functioning of ecosystems associated with such Cu-contaminated soils is generally affected, due to soil ecotoxicity and Cu dispersion through natural agencies (Bakshi et al., 2014).

Out of gentle options for remediating Cu-contaminated soils, in situ stabilization is an alternative to physico-chemical methods. It aims at reducing the pollutant linkages by managing the contaminant pathways to protect the biological receptors (Cundy et al., 2015). It relies on incorporation of amendments into the soil to immobilize metal(loid)s in the stable solid phase by sorption, precipitation, complexation, ion exchange or redox process, thereby decreasing their mobility and bioavailability (Kumpiene et al., 2008; Bolan et al., 2014). Amending soils with biochar can (1) increase the cation exchange capacity (CEC), pH, and retention of water and nutrients, (2) improve microbial soil habitats and (3) immobilize contaminants (Zhang et al., 2013). Several studies have assessed the biochar effectiveness for in situ stabilization of Cu-contaminated soils (Beesley and Marmiroli, 2011; Beesley et al., 2011). As suggested by Beesley and Marmiroli (2011), combining amendments with biochar may be more efficient than biochar alone for the remediation and revegetation of contaminated soils. Iron grit mainly consists of zerovalent Fe(0) and Mn and rapidly corrodes into the soil to form newly Fe/Mn oxi(hydro)xides. Such (hydro)xides can sorb metal(loid)s and reduce their (bio)availability (Kumpiene et al., 2011; Tiberg et al., 2016). In a previous pot experiment, a pine bark-derived biochar (1% w/w) decreased Cu^{2+} concentration in the soil pore water (SPW) of a contaminated soil (964 mg Cu kg^{-1}) from a wood preservation site (Oustriere et al., 2016a). Adding this biochar with iron grit (1% w/w) decreased both total Cu and Cu^{2+} concentrations in the SPW. However biochar alone and in combination did not promote the growth of dwarf bean (*Phaseolus vulgaris* L.). Moreover this study was limited to a 3-month reaction period followed by a 2-week plant testing. Potential changes in the phytotoxicity of biochar-amended soils over time must be considered. Long-term efficiency of soil amendments is pivotal to sustain metal(loid) immobilization in contaminated soils and to avoid their leaching out of the root zone.

Few long-term studies reported an increased immobilization of trace elements (TE) over time in biochar-amended soils (Cd, Pb: Bian et al., 2014; Ni, Zn: Shen et al., 2016). Such long-term effects on TE immobilization however depend on biochar and soil types. After 3 years, hardwood-derived biochar gradually reduced the ($CaCl_2$)-extractable Cd and Cu concentrations and uptake by rice, while these values did not change in the soil amended by corn-straw-derived biochar (Li et al., 2016). Eucalyptus wood-derived-biochar aged for 12 months into two (Cd/As)-spiked soils increased Cd and As sorption in the Inceptisol whereas it decreased As sorption in the Oxisol (Nagodavithane et al., 2014). The sorption capacity of aged biochar may depend on (1) metal(loid)s, (2) SPW pH, and (3) soil properties such as organic matter (OM) content and oxidation process through both abiotic and biotic mechanisms (Nagodavithane et al., 2014).

Among energy crops, poplar (*Populus* sp.,) and Giant reed (*Arundo donax* L.) used in short rotation coppices (SRC) have been tested for producing valuable feedstock for bioenergy productions on contaminated soils (Lucas-Borja et al., 2011; Evangelou et al., 2012; Kidd et al., 2015; Mola-Yudego et al., 2015; Gonsalvesh et al., 2016). The lignocellulosic biomass of such plants can be either used to produce heat and electricity by direct combustion, transformed by pyrolysis and gasification into biofuels and biochars or used for producing derived bioproducts notably platform chemicals (Bridgwater, 2006; Nsanganwimana et al., 2014). Poplar is a fast growing tree, producing large yields, tolerant to high TE exposures, with low contaminant accumulation in the wood (Cd/Zn are mainly accumulated in the leaves) and having high energy potential (Calfapietra et al., 2010). *Arundo donax* is a productive species, highly stress resilient (e.g. cold, drought, salinity, and extreme pH) (Mantineo et al., 2009), tolerant to high TE exposure (Elhawat et al., 2015), usable by many conversion chains, e.g. energy sector, litter, pulp and building materials (Scordia et al., 2012; Nsanganwimana et al., 2014).

In situ stabilization combined with either *Populus nigra* L. or *A. donax* cultivation is a potential option to produce a valuable biomass while stabilizing Cu-contaminated soils at wood preservation sites. Therefore, we investigated the long-term aging effect of biochar, alone and combined with iron grit, in such Cu-contaminated soil cultivated with both plant species through (1) Cu and nutrient concentrations in the soil pore water, (2) biomass production of *P. nigra* and *A. donax*, and (3) shoot Cu concentrations and removals.

2. Material and methods

2.1. Soil, amendments and soil treatments

The wood preservation site (about 10 ha, Saint-Médard d'Eyrans, Gironde, SW France; 44°43.353′ N, 000°30.938′ W) has been used for over a century, with various Cu-based salts (mainly Cu-sulphate based on the duration and amount used) as wood preservatives (Mench and Bes, 2009). Topsoil (Unt, 0–25 cm, Fluvisol - Eutric Gleysol, World Reference Base for soil resources) was collected (100 kg) at the P1-3 sub-site. This sandy soil (85.8% sand, 5.9% clay, and 8.3% silt; 1.3% OM; C/N 16), with a low CEC, is mainly contaminated by Cu (Table 1, Oustriere et al., 2016a), which largely exceeds its median and upper whisker background values in French sandy soils, but its total soil As and Cr are at background levels (Table 1, Baize, 2000; Villanneau et al., 2008). Soil was air-dried and homogenized after sieving through a 5 mm mesh.

Two treatments, a pine bark-derived biochar (B, pyrolysis of 420 °C for 180 s; not desalted; Florentaise, Saint-Mars-du-Désert, France) alone and combined with zerovalent iron grit (BZ), were trialed. Biochar was crushed, sieved at 2 mm and manually homogenized. Elemental composition, carbon (C) content and polycyclic aromatic hydrocarbon (PAH) concentrations were determined at the INRA Laboratoire d'Analyses des Sols (INRA LAS, Arras, France) with standard methods (Table 2). Zerovalent iron grit (Z, GH120, particle size <0.1 mm) was obtained from Wheelabrator Allevard, France (Bes and Mench, 2008). Amended soils were thoroughly homogenized in large plastic containers and individually prepared prior to use.

The pot experiment consisted in 3 soil treatments: (1) untreated Cu-contaminated soil (Unt); (%, w/w) (2) Unt + 1% B (B), (3) Unt + 1% B + 1% Z (BZ). Each treatment was made in 10 replicates. Soils (3 kg) were potted (5 L, plastic pots) and placed in a greenhouse. Potted soils, with a bottom cup to avoid any leaching, were watered and weekly maintained to 70% of water holding capacity (WHC, 10% of air-dried

Table 1
Main physico-chemical soil parameters.

Parameter	Mean ± SD	Background levels[a]
pH	7 ± 0.09	6.6
CEC	2.5 ± 0.2	
P_2O_5 (g kg^{-1})	0.03 ± 0.002	
Organic matter (g kg^{-1})	13 ± 0.4	
Organic C (g kg^{-1})	8 ± 0.3	14.5
Total N (g kg^{-1})	0.5 ± 0.01	
C/N	16 ± 0.2	10
Texture (g kg^{-1})		
Sand	858	≥650
Silt	83	≤350
Clay	59	≤180
Total TE (mg kg^{-1})		
Cr	21 ± 0.7	14–40
Cu	964 ± 20	3.2–8.4
Ni	5 ± 0.3	4.2–14.5
Zn	37 ± 1.6	17–48
As	7 ± 0.4	1–25[b]

[a] Frequent total concentrations in French sandy topsoils (Baize, 2000; Villanneau et al., 2008).

[b] Frequent total As concentrations for all French soil types (Bes and Mench, 2008).

Table 2

Composition of soil amendments. In bold, values exceeding the European Biochar Certificate V4.8 threshold values.

	B	Z	EBC[a]	IBI[b]	French upper critical threshold values for organic amendments[c]
pH	9.89		–	–	
CEC (cmol kg^{-1})	1.23		–	–	
Major elements (% DW)					
H	0.8		–	–	
N	0.3		–	–	
S	0.03		–	–	
Cl	0.01		–	–	
C	90.3		> 50%	Class 1: ≥ 60% Class 2: [30% - 60%] Class 3: [10% - 30]	
Nutrients (g kg^{-1})					
Ca	18.6		–	–	
K	13.8		–	–	
Mg	29.9		–	–	
Na	0.389		–	–	
P	0.105		–	–	
Elements (mg kg^{-1})					
Al	1650	600	–	–	
As	0.461	70	–	12–100	18
Cd	<0.5	0.03	1	1.4–39	3
Cr	45.9	3500	80	64–1200	300
Cu	230	1010	100	63–1500	120
Mn	–	7710	–	–	
Fe	4470	973,000	–	–	
Hg	<0.1		1	1–17	2
Ni	28.3	739	30	47–600	60
Pb	2.61	20	120	70–500	180
Zn	50.5	104	400	200–7000	600
PAHs (mg kg^{-1})					
Sum of 16 US EPA PAHs[d]	**163**		4	6–300	
Fluoranthene	7.48		–	–	4
Benzo(b)fluoranthene	0.149		–	–	2.5
Benzo(a)pyrene	<0.2		–	–	1.5
Naphtalene	101		–	–	

[a] Following Switzerland's Chemical Risk Reduction Act (ChemRRV) on recycling fertilizers.

[b] Range of Maximum Allowed Threshold values reflects different soil tolerance levels for these elements in compost, biosolids, or soils established by regulatory bodies in the US, Canada, EU and Australia (See Appendix 3 of the IBI Biochar Standards for further information).

[c] French upper critical threshold values for organic amendments (NF U 44 051, Dec. 2010).

[d] Corresponds to the PAH threshold values defined in the Swiss Chemical Risk Reduction Act (Chem RRV).

soil) with deionized water, and allowed to react for one month in May 2014. Thereafter, plant was cultivated from June 2014 to March 2016.

2.2. Plant testing

Stem cuttings of *P. nigra* and Giant reed (*A. donax*) (roughly 20 cm long) were collected in May 2013. Poplars were sampled on 4-year old trees growing at this wood preservation site. Giant reed plants were sampled from natural stand along a drainage ditch, San Remo, Italy, and cultivated since 2012 in a greenhouse. Stem cuttings were rooted in individual pots (9 * 8 * 9 cm^3) on perlite imbibed with a quarter-strength Hoagland nutrient solution (HNS, Marchand et al., 2014) for one year in a greenhouse. For each treatment, one standardized plant of either *P. nigra* or *A. donax* was transplanted in potted soils (five replicates) and cultivated during 22 months from June 2014 to March 2016 in a greenhouse. The experiment consisted in 6 treatments:

(1) untreated Cu-contaminated soil planted with *P. nigra* (*Pn*)
(2) B-amended soil planted with *P. nigra* (*Pn*B)
(3) BZ-amended soil planted with *P. nigra* (*Pn*BZ)
(4) untreated Cu-contaminated soil planted with *A. donax* (*Ad*)
(5) B-amended soil planted with *A. donax* (*Ad*B)
(6) BZ-amended soil planted with *A. donax* (*Ad*BZ).

Pots were arranged in a fully randomized block and maintained at 70% of WHC using deionized water without loss from drainage. Hoagland nutrient solution (250 mL) was applied each month in all pots to avoid nutrient deficiencies. Before the beginning of the 2015 growing season, in March, dry shoots of *A. donax* were harvested (Cut 1), 1 cm above the soil surface. In March 2016, the shoots and roots of *P. nigra* (Cut 1) and *A. donax* (Cut 2) were harvested. All harvested biomass were washed twice with deionized water, blotted with filter paper, placed in paper bags and oven dried at 60 °C to constant weight for 72 h and then weighed for determining the shoot and root DW yields.

2.3. Soil pore water and plant analysis

Dried shoots were ground (<1.0 mm particle size, Retsch MM200) then weighed aliquots (0.5 g DW pot^{-1}) were wet digested under microwaves (CEM Marsxpress 1200 W) with 5 mL supra-pure 14 M HNO_3 and 2 mL 30% (*v*/v) H_2O_2 not stabilized by phosphates. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Mineral composition (Al, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) in digests was determined by ICP-MS (Thermo X series 200) at the INRA USRAVE laboratory, Villenave-d'Ornon, France. All elements were recovered (>95%) according to the standard values and standard deviation for replicates was <5%. Shoot Cu removal was calculated as: Cu (μg plant^{-1}) = shoot DW yield (g plant^{-1}) x shoot Cu concentration (μg g^{-1} DW).

23 months after the soil amendment, the soil pore water (SPW) was collected in all pots just before the harvest of *P. nigra* and *A. donax* (March 2016, three times 10 mL) using Rhizon MOM moister samplers (Eijkelkamp Agrisearch Equipment, The Netherlands) placed in January 2016 and samples kept at 4 °C prior to their analysis. The pH, electrical conductivity (EC), and Cu^{2+} concentration in the SPW samples were determined using electrodes (Hanna instruments, pH 210, combined electrode Ag/AgCl–34, Tetracon 325 WTW, and Cupric ion electrode, Fischer Bioblock, USA), respectively. Aluminum, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn were analyzed by ICP-OES (Varian Liberty 200). Dissolved organic carbon (DOC) in SPW samples was analyzed with a Shimadzu© TOC 5000A carbon analyzer. The measurement accuracy was checked by performing calibration with a standard reference solution of potassium hydrogen phthalate (KHP) at a concentration of 1000 mg C L^{-1}. Four DOC measures were performed for each SPW solution. The SPW composition was compared to previous values reported by Oustriere et al. (2016a) for the same unplanted soil treatments 3 months after soil amendment.

2.4. Statistical analysis

Influence of soil treatments on SPW parameters, shoot DW yields, shoot ionome and element removals were tested using one-way analysis of variance (ANOVAs). Shoot DW yields and shoot ionome of both plants were tested separately. Normality and homoscedasticity of residuals were met for all tests. When significant differences occurred between treatments, multiple comparisons of mean values were made using post-hoc Tukey HSD tests. Differences were considered statistically significant at $p < 0.05$. When element concentrations were below the detection limits in the Unt samples, influence of soil treatments were not statistically tested. All statistical analyses were performed using R software (version 3.0.3, Foundation for Statistical computing, Vienna, Austria).

3. Results

3.1. Soil pore water

3.1.1. pH, EC and nutrient concentrations

The Unt soil has a neutral SPW pH (Table 3). At month 3, biochar alone (B) and combined with iron grit (BZ) significantly increased the SPW pH (i.e. 7.3 ± 0.08 and 7.6 ± 0.05, respectively) and decreased the SPW EC (μS cm^{-1}) albeit not significantly (i.e. 483 ± 51 and 477 ± 110 respectively). At month 22 with *A. donax*, the SPW pH and EC rose significantly in all soil treatments from 6.9 ± 0.1 (Unt) to 8.1 ± 0.2 (*Ad*BZ) for the pH and from 1024 ± 117 (Unt) to 2037 ± 284 (*Ad*BZ) for the EC. In the *Pn* and *Pn*BZ treatments, the SPW pH did not differ from that of the Unt soil whereas it remained higher in the *Pn*B soil (i.e. 7.5 ± 0.06). The SPW EC increased in the *Pn*B soil (Month 22) as compared to the B soil (Month 3).

The SPW Ca concentration decreased in the B and BZ soils at month 3 and in all planted soils at month 22 as compared with the Unt soil. The SPW Fe and P concentrations remained below their detection limit, except for P in the B soil. At month 3, the SPW K concentration increased in the B and BZ soils but it decreased at month 22 for both plant species in all soils, and the SPW Mg concentration as well. The SPW Na concentration was significantly lower in the soils planted with *A. donax* as compared with the Unt soil.

3.1.2. DOM, Cu and Cu^{2+}

The DOM concentration in the SPW peaked in all soils cultivated with *A. donax*, being 3–5 fold higher than in the Unt soil (Fig. 1 A, B and C). In the *Pn* and *Pn*B soils, the DOM increased significantly, i.e. (mg L^{-1}) 24 ± 7 (*Pn*) and 23 ± 2 (*Pn*B) relative to the Unt soil (13 ± 4). Total Cu concentration in the SPW (mg L^{-1}) peaked in the *Ad* and *Ad*B soils (i.e. 0.53 ± 0.18 and 0.68 ± 0.26, respectively) as compared to the Unt soil (i.e. 0.22 ± 0.05). Conversely, adding Z into the Unt soil significantly decreased the SPW total Cu concentration only in the *Ad*BZ soil relative to *Ad* and *Ad*B. All treatments, except the *Pn* soil, significantly decreased the SPW Cu^{2+} concentration (mg L^{-1}) as compared to the Unt soil (i.e. 14.9 ± 5.3).

3.2. Plants

3.2.1. Plant growth parameters

At month 22, the root and shoot DW yields of *P. nigra* and *A. donax* for the B-and BZ-amended soils did not differ from those for the Unt soil (Fig. 2 A and B). The root DW yields (mg DW plant^{-1}) ranged from 17 ± 11 to 29 ± 9 for *P. nigra* and from 57 ± 12 to 84 ± 14 for *A. donax* whereas the shoot DW yields (mg DW plant^{-1}) range from 19 ± 7 to 25 ± 11 for *P. nigra* and from 22 ± 4 to 26 ± 3 for *A. donax*. For *A. donax*, the shoot DW yield produced at month 10 (Cut 1) was lower than that harvested at month 22 (Cut 2).

3.2.2. Shoot ionome

Shoot Cu concentration of *P. nigra* was significantly lower for the *Pn*BZ plants relative to the *Pn* plants (Table 4). For *A. donax*, the shoot Cu concentration was significantly higher for the *Ad*B plants relative to the *Ad* plants. Shoot Cu removal slightly dropped for the *Pn*BZ plants relative to the *Pn* plants and significantly increased for the *Ad*B plants as compared to the *Ad* ones, mainly due to changes in shoot Cu concentration. Shoot Ca, Fe, K, Mg, Na, P, Zn and Mn concentrations of poplar and Giant reed were globally similar on the amended and untreated soils, except two significant cases: shoot Na concentration was lower for the *Pn*BZ plants relative to the *Pn* ones, and shoot Mn concentration higher for the *Ad*BZ plants as compared to *Ad* plants. For *A. donax*, shoot element concentrations for the Cut 1 were globally higher than those for the Cut 2. Only shoot Na and Mg concentrations were higher for the *Ad* and *AdBZ* plants of Cut 2 relative to plants harvested in Cut 1 (Supplemental material 1).

4. Discussion

Due to biogeochemical reactions between soil phases, amendments, and biological organisms, the behavior of chemical elements (e.g. Cu) in amended soils may change over time, which is referred as “aging” (Kookana, 2010). Plant rhizodeposition and associated microorganisms may influence soil Cu speciation, especially when cultivated in a long-time basis (Merino et al., 2015). Here, we discuss the aging effect on the efficiency of biochar, alone and combined with iron grit, and how plants and amendments may influence Cu (im)mobilization and crop productivity for this Cu-contaminated soil from a wood preservation site.

4.1. Soil pore water

4.1.1. Composition at month 3

At short-term (3 months), the B amendment decreased Cu^{2+} concentration while the BZ combination reduced both Cu^{2+} and Cu

Table 3
Comparison of physico-chemical parameters of soil pore waters, 3 and 22 months after soil amendment.

Treatments			(μS cm^{-1})	Nutrients (mg L^{-1})					
		pH	EC	Ca	Fe	K	Mg	Na	P
SPW: Month 3									
Unt	(n = 6)	6.9 ± 0.1 f	1024 ± 117 cd	143 ± 39 a	<0.02	14 ± 5 b	5 ± 2 a	12 ± 3 ab	<0.2
B	(n = 3)	7.3 ± 0.08 def	483 ± 51 d	59 ± 5 **b**	<0.02	43 ± 11 **a**	3 ± 0.3 a	13 ± 2 a	0.3 ± 0.05
BZ	(n = 3)	7.6 ± 0.05 **bcd**	477 ± 110 d	46 ± 20 **b**	<0.02	53 ± 16 **a**	3 ± 1 ab	17 ± 6 a	<0.2
SPW: Month 22									
Pn	(n = 5)	7.1 ± 0.1 ef	890 ± 171 cd	2.5 ± 0.5 **c**	<0.02	4.1 ± 0.5 **c**	1.1 ± 0.2 **c**	9.7 ± 1.9 abc	<0.2
*Pn*B	(n = 5)	7.5 ± 0.06 **cde**	1299 ± 118 bc	3.4 ± 0.3 **c**	<0.02	6.3 ± 0.8 bc	1.4 ± 0.1 **bc**	12.9 ± 1.1 a	<0.2
*Pn*BZ	(n = 5)	7.2 ± 0.1 def	755 ± 231 cd	2.1 ± 0.6 **c**	<0.02	4 ± 1.1 **c**	1.1 ± 0.2 **c**	5.7 ± 2.8 bcd	<0.2
Ad	(n = 5)	7.9 ± 0.2 **abc**	1737 ± 300 **ab**	<0.5	<0.02	1 ± 0.3 **d**	0.8 ± 0.4 **c**	< 0.5	<0.2
*Ad*B	(n = 5)	8.0 ± 0.3 **ab**	1819 ± 529 **ab**	1.1 ± 0.5 **c**	<0.02	1 ± 0.5 **de**	1.3 ± 0.2 **bc**	3.8 ± 3.9 **cd**	<0.2
*Ad*BZ	(n = 5)	8.1 ± 0.2 **a**	2037 ± 284 **a**	0.8 ± 1.1 **c**	<0.02	0.5 ± 0.3 **e**	1.1 ± 0.6 **c**	1 ± 1.5 **d**	<0.2
Common values in sandy soils (Oustriere et al., 2016b)		–	–	143 ± 66	0.05 ± 0.01	27 ± 44	21 ± 11	63 ± 12	5 ± 2

SPW: soil pore water; Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value b 0.05). Mean values followed by letters in bold are significantly different as compared to the Unt soil.

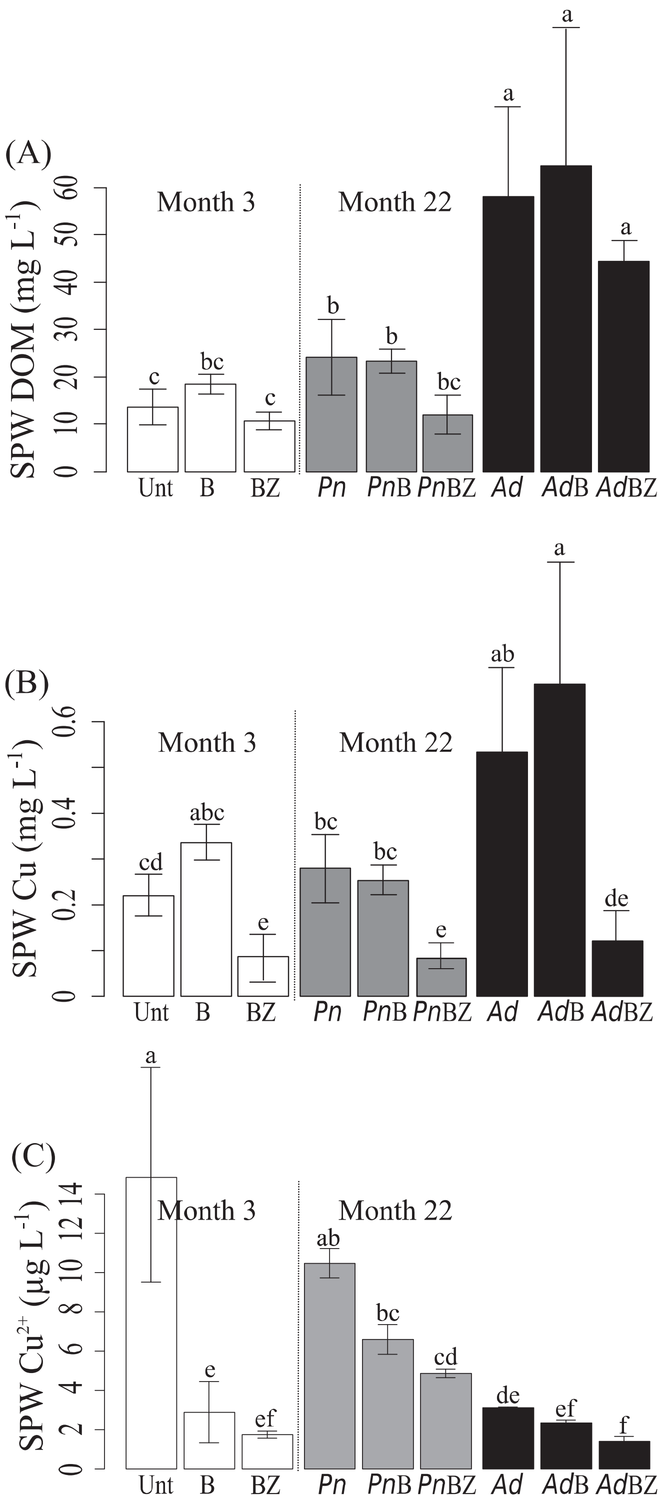

Fig. 1. (A) DOM, (B) total Cu and (C) Cu^{2+} concentrations in the soil pore water; Cu contaminated soil (Unt, white), amended with either biochar (B) or biochar plus iron grit (BZ) in the Cu contaminated soil cultivated with *Populus nigra* (*Pn*, grey) or *Arundo donax* (*Ad*, black), alone and amended with either biochar (*Pn*B and *Ad*B) or biochar plus iron grit (*Pn*BZ and *Ad*BZ). Mean values per treatment (n = 5, Unt: n = 6). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

concentrations in the soil pore water (Oustriere et al., 2016a). In the B soils, the decreased SPW Cu^{2+} concentrations were associated with increased soil pH and SPW P and DOM concentrations and decreased SPW Ca concentrations. Based on X-ray absorption fine structure spectroscopy (XAFS), Cu^{2+} sorption onto biochar is pH dependent (Ippolito et al., 2012). Free Cu^{2+} may have (1) form inner sphere complexes with the active functional groups of biochar surface in line with SPW pH, (2) precipitate with phosphates and carbonates based on biochar composition (Table 2), and (3) form soluble Cu complexes with DOM increasing total Cu concentration in the SPW. Adding Z in combination with B increase soil pH. In the BZ treatment, free Cu^{2+} ions may primarily be sorbed on Fe/Mn oxy-hydroxides, potentially by bidentate inner-sphere complexes with Fe (hydr)oxides (Oustriere et al., 2016a; Tiberg et al., 2016).

4.1.2. Composition at month 22

Poplar and aging effect poorly affected pH, DOM and total Cu concentrations in the soil pore water (Table 3; Fig. 1), which agreed with previous studies: pH and total Cu in the SPW did not change over 36 months in a poly-contaminated soil at Aznalcázar, South West of Spain, cultivated with *Populus alba* L. (Ciadamidaro et al., 2013); pH and total Cu in the SPW were similar after 5 years in the untreated and compost-amended field plots cultivated with *Populus trichocarpa* × *deltoides* cv. Beaupré at the P7 sub-site (soil contamination with Cu and PAHs) of this wood preservation site (Mench, unpublished data). Some studies on biochars supported the lack of aging effect on pH and DOM and total Cu concentrations in the soil pore water: in a PAH-contaminated soil, soil pH remained steady after artificial chemical, biological, and physical aging of corn stover residues-derived biochar (Hale et al., 2011). The soil pH was kept between 7.9 and 8.1 over a 3-year period in a (Ni/Zn)-contaminated soil, amended by broadleaf hardwood-derived biochar, Castleford, UK, (Shen et al., 2016). After 3 years, SOM and ($CaCl_2$)-extractable Cu concentration were stable in a (Cu/Cd)-contaminated soil from a former Cu mine, amended by corn-straw-derived biochar (Li et al., 2016). At month 22, the SPW Cu^{2+} concentration increased in the *Pn*B and *Pn*BZ soils as compared to the B and BZ ones (at month 3) (Fig. 2). This might be explained by: (1) less potential precipitation with phosphates in line with lower SPW P concentration in the *Pn* soil than in the B one, and (2) decrease of the sorption capacity of biochar over the time (Martin et al., 2012). Rozada et al. (2008) suggested that Cu^{2+} sorption on biochar surface was easily reversible. When biochar aged, the proportion of oxygen-containing acidic functional groups (e.g., COO^{-}, COH and OH) increases on the biochar surface, while carboxyl groups slightly decrease (Nguyen and Lehmann, 2009; Guo et al., 2014), contributing to the negative charge of the biochar. Their association with cations (i.e. Ca, K and Mg), in line with the low SPW cation concentrations (Table 3), and strong affinity for water and DOM may decrease the sorption capacity of biochar for free Cu^{2+} (Li et al., 2016). In addition, gradual coating and interactions with OM and inorganic phases (Sorrenti et al., 2016) and microbial colonization may decrease the reactivity of the biochar surface. After 4 years, aging increased biochar skeletal density and reduced the water imbibition rate within fragments as a consequence of partial pore clogging (Sorrenti et al., 2016). The cation exchange capacity (CEC) and adsorption capacity of Cu(II) on the aged biochar were smaller than those of new biochar in a 300 day incubation time pot experiment (Guo et al., 2014). Here, aged biochar particles after cultivation could be further retrieved from the amended soils to assess their composition and reactivity and evidence the main mechanisms.

Enhanced SPW pH and DOM concentration in all amended soils planted with *A. donax* (Table 3; Fig. 1) agreed with an increase in soil pH (5–9%) after a 3-month cultivation of *A. donax* on red mud-contaminated soil (Alshaal et al., 2013). Change in SPW pH may partly result from root activity, imbalance uptake of anions (e.g. NO_3^-) vs. cations increasing the rhizosphere pH in the basal root zone (Hinsinger et al., 2003; Bravin et al., 2009; Qasim et al., 2016). Calcium uptake by plant, in line with low SPW Ca concentration (Table 3), may initiate a further cation desorption from the solid phase, promote H^+ sorption and increase soil pH. In addition, increased SPW pH may be related to the higher SPW DOM concentration (Fig. 1; Zhang et al., 2014), which was previously reported in soils cultivated with *A. donax* (Riffaldi et al., 2010). DOM may originate from decayed plant litter, microbial

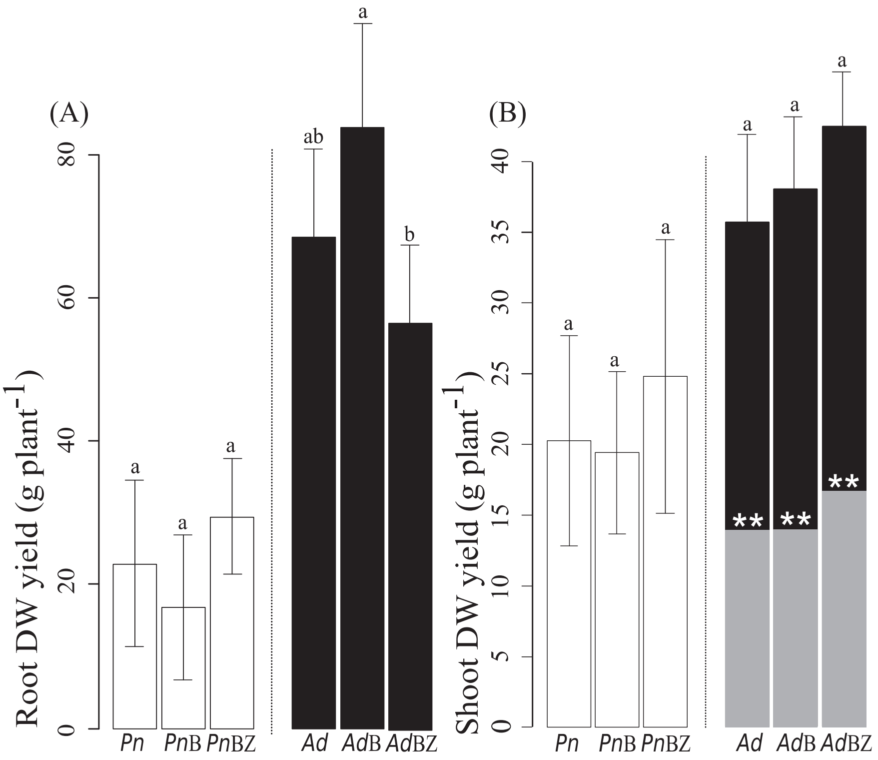

Fig. 2. (A) Root and (B) shoot DW yields of *P. nigra* (*Pn*, white, month 22) and *A. donax* (*Ad*, grey, and black) (mg DW plant^{-1}) at month 22 in the Cu-contaminated soil (Unt) and in the soil amended with either biochar (*Pn*B and *Ad*B) or biochar plus iron grit (*Pn*BZ and *Ad*BZ). The grey bars correspond to the biomass at month 10 (Cut 1). Mean values per treatment (n = 5). Values with different letters differ significantly (one way ANOVA, p-value <0.05).

and root depositions, and hydrolysis of insoluble SOM (Haynes, 2005). No leachate from plant litter was produced here as shoots were harvested, but *A. donax* formed a dense mat of roots and rhizomes in the soil (Fig. 2). Giant reed rhizodeposition can highly contribute to SOM available for microbial activity and the DOM pool (Cattaneo et al., 2014; Nsanganwimana et al., 2014; Monti and Zegada-Lizarazu, 2016). Enhanced microbial activity may stimulate both the degradation of SOM and production of microbial DOM (Kiikkilä et al., 2012; Hagedorn et al., 2015). In *A. donax* cultivated soil, pH increased between 7.9 ± 0.2 (*Ad*) and 8.1 ± 0.2 (*Ad*BZ) as compared to the Unt soil (i.e. 6.9 ± 0.1), which may promote DOM solubilization (Fang et al., 2016). At month 22, the SPW Cu concentration increased in the *Ad* and *Ad*B soils as compared to the B and BZ ones (at month 3) (Fig. 2). Enhanced DOM concentration may form soluble complexes with Cu and increase its SPW concentration (Beesley et al., 2010; Karami et al., 2011). Here, Giant reed-derived-DOM may act as competitive ligands preventing Cu retention on biochar surfaces (Beesley et al., 2014). In parallel, with aging, negative charges on biochar surface may form direct or indirect surface complexes with soil components. In addition, coating of DOM may gradually mask sorptive surfaces, thus limiting Cu sorption (Pignatello et al., 2006).

In contrast, the SPW Cu^{2+} and Cu concentrations in the *Ad*BZ and *Pn*BZ soils were lower than in the untreated and biochar-amended-soils cultivated with *A. donax* and *P. nigra*, albeit not significantly for Cu^{2+} as compared to *Ad*B and *Pn*B. The SPW Cu^{2+} and Cu concentrations in the *Ad*BZ and *Pn*BZ soils at month 22 were as low as in the BZ soil at month 3 after soil amendment, albeit not significantly for Cu^{2+} in the *Ad*BZ soil. Cationic metal species such as Cu^{2+} or DOM-Cu complex have high affinity for Fe oxides and can sorb on the newly formed Fe and Mn oxy-hydroxides after Z corrosion in the soil (Kumpiene et al.,

Table 4
Shoot ionome and shoot Cu removal of *P. nigra* and *A. donax* at month 22 (n = 5).

Treatment	**Cu**		**Shoot nutrient concentrations (g kg^{-1})**						**Shoot metal concentrations (mg kg^{-1})**	
	Shoot Cu concentration (mg kg^{-1} DW)	Shoot Cu removal (µg plant^{-1})	**Ca**	**Fe**	**K**	**Mg**	**Na**	**P**	**Zn**	**Mn**
Pn	5.1± 0.7 a	2.6 ± 0.4 a	5.3 ± 1.1 a	0.019 ± 0.005 a	5.8 ± 0.3 a	0.87 ± 0.21 a	1.06 ± 0.28 a	1.24 ± 0.48 a	25 ± 6 a	8 ± 2 a
*Pn*B	4.3 ± 0.4 ab	2.2 ± 0.2 ab	5.1 ± 0.9 a	0.015 ± 0.002 a	6.6 ± 1.1 a	0.89 ± 0.12 a	0.68 ± 0.29 ab	1.2 ± 0.16 a	25 ± 8 a	10.4 ± 4 a
*Pn*BZ	4.0 ± 0.5 **b**	2.0 ± 0.2 **b**	4.8 ± 0.9 a	0.016 ± 0.003 a	6.5 ± 1 a	0.84 ± 0.12 a	0.47 ± 0.15 **b**	1.24 ± 0.19 a	32 ± 6 a	9.8 ± 2 a
Ad	4.4 ± 0.7 **b**	2.2 ± 0.4 **b**	2.8 ± 1.6 a	0.015 ± 0.003 a	6.1 ± 1.2 a	1.49 ± 0.63 a	0.19 ± 0.05 a	0.16 ± 0.03 a	6 ± 4 a	13 ± 7b
*Ad*B	7.0 ± 2 a	3.5 ± 1.2 a	3.2 ± 0.8 a	0.016 ± 0.004 a	8.3 ± 1.4 a	1.68 ± 0.43 a	0.25 ± 0.1 a	0.25 ± 0.06 a	9 ± 5 a	14 ± 6 b
*Ad*BZ	5.2 ± 1 ab	2.6 ± 0.5 ab	3.2 ± 0.7 a	0.021 ± 0.007 a	8.7 ± 2.6 a	1.77 ± 0.28 a	0.2 ± 0.09 a	0.19 ± 0.03 a	7 ± 5 a	32 ± 2 a
Common values *	3–20	-	1–50	0.02–0.3	20–50	1.5–3.5	-	1.6–6.0		

Mean value ± SD for each treatment. Values with different letters differ significantly (one way ANOVA, p-value <0.05). Letters in bold indicated significant differences.

[*] (Tremel-Schaub and Feix,2005) .

2008; Komárek et al., 2013). The sorption of Cu on Fe and Mn oxy-hydroxides is pH-dependent with stronger sorption at high pH. The additional liming effect of biochar can favor the net negative surface charge of Fe and Mn oxy-hydroxides and enhance Cu sorption. Tiberg et al. (2016) investigated Cu-contaminated soils collected in untreated and *Z*-treated plots at our sampling sub-site after 6 years: the bound Cu was primarily associated to SOM in the Unt soil whereas with Z addition and increased soil pH, Cu sorption shifted towards metal (hydr)oxides. Based on the Cu-ferrihydrite EXAFS spectrum, Cu was found to primarily bind as inner-sphere bidentate complexes with iron (hydr)oxide (Tiberg et al., 2016).

4.2. Plants

At month 3, this biochar alone and combined with iron grit did not promote the root and shoot yields of dwarf beans (Oustriere et al., 2016a). At month 22, this result was similar for *P. nigra* and *A. donax* (Fig. 2) and agreed with some studies: root and shoot biomass produced by *Lolium multiflorum* Lam. was unchanged in a (Cd, Zn, and Pb)-contaminated soil amended with 1% miscanthus-derived-biochar after 28 days and 56 days (Houben et al., 2013). After 2 years, grain yields and biomass of wheat were not affected by both rate 10 and 40 t ha^{-1} of wheat straw-derived biochar addition in a Cd contaminated paddy soil (Cui et al., 2012). Germination of grass (mix of *Festuca rubra* L. and *Lolium perenne* L.) on site failed in a 3-year field experiment on a contaminated (Ni/Zn) soil amended with broad leaf hardwood-derived biochar (Shen et al., 2016). Here, despite HSN supply, Ca, Fe, K, Mg and P sub-deficiencies might be suggested as their concentrations in *P. nigra* and *A. donax* shoots were below or in the low ranges as compared to common values (Tremel-Schaub and Feix, 2005, Table 4). At month 22, Ca, Fe, K, Mg and P concentrations in the SPW were low as compared to common values in sandy soil (Table 3), likely due to (1) reaction with soil and amendment phases (Cheng et al., 2014), and (2) root uptake. Nutrient concentrations in *A. donax* shoot even decreased between the Cut 1 and the Cut 2 (Supplemental material 1). It may reflect a dilution effect as shoot DW yield increased. The shoot Cu concentrations of *P. nigra* and *A. donax* (mg kg^{-1}) were similar across the treatments, ranging from 4.0 ± 0.5 (*Pn*BZ) to 7.0 ± 2 (*Ad*BZ) (Table 4), and in the range of common values (3–20 mg kg^{-1}, Tremel-Schaub and Feix, 2005). Stem and foliar Cu concentrations (mg kg^{-1}) reached 3.21 and 7.17 for *A. donax* on a poly-contaminated urban stream (Bonanno et al., 2013). Shoot Cu concentration ranged between 7 and 17 mg kg^{-1} in *P. alba* grown for 3 years in a Cu-contaminated soil (Ciadamidaro et al., 2013). Giant reed and poplars are Cu excluders accumulating Cu in their roots, which may explain their low shoot Cu concentration (Kabata-Pendias, 2001; Bonanno et al., 2013; Ciadamidaro et al., 2013; Elhawat et al., 2014).

4.3. Practical implication

Effect of plant species: Giant reed was more efficient than *P. nigra* to decrease Cu^{2+} exposure in its rhizosphere but increased total Cu concentration in the soil pore water, which may potentially enhance Cu leaching from the root zone (Fig. 1). After 2 years, *P. nigra* did not contribute to Cu immobilization (Fig. 1). Without soil amendment, both plant species were unable to stabilize Cu in excess.

Effect of soil amendment: combining iron grit with biochar promoted Cu stabilization but not the growth of *A. donax* and *P. nigra*. An additional fertilization may be required. Long-term field plots must investigate the sustainability of Cu stabilization, biomass production of non-food crops and the life cycling of such phytomanagement option.

5. Conclusion

After a 22-month reaction period, the SPW Cu^{2+} concentration increased again in the B and BZ soils cultivated with *P. nigra* as compared to its value at month 3. Cultivation of *A. donax* incremented the DOM concentration in the soil pore water. Such high SPW DOM concentration in the Giant reed-planted soils, which may induce the formation of Cu-DOM complexes, matched with a decrease of Cu^{2+} concentration but increased total Cu concentration in the soil pore water. Iron grit with biochar was more effective to stabilize soil Cu than biochar alone with both *A. donax* and *P. nigra* cultivation. Biochar alone and combined with iron grit did not promote the root and shoot yields of *A. donax* and *P. nigra* as compared to the untreated soil but the shoot Cu concentrations were in the common ranges for both plants.

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