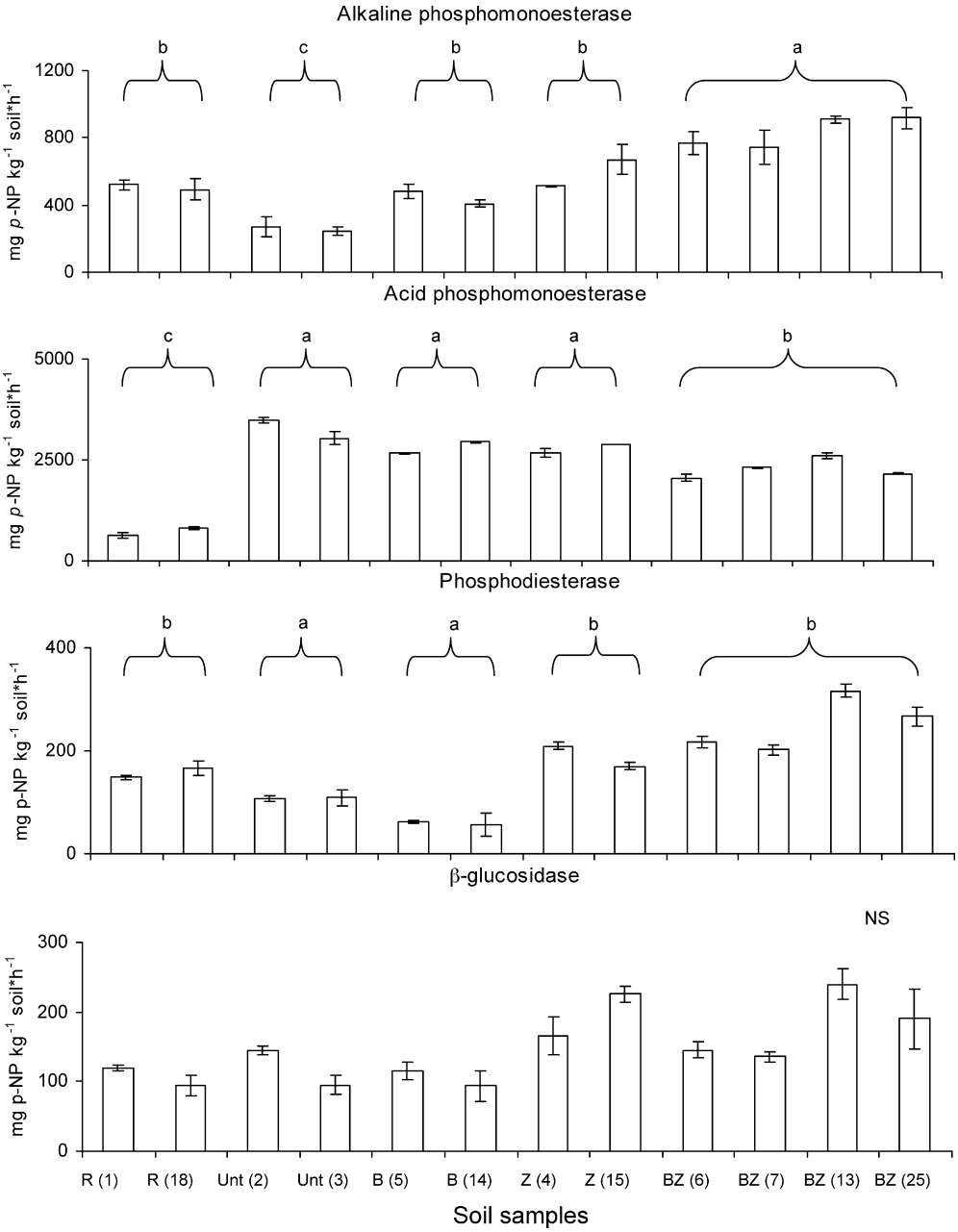

Fig. 1. Phosphatase and β-glucosidase activities of the reference (R), untreated (Unt) and treated soils (B, Z, BZ). The numbers in brackets indicate the independent plot replicates. Different superscripts upon the branches indicate significant differences between treatments ($P < 0.05$). The error bars represent the standard deviation of the means ($n = 3$) of each of the replicate lysimeters.

Table 4
Basal respiration, ATP and DNA content, qCO_2 and glucose mineralization values. Values in bracket represent the percentage of glucose net C mineralized after 48 h. LSD values are the minimum significant differences among means ($P < 0.05$) in rows.

Time (h)	Soils												
	R (1)	R (18)	Unt (3)	Unt (2)	B (4)	B (15)	Z (5)	Z (14)	BZ (6)	BZ (7)	BZ (13)	BZ (25)	LSD
Basal respiration (mg CO_2-C kg^{-1} d^{-1})													
	7.5	6.8	6.3	6.0	5.8	5.0	6.7	7.0	6.9	6.6	7.1	6.7	2.7
DNA yields (ng kg^{-1} soil)													
	860.5		444.6		425.5		706.8				658.6		245
ATP content (ng kg^{-1})													
	428.7	400.9	134.8	148.2	199.4	195.8	393.0	381.3	328.7	292.0	274.7	280.1	112
qCO_2 (mg CO_2-C mmol ATP^{-1} d^{-1})													
	0.018	0.017	0.046	0.040	0.028	0.026	0.017	0.018	0.021	0.028	0.027	0.024	0.011
Glucose mineralization (mg CO_2-C kg^{-1})													
2	75	98	36	33	44	50	76	85	48	67	74	87	16
4	270	325	60	63	155	157	282	323	148	180	173	257	45
24	899	1103	442	410	643	614	842	915	720	810	895	856	144
48	1061 (53)	1106 (56)	679 (34)	621 (31)	741 (37)	653 (33)	1003 (57)	1022 (51)	907 (45)	929 (46)	1150 (57)	1093 (55)	221

alkainization effect on Ni solubility (Tables 1 and 2). Even if chemical extractions may not reflect the labile pool of trace elements in natural situations, the $Ca(NO_3)_2^-$ extractable pool can be used as a rapid test for evaluating changes in labile elements after amendment incorporation into soil, their advantage being a limited effect on both the operative pH at the exchange sites and complexation (Lebourg et al., 1996).

Lettuce leaf As concentration was in agreement with extractable As. Correlations between soluble and exchangeable As fractions and lettuce uptake have been reported (Cao and Ma, 2004). Higher extractable As in B amended soils and foliar As concentration in B lettuces confirmed the lower affinity of B for As in this soil. Foliar As concentration in BZ plants was similar to that in plants grown on the reference soil and within the range for healthy lettuce (Mills and Jones, 1996). A slight increase in soil organic matter in BZ amended soils could also have contributed to reducing foliar Pb concentration in lettuce by forming ternary Fe oxides–metal–organic matter complexes (Table 2; Kumpiene et al., 2008). Leaf Cd concentration in Unt, B, and Z lettuces exceeded the EU maximum permitted concentration (2 mg Cd kg^{-1} DW) assuming a water content of 90%, whereas Cd and Pb concentrations in BZ lettuces were below the EU maximum permitted concentrations, and did not affect the Ca and P plant assimilation (Table 3). Foliar Cd, Zn, and As concentrations were correlated with concentrations in soil extracts (i.e. Cd $r^2 = 0.85$; As $r^2 = 0.94$; Zn $r^2 = 0.97$). The Z induced increase in extractable soil Mn could affect lettuce cultivars sensitive to Mn (Winsor and Adams, 1987). In fact, lettuce foliar concentration is an option to asses the labile element pool in these soils.

Soil functionality was significantly increased in the amended soils as compared to the Unt soil, in some cases with values higher than those of the R soil. Increase in microbial biomass, dsDNA yields and glucose mineralization capacity in the Z and BZ treated soils indicated the presence of a larger and more responsive

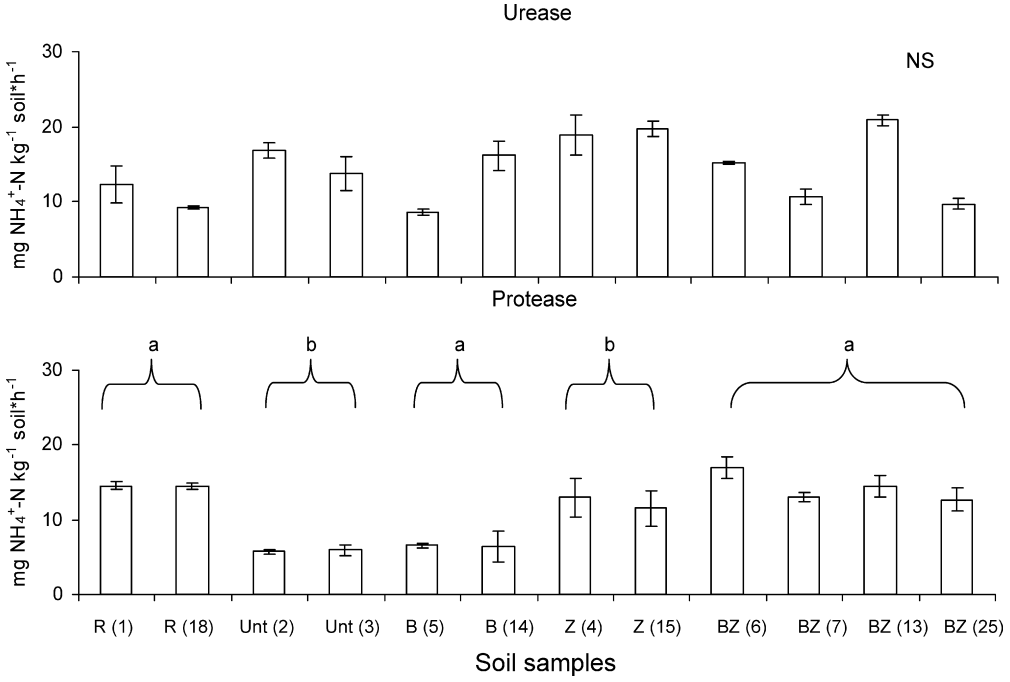

Fig. 2. Urease and protease activities of the reference (R), untreated (Unt) and treated soils (B, Z, BZ). Different superscripts upon the branches indicate significant differences between treatments ($P < 0.05$). The error bars represent the standard deviation of the means ($n = 3$) of each of the replicate lysimeters.

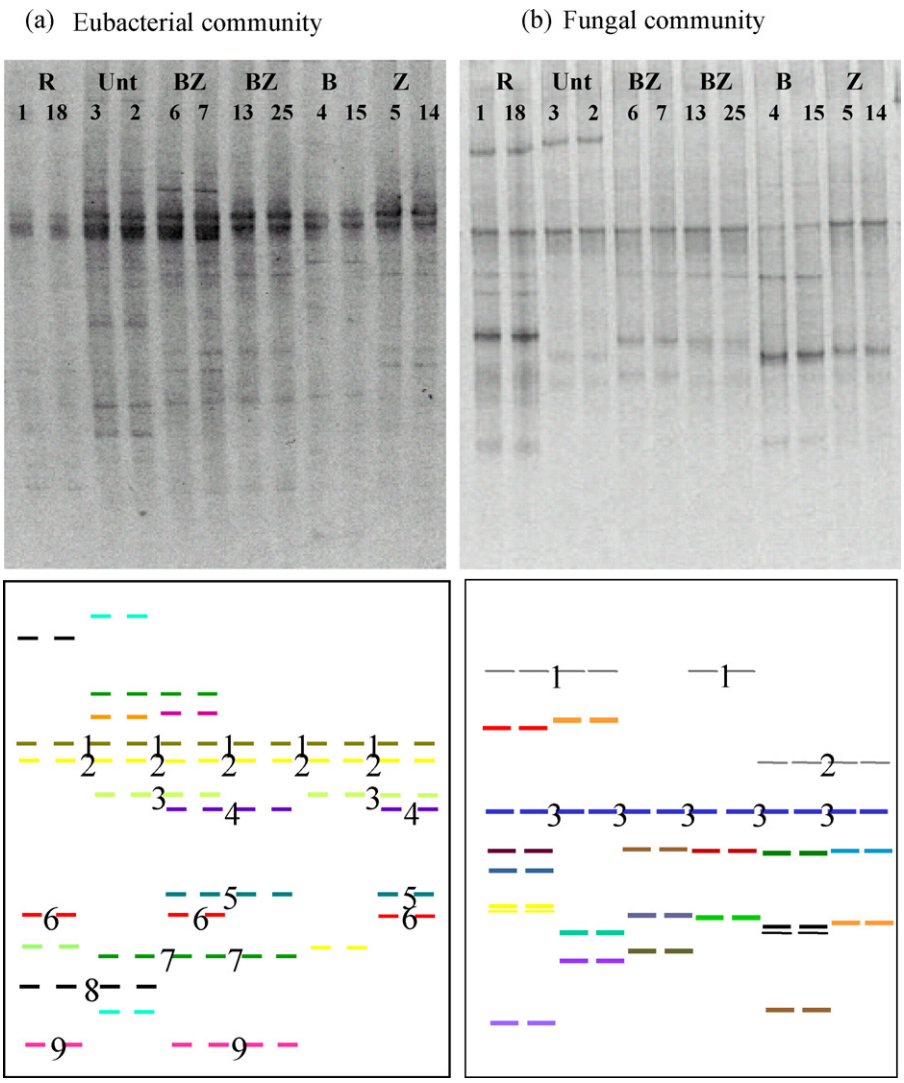

Fig. 3. Eubacterial (a) and fungal (b) community fingerprinting of the reference soil (R), untreated (Unt) and treated soils (BZ, B, Z). DGGE was performed on DNA extracted from independent replicate lysimeters as indicated by the numbers at the top of the gel. Numbers on the bands in the schematic fingerprints indicate predominant microbial populations in common between the analysed soils.

microflora (Table 4). This was likely related to the trace element stabilization in the treated soils as glucose mineralization is delayed in trace element contaminated soils (Palmborg and Nordgren, 1993) and can increase following trace element stabilization in contaminated soils (Chander and Joergensen, 2002). Reduction of qCO_2 values in the treated soils further indicated a reduction of microbial metabolic stress in the amended soils (Anderson and Domsch, 1993). These results were related to the reduced As and metal labile fractions (Table 2). Correlations between concentrations of labile As fractions and microbial As bioavailability have been reported (Turpeinen et al., 2003). Recovery of hydrolase activities was also related to both increased microbial activity and reduced direct enzyme inhibition by excessive trace element labile fractions. Impacts of labile As fractions on soil microbial activity and enzyme activities have been reported (Fernandez et al., 2005; Lorenz et al., 2006). The sensitivity of phosphatase activity to As may be due to structural similarity of phosphate and arsenate (Speir et al., 1999), and our data confirm that amendment of As contaminated soils with Fe oxy-hydroxides rich or alkaline industrial by-products may have positive effects on soil microbial biomass and activity, mainly through the reduction of the labile As pool (Lombi et al., 2002, 2004; Kumpiene et al., 2006; Mench et al., 2006b; Renella et al., 2008). A lesser increase in soil functionality in B amended soils was likely related to the higher extractability of As compared to other amended soils (Table 2) which was confirmed by lettuce As concentration (Table 3). High concentrations of exchangeable forms of several trace elements may have additive or synergistic effects on soil hydrolase activity and microbial biomass (Renella et al., 2003). Alkaline phosphomonoesterase and protease were lower in Z soils than in BZ soils likely due to acid soil pH and relatively higher extractable Cd, Ni, Mn, and Zn concentration (Tables 1 and 2).

Soil treatment with B and Z, singly and combined, did not increase eubacterial and fungal species richness but caused shifts within the community structures of the amended soils (Fig. 3). Untreated and BZ amended soils from lysimeters 6 and 7 (BZ 6–7) showed the same number of eubacterial dominant populations but shared low similarity, while eubacterial species richness was generally reduced by other treatments. Fungal communities showed lower complexity which was also not generally increased by the soil treatments. Overall, DNA-based molecular analyses of microbial communities indicated that a decrease in extractable soil trace elements in the treated soils was not followed by an increase

in microbial species richness. No increase in microbial species richness in phytostabilized soils and mine spoils has been reported by Mench et al. (2006b) and Renella et al. (2008). Variations in the eubacterial community structure in relation to decreased zinc toxicity in a Zn polluted soil were found (Brim et al., 1999). Possibly, the reduction in several extractable trace elements was not sufficient to prevent negative additive effects on the soil microbial community (Ranjard et al., 2006). Moreover, it can not be excluded that soil amendment with B and Z, singly and in combination, influenced bacterial and fungal communities by selecting predominating microbial species. Bouwman et al. (2001) reported that cultivable bacterial and fungal populations of Zn contaminated soils were increased by soil amendment with B and revegetation, whereas Chander and Joergensen (2002) reported that incorporation of a synthetic zeolite into a Pb contaminated soil reduced the soil ergosterol content (a fungal biomass marker). It can not be excluded that trace element-induced microbial selection may require a longer time after treatment for the establishment of fundamental ecological interactions necessary for successful microbial colonization of remediated soils. Dıaz-Raviña and Bååth (1996, 2001) demonstrated that selected tolerant or resistant microbial species disappear slowly when metal induced stress is removed. Moreover, the lack of increase in microbial species richness in this study could be due to the fact that soils were not amended with organic matter, which may increase soil microbial species (Kandeler et al., 2000).

5. Conclusions

Stabilization of As in a contaminated loamy sand soil can be achieved through incorporation of iron grit (Z) and B to a lesser extent, singly and in combination, with BZ being the most effective amendment to decrease soil extractable As and foliar As concentration in lettuce. Reduction of extractable As by sorption on newly formed Fe oxides and of extractable Cd, Zn, and Mn mainly by changes in soil pH, led to increased microbial biomass and soil functionality, and reduced microbial stress to levels similar to those of an uncontaminated soil kept under the same conditions. However, the decrease in soil toxicity was not followed by an increase in microbial species richness. More research is needed to understand whether this lack of recovery could be due to mineral-induced microbial selection or may require longer time after treatment for the establishment of fundamental ecological interactions, and to better understand what are the main mechanisms leading to successful microbial colonization of remediated soils.

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