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Root development of non-accumulating and hyperaccumulating plants in metal-contaminated soils amended with biochar

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HIGHLIGHTS

- Biochar induced root proliferation in an acidic contaminated soil.
- Root surface increased when the availability of metals or nutrients decreased.
- The increase of root surface was able to cause a higher plant metal uptake.

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ABSTRACT

Biochar may be used as an amendment in contaminated soils in phytoremediation processes. The mechanisms controlling plant metal uptake in biochar-amended soils remain however unclear. This work aimed at evaluating the influence of biochar on root development and its consequence on plant metal uptake, for two non-hyperaccumulating plants (*Zea mays* and *Lolium perenne*) and one hyperaccumulator of Cd and Zn (*Noccaea caerulescens*). We conducted rhizobox experiments using one acidic and one alkaline soil contaminated with Cd, Pb and Zn. Biochar was present either homogeneously in the whole soil profile or localized in specific zones. A phenomenon of root proliferation specific to biochar-amended zones was seen on the heterogeneous profiles of the acidic soil and interpreted by a decrease of soil phytotoxicity in these zones. Biochar amendments also favored root growth in the alkaline soil as a result of the lower availability of certain nutrients in the amended soil. This increase of root surface led to a higher accumulation of metals in roots of *Z. mays* in the acidic soil and in shoots of *N. caerulescens* in the alkaline soil. In conclusion, biochar can have antagonist effects on plant metal uptake by decreasing metal availability, on one hand, and by increasing root surface and inducing root proliferation, on the other hand.

1. Introduction

The use of biochar has recently been investigated for *in situ* remediation of contaminated lands in association with plants (Beesley et al., 2011; Rees et al., 2015). In general, the uptake of divalent metal by most of the plant species decreases in the presence of biochar (Namgay et al., 2010; Cui, 2011; Karami et al., 2011; Park et al., 2011; Zheng et al., 2012; Houben et al., 2013; Zhang et al., 2013). This may be caused by a decrease of soil metal mobility in the presence of biochar, due to the direct sorption of metals on biochar or to indirect effects, e.g. an increase of soil pH (Cui, 2011; Houben et al., 2013; Rees et al., 2014).

Despite this general trend, a few works have reported no change or even an increase in plant metal uptake in the presence of biochar amendments (Gartler et al., 2013; Hu et al., 2014; Fellet et al., 2014). An increase of Cd and Zn uptake by the hyperaccumulating plant *Noccaea caerulescens* with 5% (w/w) biochar was recently described (Rees et al., 2015). Such plant response in the presence of biochar may be due to direct or indirect mechanisms involving root development that have counteracted the decrease of metal availability caused by biochar and promoted a higher uptake by the plant.

Despite the existence of various studies investigating biochar's effects on plant growth (Jeffery et al., 2011; Spokas et al., 2012; Crane-Droesch et al., 2013), the influence of biochar on root development in contaminated soils has been scarcely investigated. A direct link was recorded between the decrease of Cu availability in the soil and the better development of *Zea mays* roots with biochar (Brennan et al., 2014). However, an avoidance of

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biochar-amended zones by *Solanum lycopersicum* roots was observed in an As-contaminated soil and interpreted as a preferential root development toward zones with more available nutrients (Beesley et al., 2013). A better root development toward biochar in a non-contaminated soil was associated to a higher availability of nutrients (Prendergast-Miller et al., 2014).

We hypothesized that biochar can affect the development of roots by decreasing the availability of both metals and nutrients in soil, thus influencing the uptake of metals by the plant. Plant growth experiments were conducted in rhizoboxes in order to monitor the development of roots in two contaminated soils amended with biochar. Biochar was introduced either homogeneously in the soil profile or heterogeneously to reveal a potential tropism of roots toward biochar.

2. Material and methods

2.1. Materials

Biochar and soils had the same origin as in previous works (Rees et al., 2014, 2015). Briefly, biochar was produced by pyrolysis at 450 °C for 36 h from a mix of hardwood and softwood. Two soils (A and B) were sampled near Pb and Zn smelters in the North of France (Sterckeman et al., 2002). Soils corresponded to Redoxic Cambisols (IUSS Working Group WRB, 2006). Large concentrations of Cd, Pb and Zn were recorded in the surface layer as a result of atmospheric deposition. Biochar and soil samples were air-dried, homogenized and sieved to <2 mm before use.

Water holding capacity (WHC) was measured as the water content of soil samples adjusted to a water potential of 10^4 Pa (pF 2) using a pressurized ceramic plate (Labotest 11500). Soils and biochar were analyzed by the *Laboratoire d'Analyse des Sols* of INRA-Arras using standard techniques (Rees et al., 2015). Biochar and soil properties are indicated in Table 1. Biochar had a high pH value (9.5), and Ca^{2+} and K^+ were the dominant exchangeable cations. Both soils had similar total metal content but they differed in pH value (5.9 for soil A and 8.0 for soil B) and $CaCO_3$ content. Exchangeable metals were considerably larger in soil A.

Two non-metal accumulating plant species were used: maize (*Z. mays*, hybrid MB362 created by INRA) and ryegrass (*L. perenne*, Prana). The third plant species was a hyperaccumulator of Cd and Zn, alpine pennycress (*N. caerulescens* (J. Presl & C. Presl) F.K. Mey, Ganges population).

2.2. Methods

Twenty-four soil profiles (22 × 11 × 8 cm) were built in the upper part of rhizoboxes, the lower part being used to allow free drainage of the bottom of the profile. Two designs were used (Fig. 1). In the first profiles, soil A profiles (P1-A) or soil B profiles (P1-B) were divided in eight cubic zones, with four containing 5% (w/w) biochar and four remaining unamended. The two types of zones were alternated as on a chessboard. In the second profiles, only soil B was used as a homogeneous substrate with 0% (P2-$B_{0\%}$) or 5% biochar (P2-$B_{5\%}$). The total mass of soil introduced in each profile varied according to the treatment: 1050 g of unamended soil + 950 g of amended soil in profiles P1, and 2100 g of unamended soil and 1900 g of amended soil in profiles P2-B. Soil–biochar mixtures were prepared by thoroughly mixing dry soil and biochar by hand for 10 min. The same mixing procedure was kept for soil alone to ensure homogeneity. Rhizoboxes were filled horizontally with soil or soil–biochar mixtures. In profiles P1-A and P1-B, the eight cubic zones were filled in parallel using a plastic grid. The same method was used for filling profiles P2-B. Each visible soil profile was covered by a glass window, 2 mm thick (Fig. 1).

Table 1
Properties of soil A, soil B and biochar. Values slightly differ from previous works (Rees et al., 2014, 2015) because of the difference of sampling points. n.a.: non available.

Group	Measure	Soil A	Soil B	Biochar
Particle size distribution (%)	<2 μm	20.1	15.5	n.a.
	2–20 μm	18.1	20	n.a.
	20–50 μm	29.7	40	n.a.
	50–200 μm	19.4	21	n.a.
	200–2000 μm	12.7	3.5	n.a.
pH in water	pH	5.90	8.04	9.46
Main content	Organic C ($g\ kg_{DW}^{-1}$)	31	19	680
	$CaCO_3$ ($g\ kg_{DW}^{-1}$)	0	13	28
	Total N ($g\ kg_{DW}^{-1}$)	1.2	1.1	1.8
	C/N	26	18	372
Available P	P_2O_5 (Olsen) ($mg\ kg_{DW}^{-1}$)	0.025	0.159	0.102
CEC	CEC ($cmol^+\ kg_{DW}^{-1}$)	12.6	9.7	4.6
Exchangeable Na, Ca, Mg, K	Na ($cmol^+\ kg_{DW}^{-1}$)	0.092	0.041	0.30
	Ca ($cmol^+\ kg_{DW}^{-1}$)	7.5	30	28
	Mg ($cmol^+\ kg_{DW}^{-1}$)	1.24	0.54	2.25
	K ($cmol^+\ kg_{DW}^{-1}$)	0.57	0.55	6.86
Total elements	Cd ($mg\ kg_{DW}^{-1}$)	18.2	19.4	0.39
	Cu ($mg\ kg_{DW}^{-1}$)	91.3	38.2	13.7
	Ni ($mg\ kg_{DW}^{-1}$)	23.1	16.9	6.1
	Pb ($mg\ kg_{DW}^{-1}$)	1850	964	7.18
	Zn ($mg\ kg_{DW}^{-1}$)	3350	1440	136
Exchangeable elements with $CaCl_2$ 0.01 M	Cd ($\mu g\ kg_{DW}^{-1}$)	5730	275	0
	Cu ($\mu g\ kg_{DW}^{-1}$)	251	285	0
	Ni ($\mu g\ kg_{DW}^{-1}$)	592	88.4	0
	Pb ($\mu g\ kg_{DW}^{-1}$)	1660	73.3	0
	Zn ($\mu g\ kg_{DW}^{-1}$)	583000	2660	30

Rhizoboxes were installed in randomized blocks in a growth chamber (day: 16 h at 23 °C with 50% air humidity; night: 8 h at 15 °C with 85% air humidity). They were set on a rack with an angle of 45° to force the growth of roots along the glass window in the darkness (Fig. 1). Soil moisture was daily adjusted by a vertical watering to 85% of average field capacity of each soil profile. In profiles P1-A and P1-B, three plant species (*Z. mays*, *L. perenne* and *N. caerulescens*) were grown in two rhizobox replicates. In profiles P2-B, two plant species (*L. perenne* and *N. caerulescens*) were grown in three rhizobox replicates. In order to allow a sufficient equilibration time, *N. caerulescens* was sown after two weeks of incubation, while *Z. mays* and *L. perenne* were introduced after a six-week incubation. One 60 h-seedling of *Z. mays*, three seeds of *N. caerulescens* (to keep one seedling after two weeks) or nine seeds of *L. perenne* were placed on the soil surface of each of the four upper squares in profiles P1-A, P1-B and P2-B. Contrary to the two other species, the number of seedlings of *L. perenne* per zone depended on the rate of germination.

Before harvest, soil profiles were scanned using a scanner Epson Perfection C750 Pro. *Z. mays* was harvested after 17 d of growth, *L. perenne* after 6 weeks and *N. caerulescens* after 9 weeks. Shoots were collected separately at the surface of each of the four upper squares. Roots were harvested by sieving to 2 mm each of the eight cubic zones of profiles P1-A and P2-B and each of the four vertical zones of profiles P2-B (Fig. 1). Soil subsamples were collected in each zone near glass surface and dried at 55 °C for 72 h. Soil pH was measured in water (1:5 (v/v), NF ISO 10390). Soil extractible elements were measured in $CaCl_2$-extracts (0,01 M; 1:10 (w/v) after NEN 5704 standard); suspensions were centrifuged and filtered to 0.2 μm to measure dissolved C (TOC-VSCN equipment, Shimadzu) and elemental composition by ICP-AES (aiCAP6300 Duo, ThermoScientific). Shoots and roots were washed with